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EFFECT OF SUBLETHAL METAL POLLUTANTS ON THE FIDDLER CRAB *Uca pugilator*

Environmental Research Laboratory
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EFFECT OF SUBLETHAL METAL POLLUTANTS ON THE FIDDLER CRAB UCA PUGILATOR

by

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ABSTRACT

Studies have been carried out on the synergistic effects of sublethal concentrations of mercury (Hg) and/or cadmium (Cd) in conjunction with temperature and salinity stress on larval and adult fiddler crabs, Uca pugilator. Six biological parameters of the adult organism were monitored including survival, tissue uptake, metabolism, behavior, microscopic anatomy, and enzymatic activity, using metal concentrations of 0.18 ppm Hg and 1.0 ppm Cd. Studies with larval stages (zoeal stages I, III, V and megalops) considered survival, metabolism and behavior under conditions of 1.8 ppb Hg and 1.0 ppb Cd.

The effect of mercury or cadmium on Uca pugilator depends upon a number of factors, including stage of the life cycle, sex, thermal history, and environmental conditions. Adults survive for at least six weeks in 0.18 ppm Hg, while larval stages are much more sensitive and survive only a few hours in this concentration. Adult males are more sensitive to chronic mercury exposure than are females, and mercury is most toxic at low temperatures and low salinities. Tissue concentration of mercury is dependent on the environmental regime, but total body burden is not. Larvae are also more sensitive to Cd than are adults, but no mortality difference between sexes is seen; cadmium is most toxic at high temperatures and low salinities. Both tissue distribution and total body burden of cadmium are dependent upon the temperature-salinity regime. Data presented here suggest that the mode of action of the two metals is not the same.

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CONTENTS

FIGURES

FIGURES (cont.)

FIGURES (cont.)

TABLES

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SECTION I

CONCLUSIONS

The effects of cadmium and mercury on Uca pugilator vary, depending upon a number of factors including stage of the life cycle, sex, thermal history, and environmental regime. Larvae are at least two orders of magnitude more sensitive to mercury than are adult stages. While concentrations of 0.18 ppm Hg and 1.0 ppm Cd were sublethal to adult animals, levels of only 1.8 ppb Hg and 1 ppb Cd were sublethal to larvae. Adult males are more sensitive to chronic mercury stress than are females. Mercury is most toxic at low temperatures and low salinities. Warm-acclimated animals (summer animals) are less tolerant of mercury at low temperatures than cold-acclimated (winter) ones, and concentrations of mercury that are sublethal under optimum conditions of temperature and salinity are lethal when temperature-salinity regimes become stressful. Distribution of mercury in the tissues of the crab is dependent on the environmental regime but the total body burden is not.

Adult crabs are less sensitive to cadmium poisoning than are larvae, but in contrast to mercury toxicity, there are no observed differences in mortality between males and females. Cadmium is most toxic at high temperature and low salinity, and both tissue distribution and total body burden of cadmium are dependent upon environmental conditions.

SECTION II

RECOMMENDATIONS

Under optimum temperature and salinity regimes, adult fiddler crabs tolerated mercury or cadmium concentrations in excess of values reported in polluted waters. Under stressful temperature-salinity conditions, however, sharply increased mortality occurred with both metals. Even under stressful conditions, however, adults tolerated much higher levels of cadmium or mercury than the larvae. Thus mortality studies with adults appear of limited value as a pollution bioassay, especially if the studies are conducted without due consideration of environmental conditions.

The additional response parameters used in these studies provided indices of the toxic properties and physiological fate of the metals in adults, but none readily lend themselves to use in routine bioassays. For example, the metabolic and behavioral studies on adults were time-consuming and required large numbrs of replicates before evaluation.

With all parameters tested larval stages were considerably more sensitive to heavy metal poisoning than were the adults. Modification of the activity pattern of larvae in the presence of toxic metals holds promise as an efficient and quick bioassay tool.

SECTION III

INTRODUCTION

In recent years there has been increased awareness of the growing competition between fisheries, industries and recreational uses of estuaries. As a result, water quality control for multiple use has become a vital problem. A key issue in assessing and subsequently controlling water quality has been the development of adequate monitoring measures. Bioassay methods offer possibilities but have not yet been widely exploited. Data presented in this report are the results of bioassay studies on a common species of Atlantic estuarine systems, the fiddler crab, Uca pugilator.

To provide realistic water quality standards it is necessary to know the effects of a pollutant over the full range of normal environmental fluctuations. One of the chief characteristics of estuaries is the large number of dynamic environmental variables; temperature, salinity, and light intensity are only a few of the factors which change from hour to hour. While organisms living in an estuarine environment are generally adapted to tolerate a wide range of such physical changes, the combination of a sublethal pollutant and a stressful environmental regime may prove lethal. This project delineates synergistic effects of sublethal levels of mercury (Hg) and cadmium (Cd) pollution upon U. pugilator over a range of temperature-salinity conditions. As noted by Waldichuk (1974), cadmium and mercury pollution studies have been primarily related to effects on humans, despite the fact that these metals are known to be toxic to aquatic organisms. Freshwater systems and estuaries receive much of the mercury released from industrial processes, including chloroalkali production, electroplating and paper manufacturers (Peakall and Lovett, 1972). Toxic cadmium salts contribute to environmental pollution via the waste discharge of electroplating and chemical plants. From these sources cadmium is flushed into the estuarine and marine environments (Holmes et al., 1974).

The fiddler crab is a major component of the intertidal fauna. Like many marine species, it has a sequence of planktonic developmental stages which occupy different niches. Larval U. pugilator are planktonic while adults occupy intertidal sand flat regions. Since the presence of a species is dependent upon the successful completion of its life cycle, it was considered crucial to assess both larval and adult responses in these studies.

Six main categories of biological response were analyzed in the adult: survival, heavy metal uptake rate, metabolic rate, swimming behavior, gill tissue anatomy and enzyme activity of selected tissues. The survival studies determined TLm of chronically-dosed adults as well as survival of gravid females and of their eggs after short exposures. Uptake of cadmium and mercury was determined for adults following chronic exposure to Hg and/or Cd under selected temperature-salinity regimes. In addition, the rate of loss of Hg from the tissues of adults which had been acutely slug-dosed and then removed to untreated sea water was also measured. Determinations were made of whole animal metabolism under Hg and Cd, and of tissue metabolism exposed to Hg in response to various temperature-salinity regimes. Behavioral studies assessed effects of Hg on total locomotor activity as well as on rhythmic components of activity. The effect of sublethal concentrations of Hg on gill tissue structure was examined by means of electron microscopy, using standard photographic techniques as well as scanning probe techniques for measuring the cellular distribution and concentration of these metals. Since previous studies on cytochrome c oxidase activity in the tissues of U. pugilator and other crabs indicated that this enzyme is important to acclimatization to temperature (Vernberg and Vernberg, 1967, 1968), enzymatic activity was determined in the hepatopancreas and gill of the crabs.

The larval studies paralleled adult studies wherever possible. First, the effects of three different chronic sublethal concentrations of Hg were determined for zoeal stages I, III, V, and megalops. Percent survival to megalopa stage and 50% survival time was measured. Other measurements included metabolic rate and swimming ability. Secondly, the synergistic effects of Hg in conjunction with temperature and salinity stress were evaluated using the criteria of survival, metabolism and phototactic response in a wide range of temperature-salinity conditions. Finally, the effects of sublethal cadmium on the same three parameters of survival, metabolism and behavior were considered with these larval stages.

SECTION IV

MATERIALS AND METHODS

Adult Uca pugilator were collected at the Belle W. Baruch Coastal Research station near Georgetown, South Carolina. In the laboratory they were maintained in plastic boxes containing approximately 100 cc filtered sea water. Boxes were tilted so that crabs could freely select total or partial immersion. Stock animals were routinely placed about 40 per box in environmental control chambers set at 25°C in 30 °/oo filtered sea water, with a 12L:12D light schedule for at least two weeks before use in an experimental program; they were fed on commercial fish pellets 3 times a week and the water was changed after each feeding.

For survival studies the adult crabs were maintained in environmental control chambers in plastic boxes containing 100 cc of the test solution. The solutions were changed three times weekly and crabs were checked daily to tally mortality until 50% had died.

For egg survival studies, crabs were brought into the laboratory in a non-gravid state in summer, kept at the control condition cited above, then examined daily until the egg sponge appeared. If the sponge did not appear in two weeks, the crabs were discarded. Sponge crabs were then placed individually in finger bowls with the desired solution for seven days, then returned to control sea water. Daily checks were made to determine the condition of the egg sponge, date of hatch, and condition of zoeae upon hatching.

Metabolic rates for whole animals and for hepatopancreas and gill tissues were measured in a Gilson apparatus. Acclimation and test temperatures are presented in the observation section. Results are expressed as microliters of oxygen consumed per hour per gram/wet weight. Significant difference of means was calculated by the method of Simpson et al. (1960) for small samples.

Locomotor activity of adults was measured quantitatively in various types of actographs. The chief type used was a Plexiglas® carousel suspended by delicate bearings on an axle; activity of the crab caused revolutions of the drum which activated a magnetic sensor. Activity counts were totaled either on an Esterline-Angus operations recorder or by numerical printout recorders. Printout data were used for calculating hourly and daily rates. For rhythmic analyses the printout data were processed by a computer periodogram program (Suter and Rawson, 1968).

Tissue uptake of mercury was monitored in gill, hepatopancreas, muscle, and carapace tissues. Tissues were removed from a group of crabs and

frozen immediately. The concentration of mercury in each tissue was determined using a Mercury Analyzer System 50 or a Coleman MAS 30. In addition, the radioisotope ^{203}Hg was utilized for measuring uptake in gill and hepatopancreas. Cadmium uptake was determined in gill, hepatopancreas, green gland and muscle using the radioisotope ^{109}Cd. Tissues exposed to ^{203}Hg or ^{109}Cd were digested in soluene and concentrations were determined by liquid scintillation on a Packard Tricarb Model 3320 counter.

For study of the effects of toxicants on the microanatomy of crab tissues, gill tissue from control and treated animals were examined under the electron microscope. The tissue was fixed in glutaraldehyde, then sectioned and examined with an electron transmission microscope. In addition, toxicant concentrations in the tissues were assessed using the scanning probe of an electron scanning microscope.

Enzymatic studies were conducted on the hepatopancreas and gill from crabs that had been cold-(10°C) or warm-acclimated (25°C) for periods of not less than 2 weeks or more than 4 weeks. Following acclimation these crabs were exposed to 0.18 ppm Hg as described above for periods of 1, 7, 14, 21 or 28 days. During this time the crabs were maintained at their acclimation temperatures (10 or 25°C). The tissues were dissected out from 5 crabs after scheduled exposure times and homogenized in a Tris (0.01M)-sucrose (0.25M)-EDTA (0.0001M) solution at a pH of 7.4. After homogenization the debris was spun down at 600 g at 0°-5°C, and the supernatant was used for the assays. The cytochrome c oxidase was then assayed spectrophotometrically by the method of Smith (1955), using a Gilford 2400 spectrophotometer. Assays on the test material from both cold- and warm-acclimated animals were made at 15°, 20°, 25° and 35°C. Four to five determinations were made for each tissue in the specified conditions. Results are expressed as m moles cytochrome c oxidized/mg protein/sec. Protein determinations were made on an aliquot sample of the supernatant by the method of Wadell (1956).

To obtain larvae, gravid females were collected from the intertidal flats of the Baruch Foundation property near Georgetown, South Carolina. The crabs were then brought into the laboratory, placed singly in finger bowls containing 30 °/°° filtered sea water and maintained in a constant temperature box at 25°C with a 12:12 LD light schedule. In a series of preliminary experiments, considerable variation in viability of larvae and general vigor of different hatches was noted. Therefore, new hatches were qualitatively rated and only active, vigorous groups having less than ten percent mortality at hatching were used for experiments. The larvae were reared in groups of 10 per finger bowl under various temperature-salinity regimes with and without Hg or Cd. Solutions were changed daily, and the food source of newly-hatched Artemia replenished at that time. Larvae were staged under 32 X magnification, using the morphological criteria of Hyman (1920). In the survival studies, newly-hatched zoeae were maintained at specified temperature, salinity and water conditions. At the time of daily feeding and water

change, mortality counts were made. Metabolic measurements were made on single larvae with a Cartesian Diver Respirometer in divers having a total volume of 10-13 microliters. Determinations were made on larvae under a variety of experimental conditions. To determine rate and pattern of swimming by the larvae, they were reared under specified conditions to the desired stage then tested in swimming chambers. Single larvae were transferred to a transparent 10 x 10 x 3 cm deep chamber having a grid of lines 0.5 cm apart etched on the baseplate. The number of lines crossed/min served as an index of activity. Ontogenetic changes in phototaxis were measured in a light intensity gradient apparatus modified from Ryland (1960) and Bayne (1964).

The toxicant solutions were made up in 30 °/oo filtered sea water. Mercury was provided in the experimental conditions as a known concentration of $HgCl_2$. A stock solution of 9×10^{-3} M $HgCl_2$ in 30 °/oo filtered sea water was prepared. This stock solution was checked at 4-6 week intervals with a Perkin-Elmer Atomic Absorption Spectrophotometer Model 303; no changes in the mercury concentration were found. Shortly before use, the stock was diluted to the three mercury test solutions: 9×10^{-7} M $HgCl_2$ (0.18 ppm Hg), 9×10^{-9} M (0.0018 ppm Hg), or 9×10^{-11} M (0.000018 ppm Hg). The cadmium stock for all experiments was reagent grade $CdCl_2$ x 2-1/2 H_2O made up to a stock solution of 1 mg Cd^{++}/ml H_2O. Dilutions to the desired concentration were made using filtered sea water (30 °/oo) and/or distilled H_2O.

SECTION V

EXPERIMENTAL RESULTS

ADULTS

Survival

Preliminary studies established that under optimum conditions of temperature (25°C) and salinity (30 °/oo) adult crabs could survive for prolonged periods of time, in sea water having an initial concentration of 9×10^{-7} M $HgCl_2$ (0.18 ppm Hg). Under temperature and salinity stress, however, this concentration of mercury significantly shortened survival time (Vernberg and Vernberg, 1972a). For example, under conditions of low temperature (5°C) and low salinity (5 °/oo), such as could occur following heavy winter rains, the crabs could not survive as long as under conditions of high temperature and low salinity. In winter animals without the added stress of a pollutant, 50% of the females died by day 8 (Fig. 1).

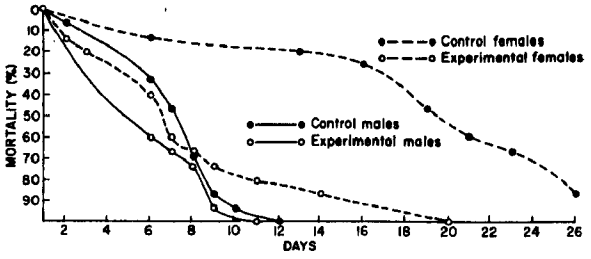


Figure 1. Mortality of Uca pugilator in 5 °/oo seawater at 5°C with and without the addition of 9×10^{-7} M $HgCl_2$ (0.18 ppm Hg)(From Vernberg and Vernberg, 1972a).

Under conditions of low salinity (5 °/oo) and high temperature (35°C), conditions apt to occur following the heavy rains associated with a summer hurricane, both male and female U. pugilator can survive with very little mortality for at least 28 days (Fig. 2). With the addition of 0.18 ppm mercury, however, survival times of both males and females are reduced. Under conditions in which crabs were maintained at this high temperature and low salinity in water containing mercury, 50% of the males had died by day 17, while 50% mortality of the females occurred by day 26 (Fig. 2).

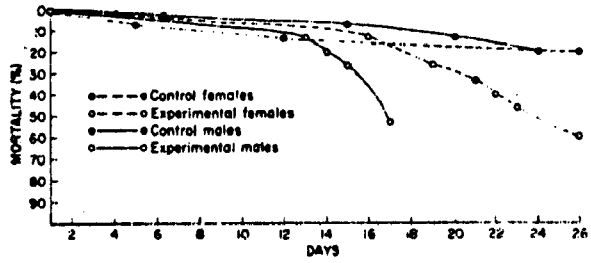


Figure 2. Mortality of Uca pugilator in 5 °/oo seawater at 35°C with and without the addition of 9 x 10^{-7} M $HgCl_2$ (0.18 ppm Hg) (from Vernberg and Vernberg, 1972a).

In contrast to Hg, the effect of Cd on the survival of adult fiddler crabs is most pronounced at high temperature (O'Hara, 1972). The susceptibility of fiddler crabs to Cd was most pronounced in the thermosaline regime of 30°C and 10 °/oo. The concentration fatal to 50% of the organisms in 240 hr (TLm-240 hr) was calculated to be 2.9 ppm Cd under these conditions, whereas at 10°C, 30 °/oo the concentration level was 47.0 ppm. At higher concentrations, the time required to kill 50% of the crabs was considerably reduced. Table 1 shows the influence of temperature, salinity, and cadmium concentration on the level of toxicant which kills 50% of the crabs in different time periods. Values generally were more influenced by temperature than by salinity within a given thermal regime. No differences were noted between survival rates of male and female crabs.

Table 1. CADMIUM CONCENTRATIONS (Cd^{++} in ppm) LETHAL TO 50% OF TEST ORGANISMS (TLm) AT DIFFERENT SALINITIES, TIMES, AND TEMPERATURES (from O'Hara, 1973).

Salinity (°/oo)	Time (hr)	Temperature 10°C (ppm)	20°C (ppm)	30°C (ppm)
10	48	--	--	11.0
	96	--	32.2	6.8
	144	51.0	21.3	4.0
	192	28.5	18.0	3.0
	240	15.7	11.8	2.9
20	48	--	--	28.0
	96	--	46.6	10.4
	144	--	23.0	5.2
	192	52.0	16.5	3.7
	240	42.0	9.5	3.5
30	48	--	--	33.3
	96	--	37.0	23.3
	144	--	29.6	7.6
	192	--	21.0	6.5
	240	47.0	17.9	5.7

Since all stages of development are vital to the completion of the life cycle, the effect of Hg on the egg sponge stage which links the adult stage with the planktonic larval stages was considered. Females carry an external egg sponge containing several hundred to 5000 or more eggs for approximately 3 weeks. The newly-hatched (stage I) zoeae actively swim and feed. Freshly gravid crabs were dosed for 7 days in 0.18 ppm Hg and subsequently removed to untreated sea water until hatching of the eggs. Comparable numbers of control crabs were maintained throughout in untreated sea water. The conditions of each female and her egg sponge were recorded daily in both control and experimental crabs. If eggs were dropped during the Hg treatment the animals were not sacrificed until 1 week of the untreated sea water post-control period had been completed.

Results are summarized for percent survival, percent sponges dropped before hatching, and average number of days before dropping (Fig. 3).

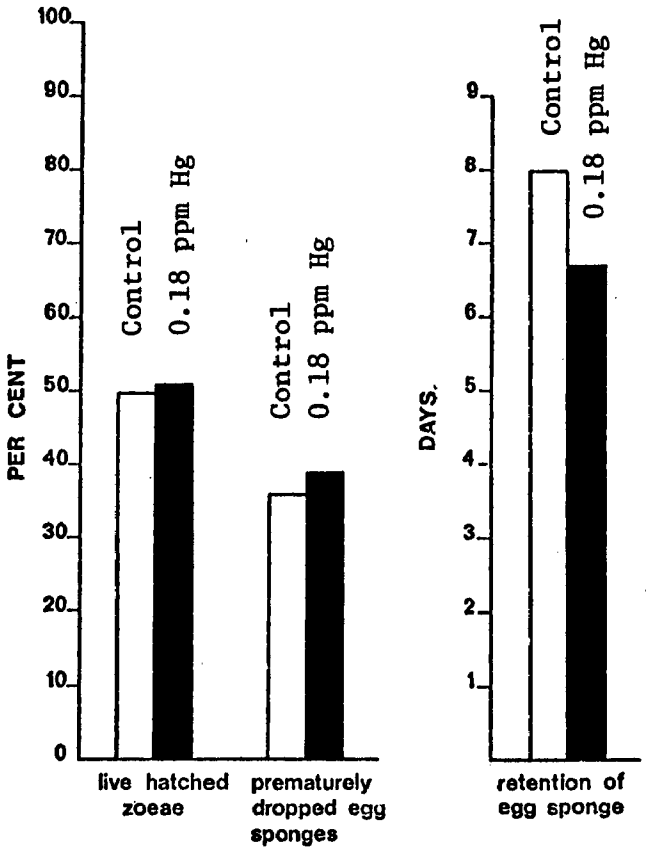


Figure 3. Egg sponge survival in Uca pugilator.

These data indicate that of all the egg sponge potential, 50% survival was realized in the control group versus 50.1% in the experiment. Considered as percent of zoeae which were alive on the day of hatching the survival percentages are 78% and 80%, respectively. Percent of females which dropped sponges prematurely is 36% in the control and 39% in the

experimental. The unexpected number of control animals which dropped sponges can possibly be correlated with the unusually low salinity conditions in the estuary during the summer due to prolonged periods of heavy rain. Average number of days that sponges were carried before dropping was 8 for the control and 6.7 for the mercury-treated. Thus, relatively little difference is seen between the control and experimental groups (Fig. 3) except for the tendency of Hg-treated females to drop their eggs slightly earlier.

Uptake

Since neither the male nor the female crabs survived well when exposed to mercury under conditions of environmental stress, it seemed possible that increased mercury uptake occurred in the tissues of the crabs under suboptimal temperature-salinity regimes. Therefore, the amount of mercury in selected tissues of crabs living under various conditions of temperature and salinity was determined.

Mercury uptake rates were first measured in tissues of crabs maintained under an optimal temperature-salinity regime and chronically exposed to 0.18 ppm Hg over a 28-day period (Vernberg and Vernberg, 1972a). Five tissues including gill, hepatopancreas, green gland, muscle and carapace were assayed for Hg concentration; gill tissue was found to have the highest concentration. Within the first 24 hours gill tissue contained 1.73 ppm mercury; the amount of mercury in this tissue increased steadily with continued exposure (Fig. 4). Mercury also accumulated in the hepatopancreas and green gland, although much less rapidly and at a lower concentration (Fig. 4). Small amounts of mercury were found in abdominal muscle tissues and in the carapace; after 28 days exposure to water containing mercury, levels were approximately 1 ppm in both the muscle and carapace.

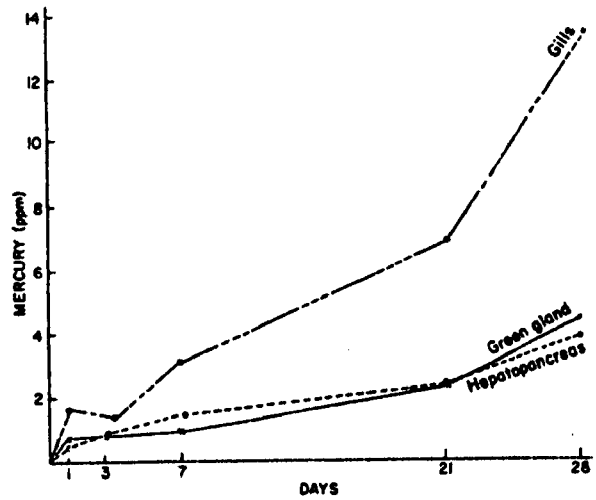


Figure 4. Mercury in tissues of Uca pugilator after exposure of the crabs to 9 x 10^{-7} M $HgCl_2$ (0.18 ppm Hg) in 30 °/₀₀ seawater at 25°C for varying lengths of time (from Vernberg and Vernberg, 1972a).

Uptake of mercury was very rapid. One group of crabs was exposed to 0.18 ppm Hg in 30 °/oo at 25°C for 1-7 hrs. The uptake by tissues was higher compared to Hg content of control crabs in untreated sea water. Results are summarized in Figure 5.

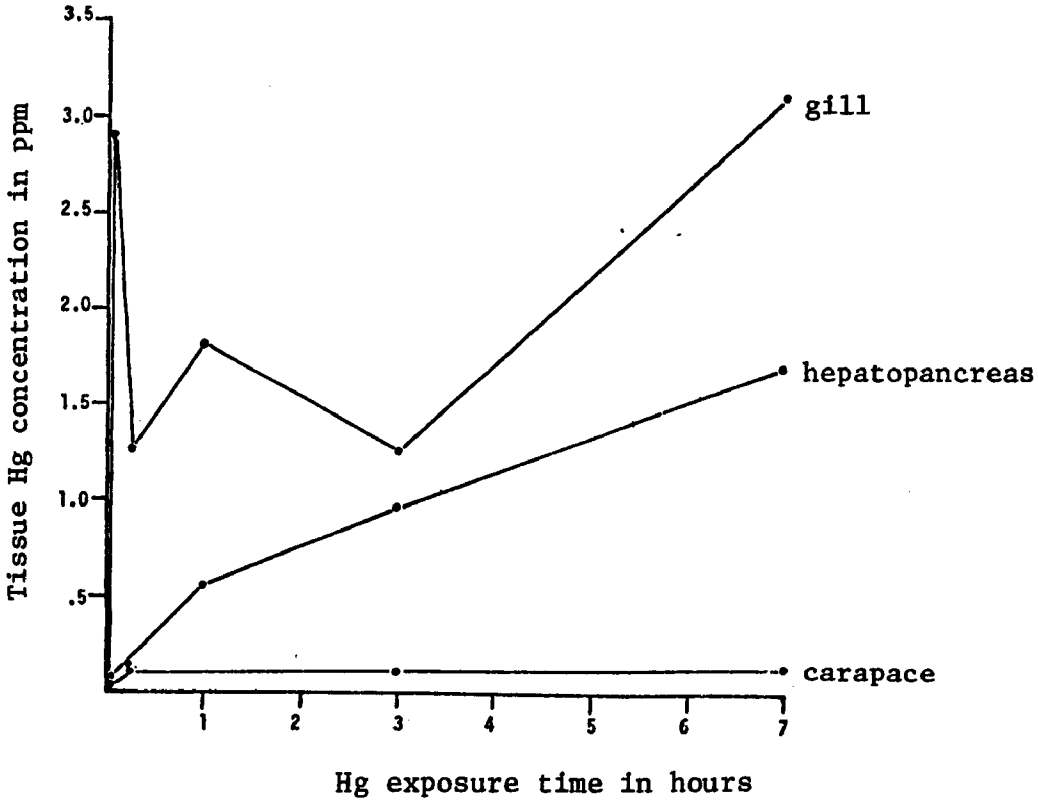


Figure 5. Tissue uptake of mercury after short-term exposures of adult Uca pugilator to 0.18 ppm Hg.

A further series of preliminary experiments was designed to measure the rate of loss of mercury after exposure. Four groups of crabs were exposed to 0.18 ppm Hg under a regime of 30 °/oo, 25°C for 1, 3, and 4 weeks, respectively. After the required dosage they were removed to control sea water (30 °/oo, 25°C) for a 7-day recovery period, and then gill, hepatopancreas, carapace, and muscle tissues were removed for analysis of Hg content. While data are incomplete, they clearly suggest (Table 2) that mercury is stored in the hepatopancreas and gill for considerable periods of time, and not eliminated immediately from the body.

Table 2. TISSUE CONCENTRATION AFTER EXPOSURE TO 0.18 ppm Hg IN 30 °/oo AT 25°C FOR VARYING PERIODS OF TIME, FOLLOWED BY A 7-DAY RECOVERY PERIOD.

	Control	Experimental		
		Exposure time		
Tissue	ppm Hg	1 wk	3 wk	4 wk
Carapace	0.015	0.262	0.282	0.346
Hepatopancreas	0.015	0.885	0.930	0.725
Gill	0.045	15.025	18.708	21.86

In another series of studies, experiments were designed to assay Hg uptake by gill and hepatopancreas tissues of crabs exposed to various conditions of temperature and salinity (Vernberg and O'Hara, 1972). Radioisotopes were used in these experiments.

Gill tissue accumulated much greater amounts of mercury than did the hepatopancreas regardless of the experimental conditions (Fig. 6A). Over 82% of the mercury accumulation in the gill tissue occurred within the first 24 hours in all thermal-salinity regimes, with only slight additions after 48- and 72-hour exposure to all experimental conditions except 5°C, 5 °/oo. Under a thermal-salinity regime of 5°C, 5 °/oo the mercury content declined slightly after 72 hours, probably due to necrosis and sloughing of the gill epithelium.

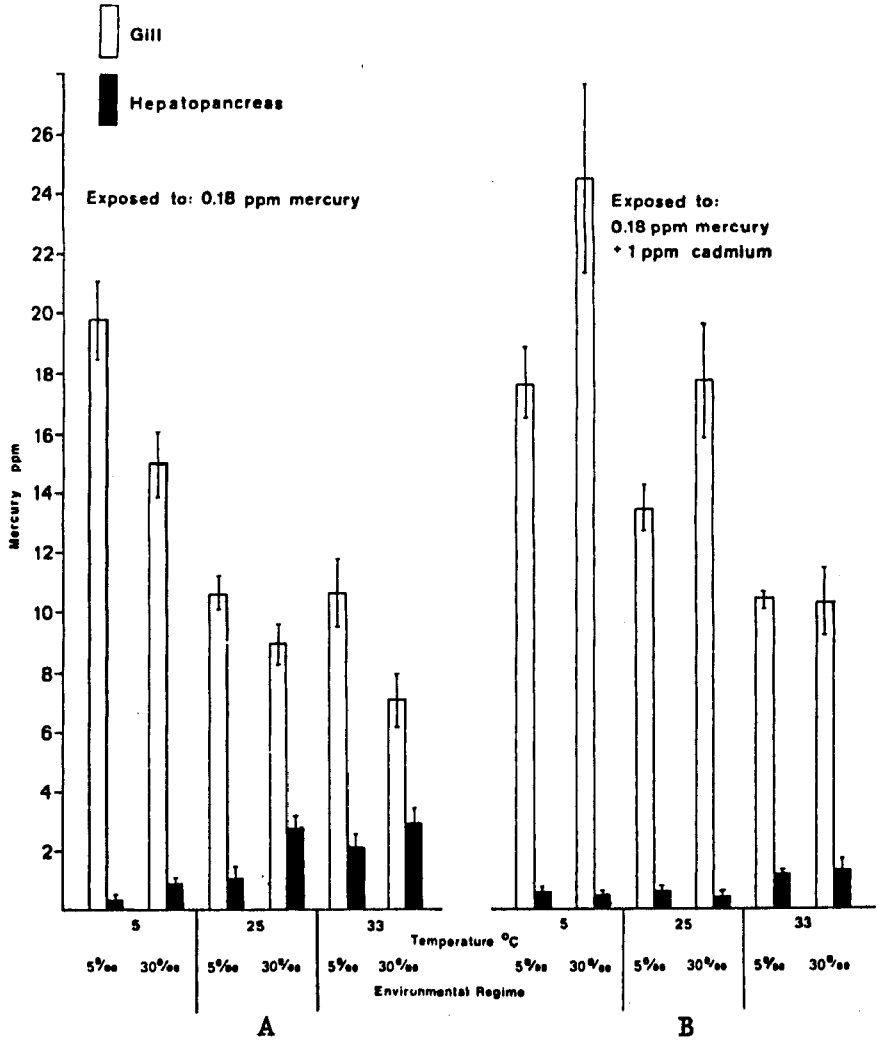


Figure 6. Tissue uptake of Hg by Uca pugilator under different temperature-salinity conditions after 24-hr exposure (from Vernberg et al., 1974).

The amount of mercury accumulated in gill tissue within the first 24 hours was significantly higher ($p<0.01$) under the thermal-salinity regime of 5°C, 5 °/oo, than in tissues of crabs maintained in any other set of experimental conditions. Under experimental conditions of 5°C, 30 °/oo, mercury accumulation in gill tissue was significantly greater than at 25°C, 30 °/oo, or at 33°C with either 5 °/oo or 30 °/oo. The least amount of mercury was accumulated in gill tissue of crabs maintained at 33°C, 30 °/oo. Thus, low salinity increased mercury accumulation in gill tissue at all temperatures; low temperature further enhanced gill tissue accumulation at low salinity (Fig. 6A).

In hepatopancreas tissue the concentration of mercury increased throughout a 72-hour exposure period in all experimental conditions except 5°C, 5 °/oo. In crabs maintained under these low temperature and salinity conditions, mercury concentration was significantly lower than in crabs held at any of the other experimental regimes. After 72 hours there was less mercury in the hepatopancreas of 5°C crabs than in any other group, regardless of the salinity. Crabs exposed to 33°C, 30 °/oo, contained 14 times more mercury in the hepatopancreas than crabs exposed to 5°C, 5 °/oo (Fig. 6A). However, the aggregate burden of mercury in the gills and hepatopancreas, when calculated as grams metal present in both tissues, was found to be relatively constant under all experimental conditions, although the relative amounts in each tissue were very different. There was no sex difference in mercury uptake in these tissues under either optimal or suboptimal conditions.

Preliminary screening with cadmium indicated that a concentration of 1 ppm Cd was sublethal for prolonged periods of time to crabs maintained under optimum temperature-salinity conditions (25°C, 30 °/oo). In a series of experiments parallel to the Hg uptake studies, cadmium uptake was determined for the hepatopancreas and gill tissue of crabs maintained under different temperature-salinity ranges (Vernberg et al., 1974).

In gill tissue, cadmium levels were essentially the mirror image found for mercury levels. Whereas the highest Hg levels were found at low temperatures, the highest cadmium levels were found at high temperatures (Fig. 7A). Cadmium was transferred rapidly and in relatively high amounts to the hepatopancreas. Under optimum environmental conditions, the amount of cadmium in the gill and hepatopancreas was approximately equal. But at either high (33°C) or low (5°C) temperatures, relatively small amounts of cadmium were found in the hepatopancreas of crabs at optimum salinity.

In another series of experiments, higher concentrations of Cd were used to determine uptake levels over a period of time (O'Hara, 1972; 1973). In the first 12 hours of exposure, gill tissue accumulated cadmium in proportion to the exposure concentration (Fig. 8). Thus, gill tissue from crabs exposed to 25 ppm Cd contained 110 ppm; gill tissue from those exposed to 15 ppm Cd contained 59 ppm, while such tissue from those exposed to 5 ppm Cd contained 18 ppm. Each accumulation in gill tissue was about four times the concentration of cadmium in the surrounding water.

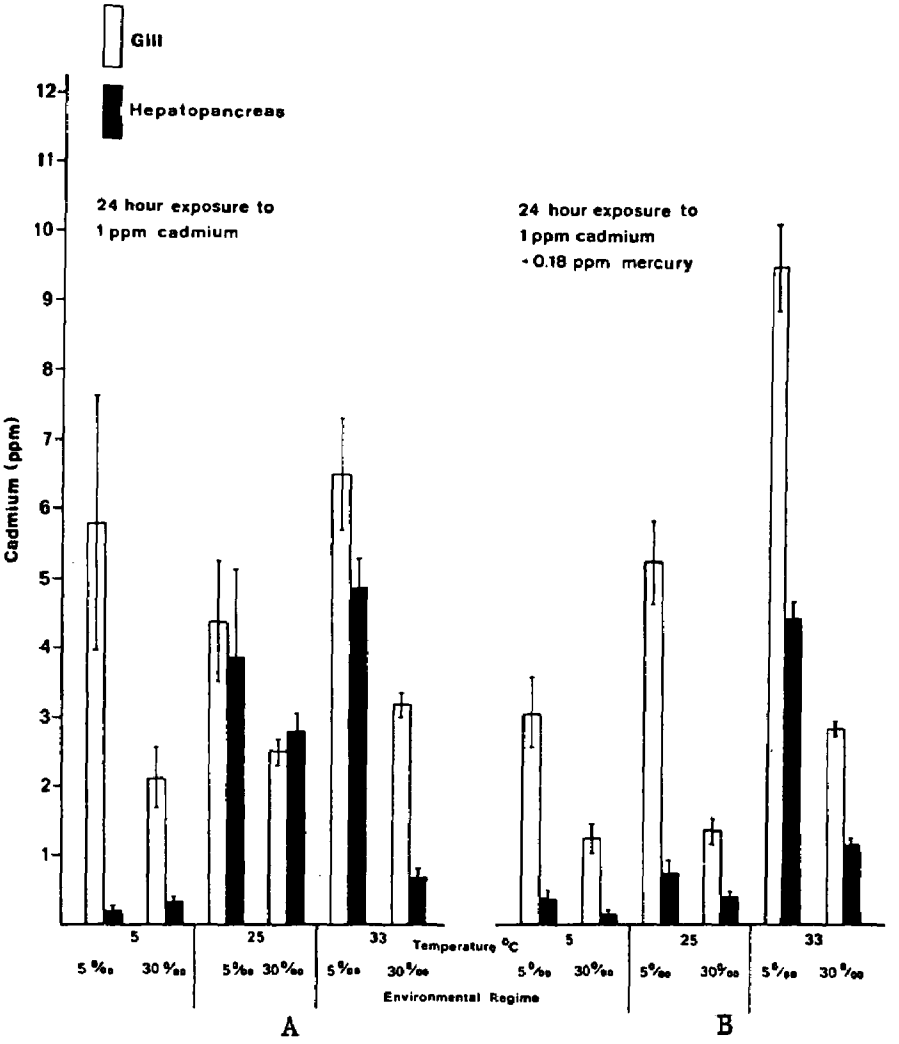


Figure 7. Tissue uptake of Cd by Uca pugilator under different temperature-salinity conditions after 24-hr exposure to cadmium alone (A), or cadmium plus mercury (B) (from Vernberg et al., 1974).

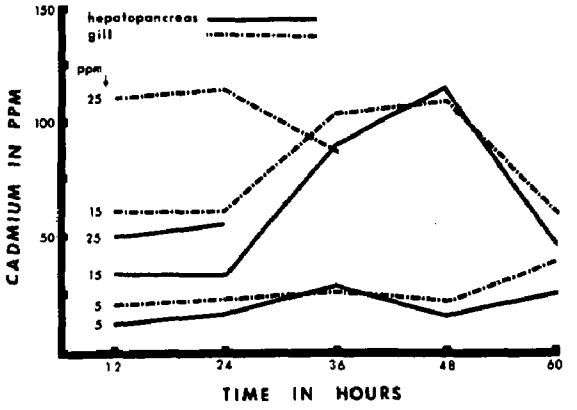


Figure 8. Concentration of cadmium in gill and hepatopancreas of crabs in 5, 15 and 25 ppm Cd^{++} at 30°C, 20 °/oo (from O'Hara,1972).

Gill tissues from crabs in 25 ppm Cd did not increase in cadmium concentration appreciably over 110 ppm in 24 hours and exhibited a decline in tissue concentration at 36 hours. High mortality at 48 hours precluded additional reliable sampling. Gill tissue from crabs exposed to 15 ppm Cd showed an increase in cadmium content between 24 and 48 hours with a maximum accumulation of 109 ppm. The significance of the value around 110 ppm is unclear; it could represent a maximum tissue burden in terms of equilibrium with the external medium. The cadmium concentration in gill tissues from crabs sacrificed at 60 hours showed a marked reduction in cadmium content. As there was high mortality of crabs in this concentration, the lower cadmium content in the tissues might represent reduced binding of the metal and loss due to the destruction of tissue. Crabs exposed to 5 ppm Cd continued to concentrate cadmium in their gill tissue, attaining a maximum of 39 ppm after 60 hours.

After 12 hours exposure, the hepatopancreas concentrated cadmium about two times greater than exposure level; 25 ppm in the medium concentrated to 50 ppm in tissue, 15 ppm to 32 ppm, and 5 ppm to 11 ppm. After 24 hours the hepatopancreas of crabs exposed to the highest concentration was almost completely destroyed; it changed from a firm glandular tissue to an amorphous and liquified condition, thus precluding samples from these specimens. Crabs exposed to 15 ppm Cd for longer periods showed an increase in hepatopancreas cadmium level to about 116 ppm in 48 hours, followed by a rapid decline. This decline might be associated with the breakdown of hepatopancreas tissue. Crabs exposed to 5 ppm showed the same gradual increase in Cd concentration that was evident in gill tissue, attaining a maximum of 25 ppm after 60 hours.

Cadmium accumulation was highest in green gland tissue (Fig. 9), with maximum concentrations of 380 ppm in tissue from crabs exposed to 25 ppm, 171 ppm from crabs in 15 ppm, and 118 ppm from crabs in 5 ppm. These values are 12 to 20 times the exposure concentrations.

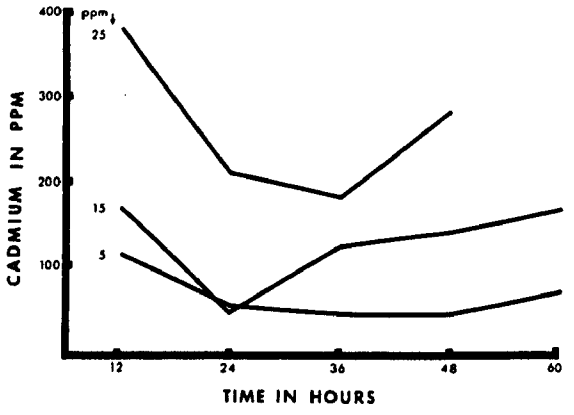


Figure 9. Concentration of cadmium in green gland tissue of crabs in 5, 15 and 25 ppm Cd^{++} at 30°C, 20 °/₀₀ (from O'Hara,1972).

Cadmium levels in muscle tissue remained almost constant throughout the experiment. Cd tissue concentrations were only slightly above the exposure levels, with maximum concentrations of 29.3 ppm in crabs exposed to 25 ppm, 17.3 ppm from crabs in 15 ppm, and 8.9 ppm from crabs exposed to 5 ppm.

To quantitatively assay the total body burden of metal in the gills and hepatopancreas of fiddler crabs, Cd levels were calculated as µg present in each tissue and added together. It is clear that this aggregate value increased over time for all temperature-salinity regimes tested. The maximum uptake was 17.44 µg Cd which occurred at 33°C, 10 °/₀₀ (Table 3). The translocation of cadmium from gills to hepatopancreas is indicated by the percent of metal in each tissue and was most pronounced at high temperatures.

Table 3. CADMIUM CONTENT IN GILL (G) AND HEPATOPANCREAS (H) FROM FIDDLER CRABS EXPOSED TO 10.0 ppm CADMIUM OVER A 72-HR PERIOD UNDER VARIOUS TEMPERATURE-SALINITY REGIMES. VALUES ARE MEANS OF CONCENTRATIONS IN TISSUES OF FOUR ANIMALS ± STANDARD ERROR (from O'Hara, 1973).

	24 hr			48 hr			72 hr		
Temperature-salinity regime	Total µg Cd^{++} in G and H	% in G and H	ppm Cd^{++} in G and H	Total µg Cd^{++} in G and H	% in G and H	ppm Cd^{++} in G and H	Total µg Cd^{++} in G and H	% in G and H	ppm Cd^{++} in G and H
10 C, 10‰	.78	G-68.8	20.8±3.3	1.03	G-55.3	28.1± 1.4	1.86	G-29.9	25.1± 3.7
		H-31.2	6.8±0.8		H-44.7	16.2± 0.8		H-70.1	30.1± 2.2
10 C, 30‰	.39	G-69.0	9.1±1.1	.44	G-44.2	10.6± 0.9	.75	G-38.6	10.2± 0.8
		H-31.0	3.6±0.8		H-55.8	8.8± 1.2		H-61.4	6.5± 2.9
25 C, 10‰	2.08	G-30.6	27.9±3.5	3.72	G-26.5	37.1± 5.6	8.65	G-19.9	70.8± 8.8
		H-69.4	34.2±4.0		H-73.5	78.3± 5.5		H-80.1	133.8±30.2
25 C, 30‰	.77	G-32.9	10.2±1.4	1.34	G-24.3	12.8± 1.8	1.85	G-20.2	15.5± 1.1
		H-67.1	14.5±2.2		H-75.7	23.3± 2.4		H-79.8	33.7± 6.3
33 C, 10‰	4.98	G-38.0	65.1±3.6	10.10	G-21.3	98.7±11.1	17.44	G-16.2	92.0±15.1
		H-62.0	74.1±2.8		H-78.7	198.2±14.7		H-83.8	200.2±22.1
33 C, 30‰	1.67	G-22.1	11.2±0.7	2.50	G-22.3	26.5± 2.8	4.90	G-11.3	24.1± 3.5
		H-77.9	30.8±7.0		H-77.7	88.0±13.7		H-88.7	77.5±13.1

Since these studies established that temperature and salinity differentially affected the uptake of cadmium and mercury in tissues of U. pugilator, further work was initiated to consider the effect of dual exposure to these two metals. Mercury was added in the form of $HgCl_2$ at an initial concentration of 0.18 ppm Hg for a 72-hour period. Cadmium was added in the form of $CdCl_2$ plus one µCi of ^{115}Cd to bring the total initial concentration to 1 ppm Cd.

In both the gill and hepatopancreas, mercury uptake was more influenced by the presence of cadmium than was cadmium uptake by the addition of mercury (Figs. 6B, 7B). Generally, where a statistically significant change in uptake did occur, the uptake of each metal was greater in the gills and showed a decrease in the hepatopancreas.

When Hg alone was present in the water, the crabs effectively transported Hg from the gills to the hepatopancreas. For example, after 72-hr at 33°C, 30 °/₀₀, the percentages of Hg in the gill and hepatopancreas were 35.1 and 64.9, respectively but 96.3 and 3.7 at 5°C, 5 °/₀₀ (Vernberg and O'Hara, 1972). When both Cd and Hg were present, the crabs seemingly lost this transport ability (Fig. 6B), and the percent Hg in the gills remained high (90-98%) regardless of the temperature-salinity regime. Although TLm values were not determined for the crabs, mortality rates among the experimental animals were considerably higher than observed mortalities in crabs subjected to only one metal. The inability of the crabs to survive for long periods of time in the presence of both metals may well be associated with the inability to transport the Hg from the gills to the hepatopancreas. Figure 7B indicates that the uptake of Cd by the gills was increased by the addition of Hg only at high temperature and low salinity (30°C, 5 °/₀₀). At low temperature and high salinity the rate of gill uptake decreased, but only to statistically significant levels after 48 hours. There was one case in which Hg significantly affected Cd uptake in the hepatopancreas; this occurred at 25° and salinity was not a factor.

Metabolism

Metabolic base-line rates were first established for adult male and female fiddler crabs at 25°C in 30 °/₀₀ sea water (Fig. 10). These rates were essentially the same for both sexes. After the base-line rates were determined, the same animals were maintained at 25°C in 30 °/₀₀ seawater with the addition of mercury, and metabolism of the crabs were measured after 1, 3, 7, 14, 21 and 28 days exposure. Although a low level concentration of mercury was not lethal to the crabs under optimum environmental conditions, metabolic rates were affected, especially for males. The rate of oxygen uptake of males was significantly lower than that of the females after 21 days in this

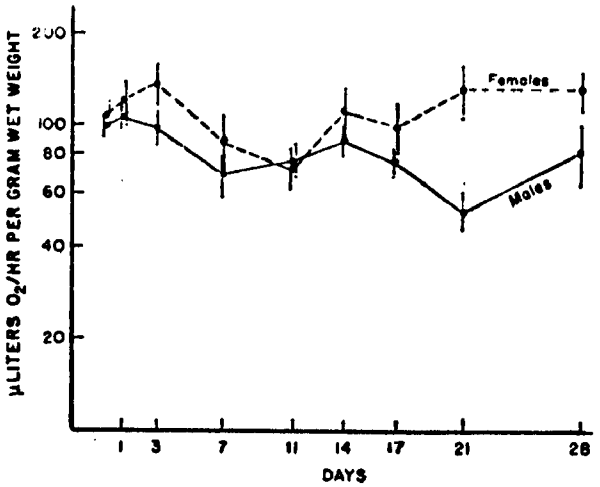


Figure 10. Oxygen uptake rates of male and female Uca pugilator maintained in 30 °/₀₀ seawater containing 0.18 ppm Hg at 25°C. The base-line rate is represented by the first set of data points on the left. The vertical bar through each mean value is the standard error (from Vernberg and Vernberg, 1972a).

Table 4. SUMMARY OF THE METABOLIC RESPONSE OF GILL AND HEPATOPANCREAS TISSUES FROM FIDDLER CRABS EXPOSED TO 0.18 ppm Hg UNDER DIFFERENT COMBINATIONS OF TEMPERATURE AND SALINITY. RESPONSES WERE MONITORED AT WEEKLY INTERVALS FOR 21 DAYS.

Acclimation temperature (°C)	Environmental regime	Determination temperature (°C)	Fig. no.	Metabolic response of tissues from exposed crabs in comparison to response of tissues from control crabs
25	5°C, 5 °/oo	5	(Fig.17)	After 7 days, rates of both exposed tissues significantly depressed (only female crabs survived).
	5°C, 30 °/oo	5	(Fig.16)	No significant difference in metabolism of gill tissue from female crabs after 7-day exposure; gill tissue from males markedly lower than that from control animals. Hepatopancreas from both males and females significantly lower.
	25°C, 30 °/oo	25	(Fig.13)	Rates of both tissues depressed in both sexes.
	33°C, 5 °/oo	33	(Fig.14)	Patterns of response generally similar in tissues of control and experimental crabs. Significant differences between control and experimental animals were observed only after 14 and/or 21-day exposure.
10	5°C, 5 °/oo	5	(Fig.18)	Rates of both tissues depressed initially (day 7); after 21-day exposure to Hg, markedly higher than those tissues from control crabs.
	5°C, 30 °/oo	5	(Fig.19)	Metabolic rate of gill tissue lower throughout; hepatopancreas initially lower, but significantly higher after 21-day exposure to Hg.
	33°C, 5 °/oo	33	(Fig.15)	Patterns of response and actual rates both different in gill tissue. In hepatopancreas pattern of response similar, but rates of experimental hepatopancreas tended to be lower than control.

sublethal concentration of mercury (Fig. 10). The metabolic rate of the males had not returned to the base-line level by the end of the 28-day experimental period. Both males and females, however, continued to survive for another month under the same mercury regime as before without any significant increase in mortality.

Under conditions of low temperature (5°C) and low salinity (5 °/₀₀) stress, females not only survived much longer than males, but also maintained a steadier rate of oxygen uptake (Fig. 11). The metabolic rate and pattern of the experimental female crabs were similar to those of the control female crabs. The metabolic rate of male experimental crabs after a 1-day exposure to mercury was not significantly different from that of the female experimental or male and female control crabs, but by day 3 the rate dropped markedly.

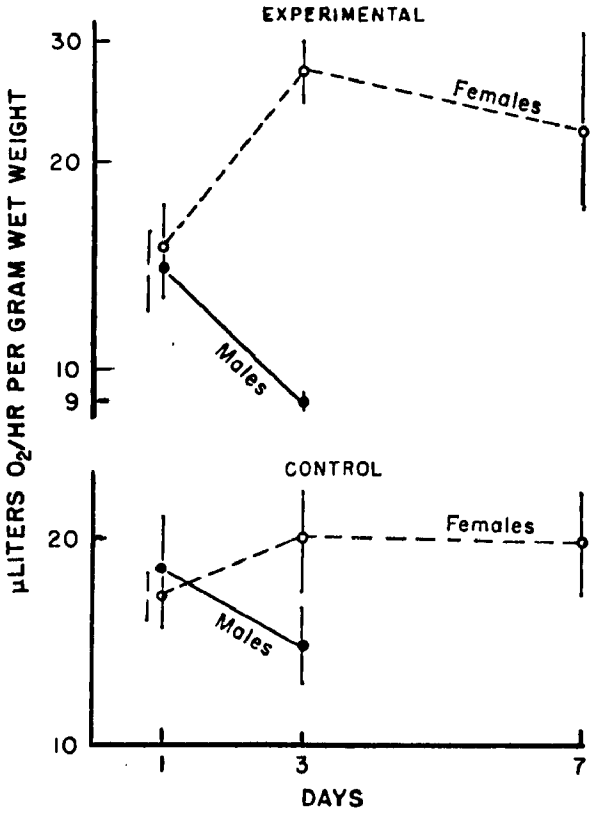


Figure 11. Oxygen uptake rates of male and female Uca pugilator maintained at 5°C in 5 °/₀₀ seawater with and without the addition of 0.18 ppm Hg. The vertical bar through each mean value is the standard error (from Vernberg and Vernberg, 1972a).

Oxygen uptake rates of female control crabs maintained in low salinity water (5 °/₀₀) and at high temperature (35°C) were relatively constant over a 28-day period and tended to be higher than that of control male crabs (Fig. 12). The metabolic rates of mercury-treated female crabs remained fairly constant for the first 7 days and then declined rapidly. The uptake rates of experimental male crabs declined steadily from day 1 and tended to be lower than those of the females throughout the remainder of the test period.

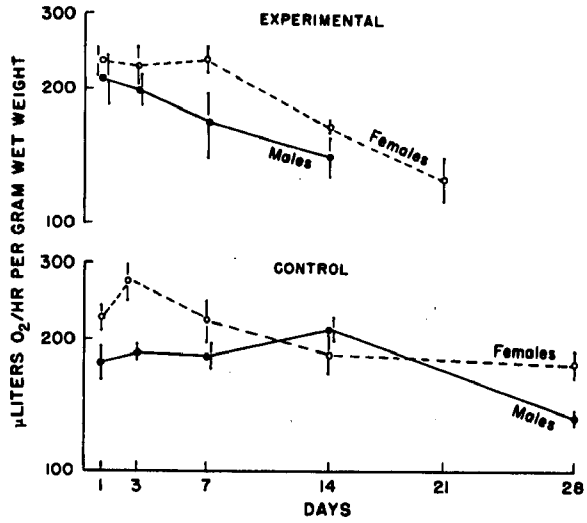


Figure 12. Oxygen uptake rates of male and female Uca pugilator maintained at 35°C in 5 °/oo seawater with and without the addition of 9 x 10^{-7} M $HgCl_2$ or 0.18 ppm. The vertial bar through each mean value is the standard error (from Vernberg and Vernberg, 1972a).

To further examine why Hg-treated males died sooner than females, a series of tissue metabolism studies was carried out (F.J.Vernberg and W.B.Vernberg, 1976). Crabs were collected in winter or early spring, then either warm-acclimated at 25°C or cold-acclimated at 10°C in the laboratory for a minimum of two weeks. Each group was then subdivided into temperature-salinity groups:

5°C, 5 °/oo
5°C, 30 °/oo
33°C, 5 °/oo
33°C, 30 °/oo
25°C, 30 °/oo (warm-acclimated crabs only)

Half the animals in each of these groups were exposed to 0.18 ppm Hg and the other half placed in untreated sea water. From 10-20 metabolic determinations were made with gill or hepatopancreas of both male and female crabs after 1, 7, 14, 21 days exposure to the specified conditions. Results of these studies are summarized in Table 4 and Figures 13-19.

Changes in whole animal metabolism induced by cadmium are also indicated by thermal-metabolic acclimation patterns (Vernberg, 1975). Respiration rates were determined on cold- and warm-acclimated crabs that had been exposed to a sublethal concentration of $CdCl_2$ (1 ppm Cd) for 24 hours or 14 days. Rates were measured at temperatures ranging from 10°C to 30°C at 5°C intervals; the salinity was maintained at 30 °/oo. After a 24-hour exposure to cadmium, the pattern of response of the experimental crabs was modified, with metabolism significantly suppressed in Cd-exposed crabs for most test conditions (Fig. 20). In the

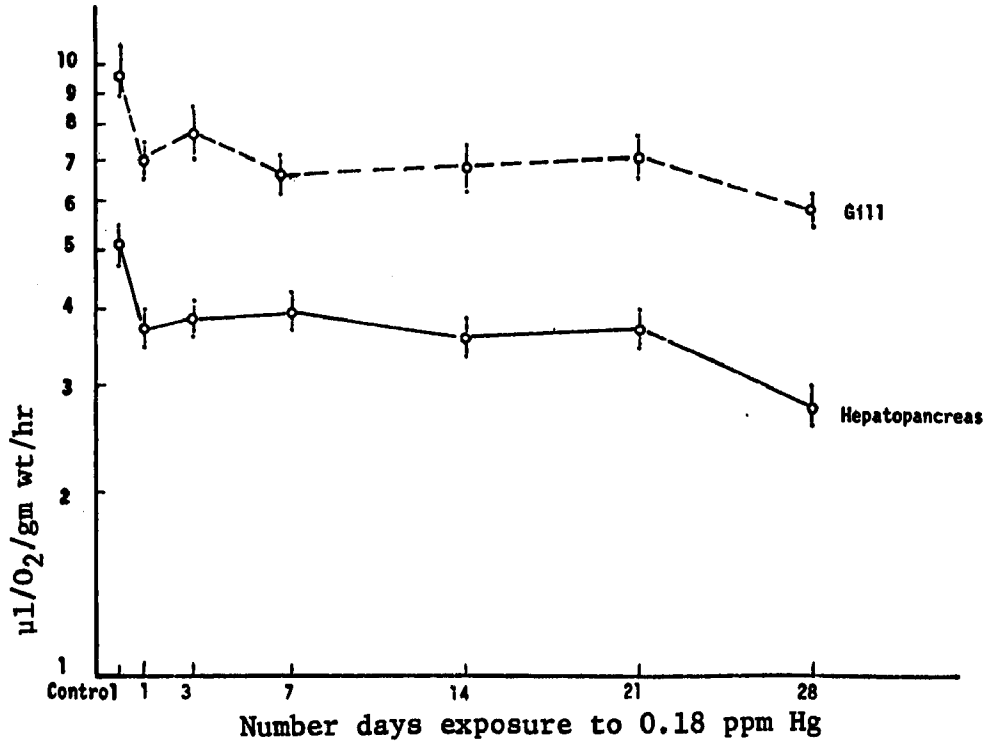


Figure 13. Metabolic rates of gill and hepatopancreas tissue from warm-acclimated male and female crabs exposed to 0.18 ppm Hg under an optimum temperature-salinity regime of 25°C, 30 °/oo. Vertical bars indicate ± one standard error.

Figure 14. Metabolic rates of gill and hepatopancreas tissue from warm-acclimated crabs maintained at 33°C, 5 °/oo for varying lengths of time with and without 0.18 ppm Hg. Vertical bars indicate ± one standard error.

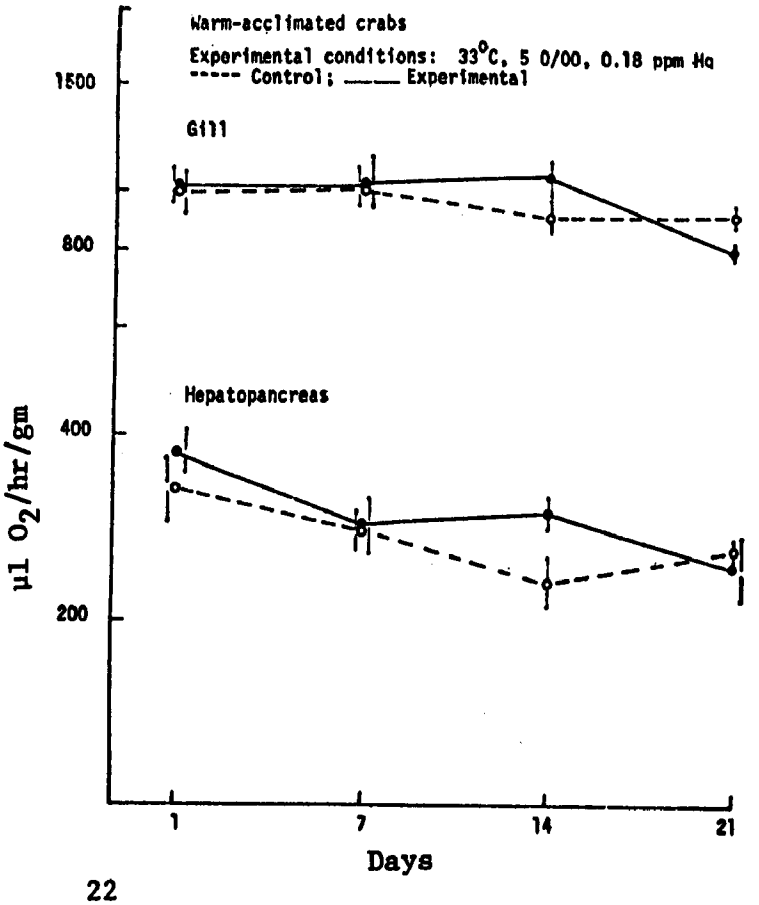
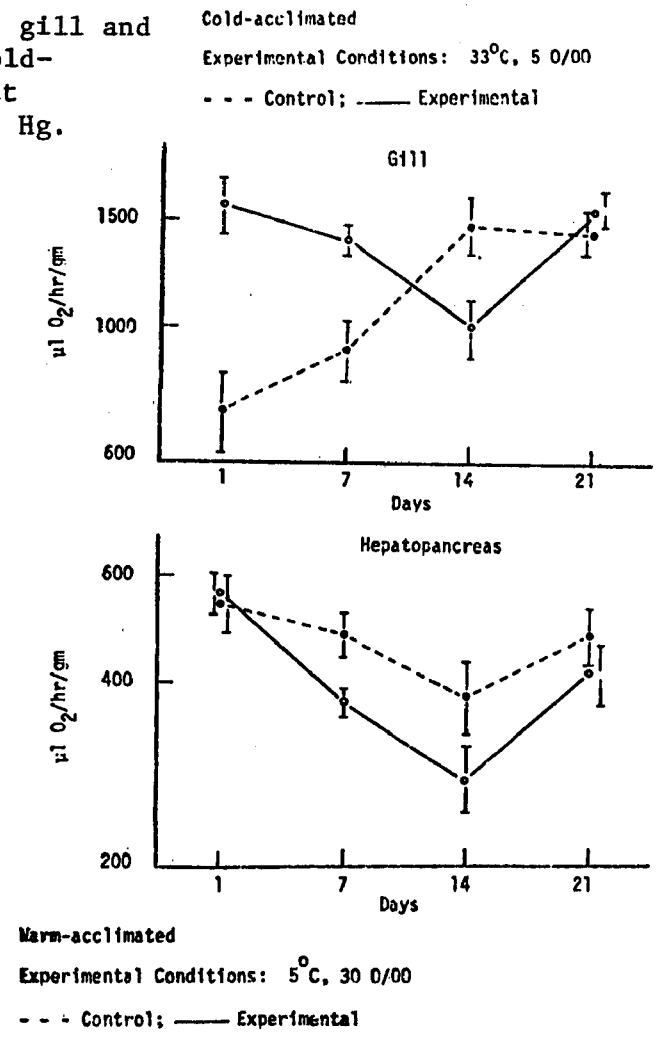


Figure 15. Metabolic rates of gill and hepatopancreas tissue from cold-acclimated crabs maintained at 33°C, 5 °/₀₀ with and without Hg. Vertical bars indicate ± one standard error.



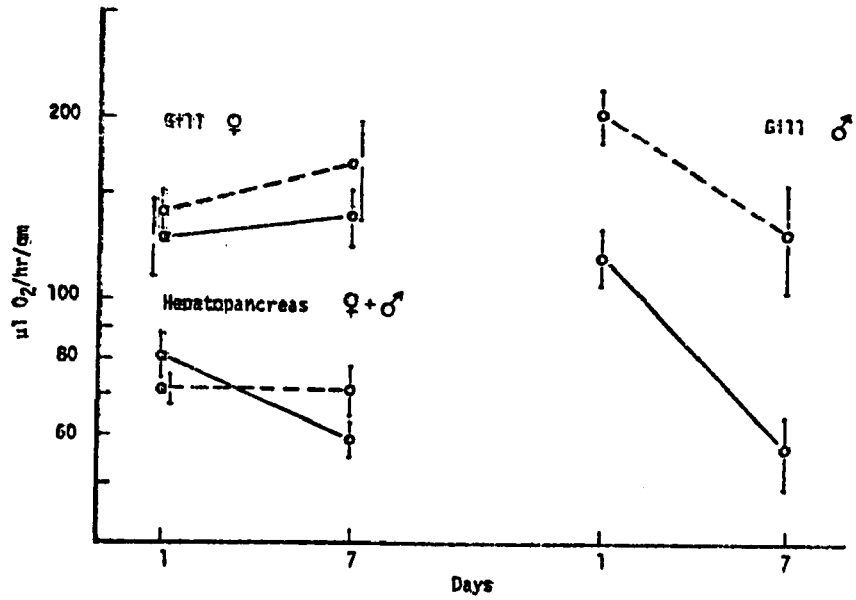


Figure 16. Metabolic rates of gill and hepatopancreas tissue from warm-acclimated crabs maintained at 5°C, 30 °/₀₀ with and without 0.18 ppm Hg. Vertical bars indicate ± one standard error.

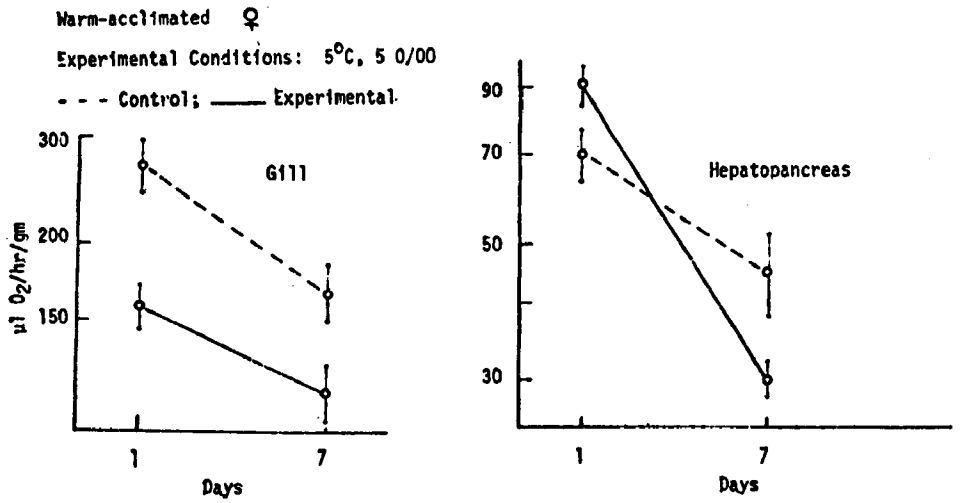


Figure 17. Metabolic rates of gill and hepatopancreas tissue from warm-acclimated crabs maintained at 5°C, 5 °/oo with and without 0.18 ppm Hg. Vertical bars indicate ± one standard error.

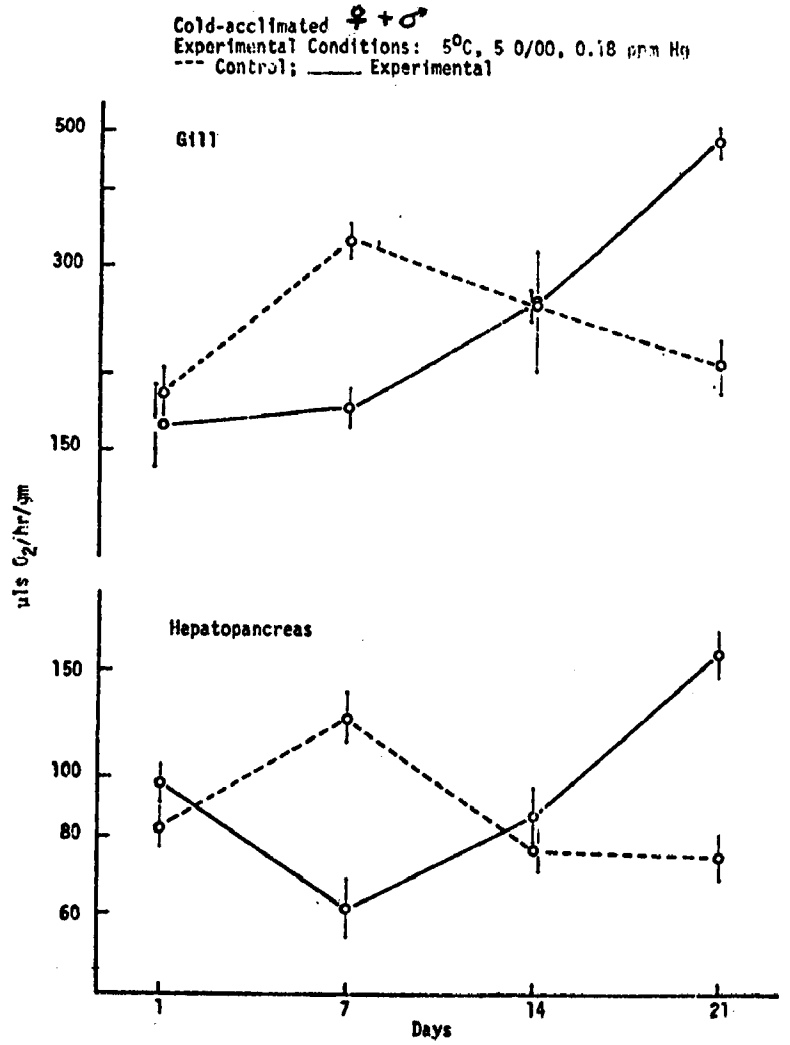


Figure 18. Metabolic rates of gill and hepatopancreas tissue from cold- acclimated crabs maintained at 5°C, 5 °/oo with and without 0.18 ppm Hg. Vertical bars indicate ± one standard error.

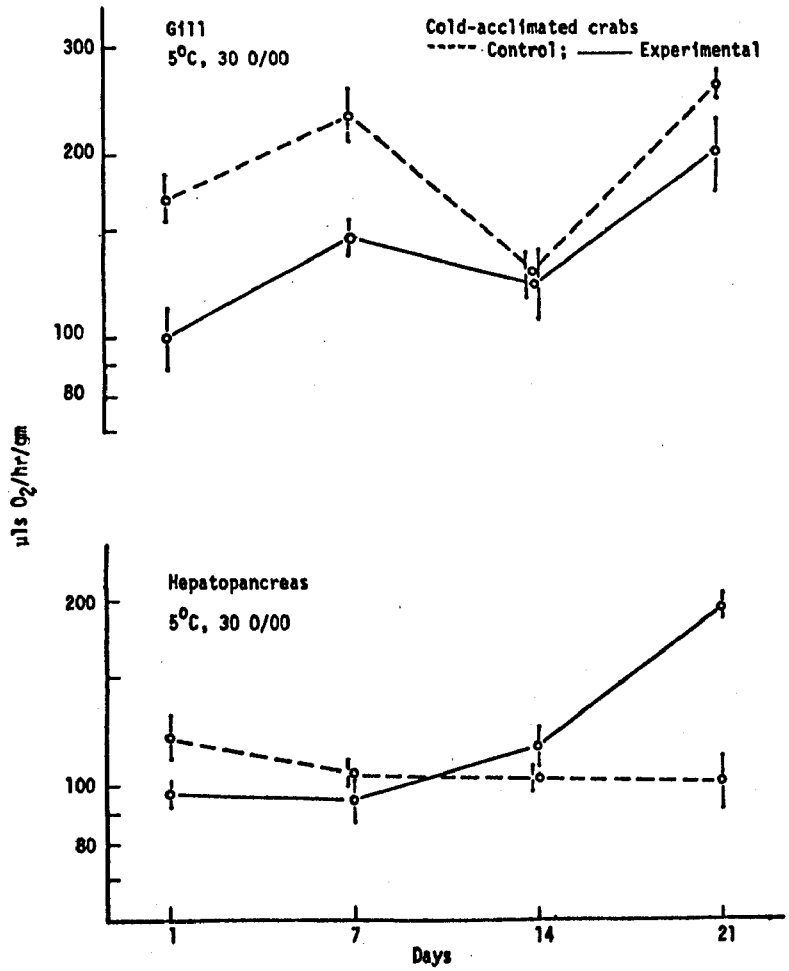


Figure 19. Metabolic rates of gill and hepatopancreas tissue from cold-acclimated crabs maintained at 5°C, 30 °/₀₀ with and without 0.18 ppm Hg. Vertical bars indicate ± one standard error.

warm-acclimated crabs, oxygen consumption appeared to be independent of temperature above 15°C. Following exposure to cadmium for 14 days, the pattern of response of cold-acclimated animals was altered to a greater extent than the patterns of warm-acclimated animals (Fig. 21). Following exposure to cadmium for 14 days rates of the experimental crabs were significantly depressed over those of controls at all temperatures above 10°C (Fig. 21). Again, there was a period of temperature insensitivity. Significantly decreased rates in the warm-acclimated experimental crabs were also noted at the higher temperature.

Behavior

Qualitative observations of adult crabs in sublethal concentrations of mercury indicated that after several weeks exposure, sluggishness and lack of responsiveness set in, followed by a state of torpor before

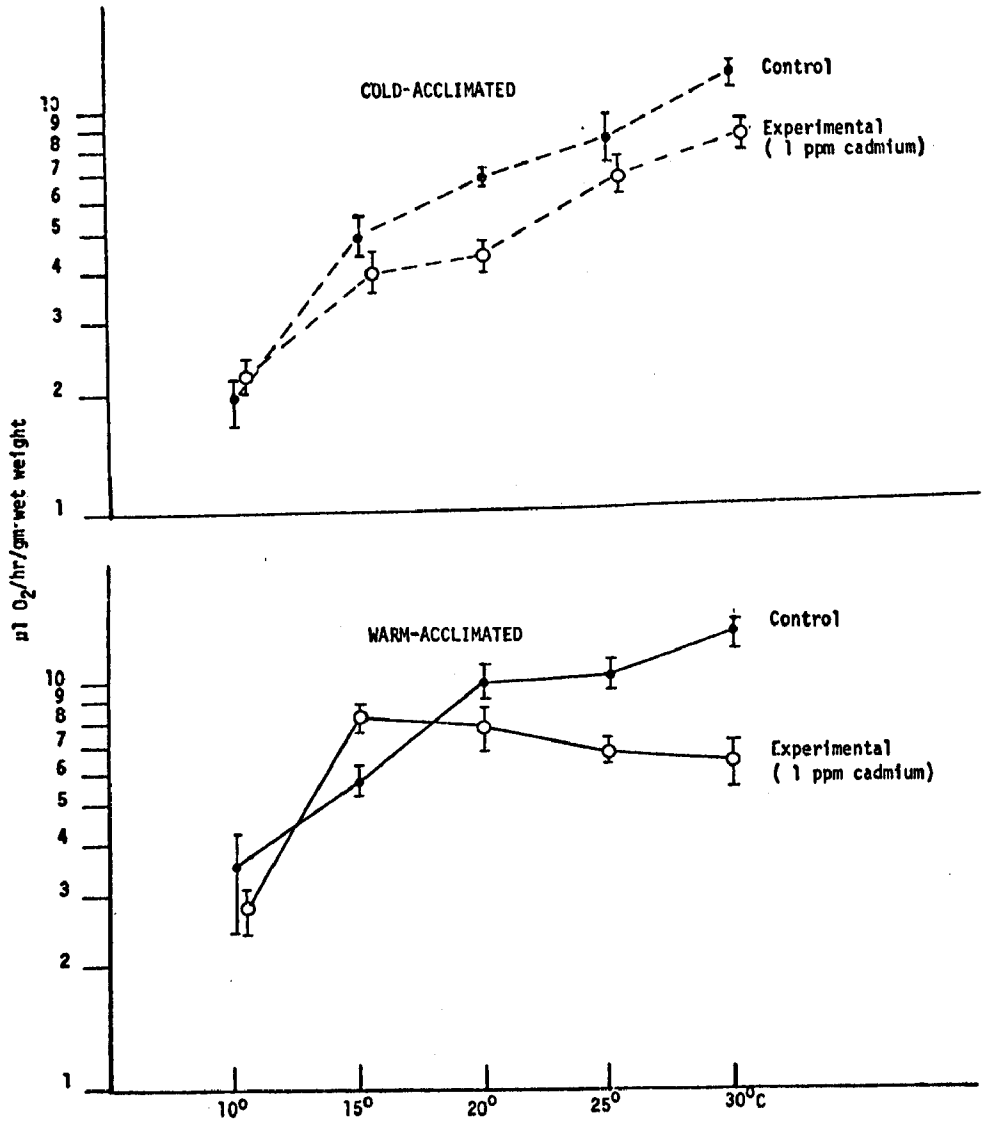


Figure 20. Uca pugilator adult metabolism, 24 hour exposure.

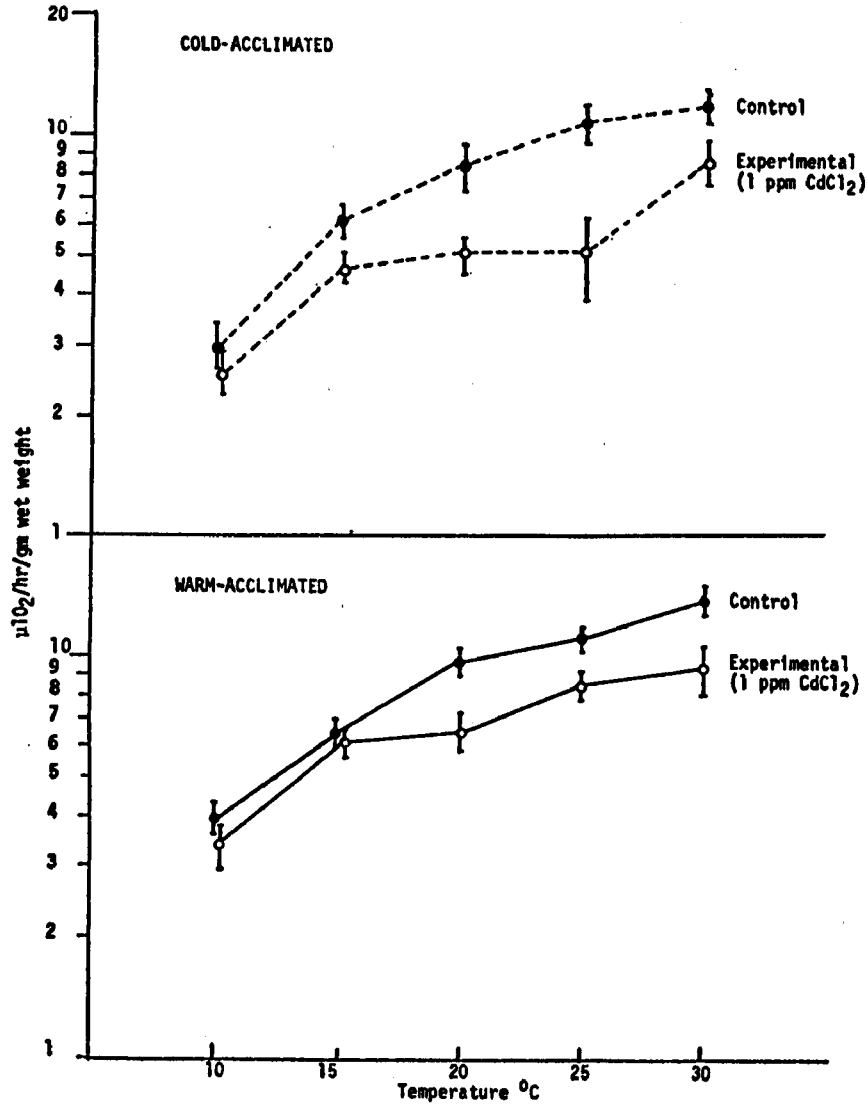


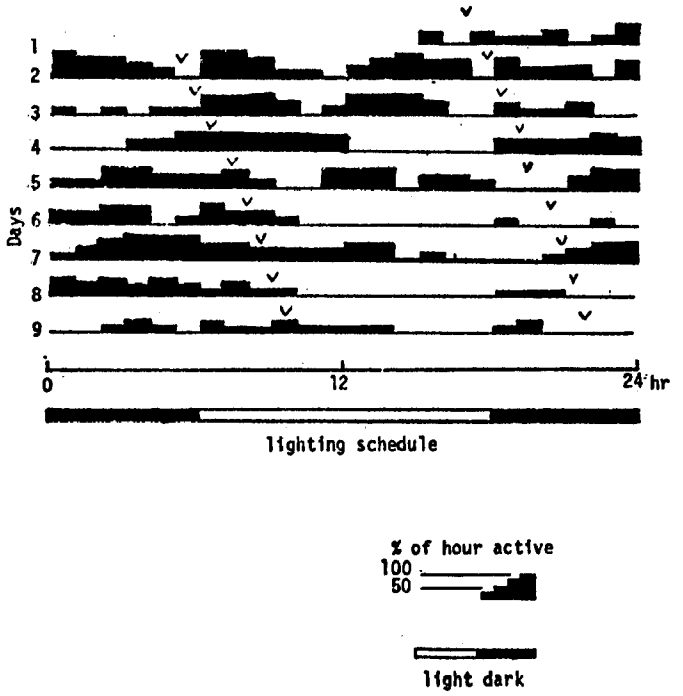
Figure 21. Uca pugilator adult metabolism, 14 day exposure.

death. Experiments were therefore undertaken using activity to measure the effects of sublethal $HgCl_2$ on normal function. An actograph patterned after Naylor (1958) was used in conjunction with an operations recorder and the data processed as chronological daily time scans (Barnwell, 1966). It seemed possible that both rhythmic pattern of locomotor behavior as well as daily intensity of activity might reflect Hg toxicity in the crabs.

In a preliminary series, groups of 10 males and 9 females were measured under control conditions of temperature and salinity, with a 12L:12D light schedule. Great variability in pattern and amount of activity occurred. A typical example is shown in Figure 22.

Figure 22. Activity of Uca pugilator adult in untreated seawater 30 °/₀₀, 25°C, 12L: 12D, using a Naylor-type actograph.

v = time of mean low tide



Further modifications of the recording system, experimental conditions, and methods of data analysis were made to overcome some of the difficulties in the system. For 10 control crabs and for 9 crabs in 0.18 ppm Hg a very high level of background activity was seen in all crabs but the crabs were predominantly nocturnal, with the greatest peaks of activity at the light transitions. Activity in the majority of mercury-treated crabs was reduced compared with control crabs, especially in the females. Yet the unexpectedly high level of background activity interferred with processing and interpreting the data, necessitating the development of a technique to analyze more quantitatively the rhythmic pattern and amount of activity. Braked wheels were subsequently fabricated which permitted movement only when the crab walked and, furthermore, which counted in direct relationship to distance traveled.

The rhythmicity and pattern of activity were first examined. Hourly counts were plotted in daily scans to give a summary of activity. In addition, 5-minute counts were processed by a computer periodogram program for frequency of rhythmic components in the range 6-15 hours and 20.5 -26.5 hours to detect tidal and circadian rhythmicity, respectively. Figures 23 and 24 show such data scan-periodograms for 2 crabs under control conditions of LL, 25°C, and 30 °/₀₀ for 8 days. A strong rhythmic component is seen in the data at 23.9 hrs for Figure 23 and at 24.3 for Figure 24. Although a large amount of background noise is apparent in the activity scan of Figure 23, the periodogram was able to detect dominant frequencies. Secondly, 24-hr totals for activity were obtained in these experiments (Fig. 25). Considerable differences were noted in the average daily amount of activity of these

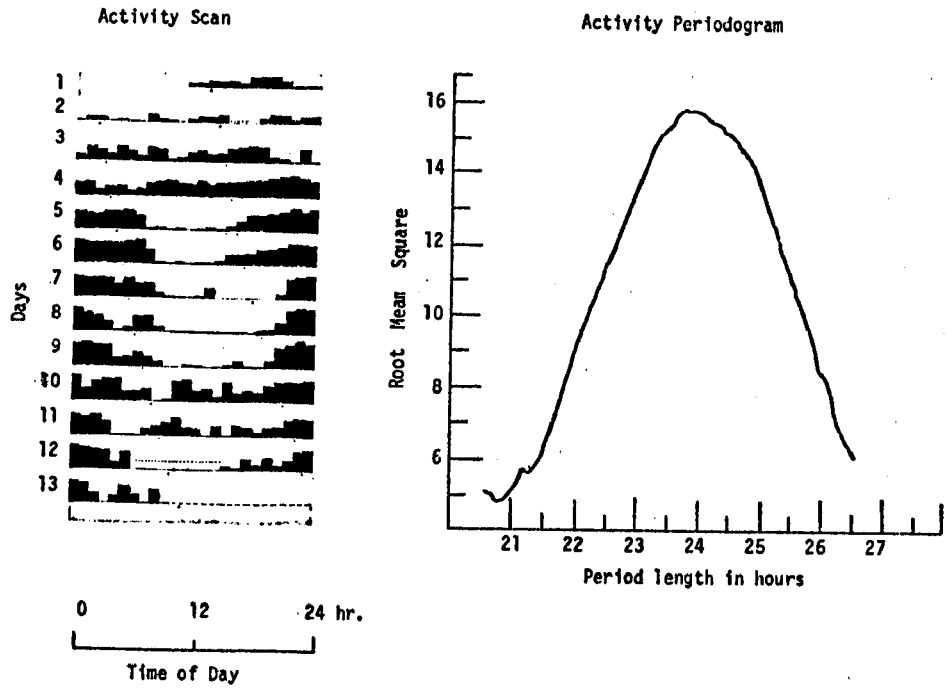


Figure 23. Activity of 1 Uca pugilator in LL, 30 °/₀₀ control sea water, 25°C, in a wheel actograph.

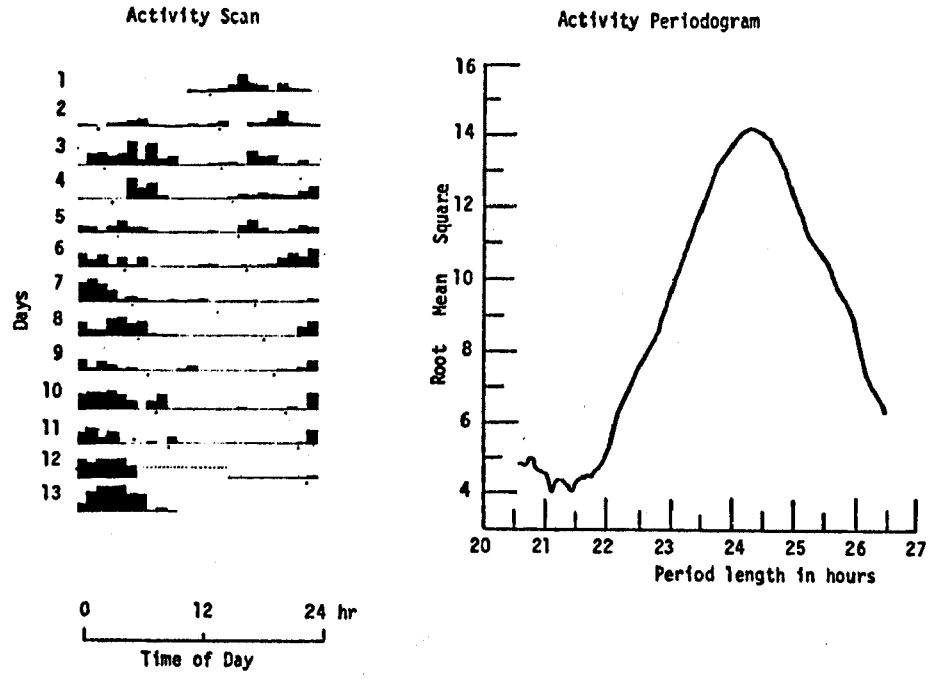


Figure 24. Activity of 1 Uca pugilator in LL, 30 °/₀₀ control sea water, 25°C, in a wheel actograph.

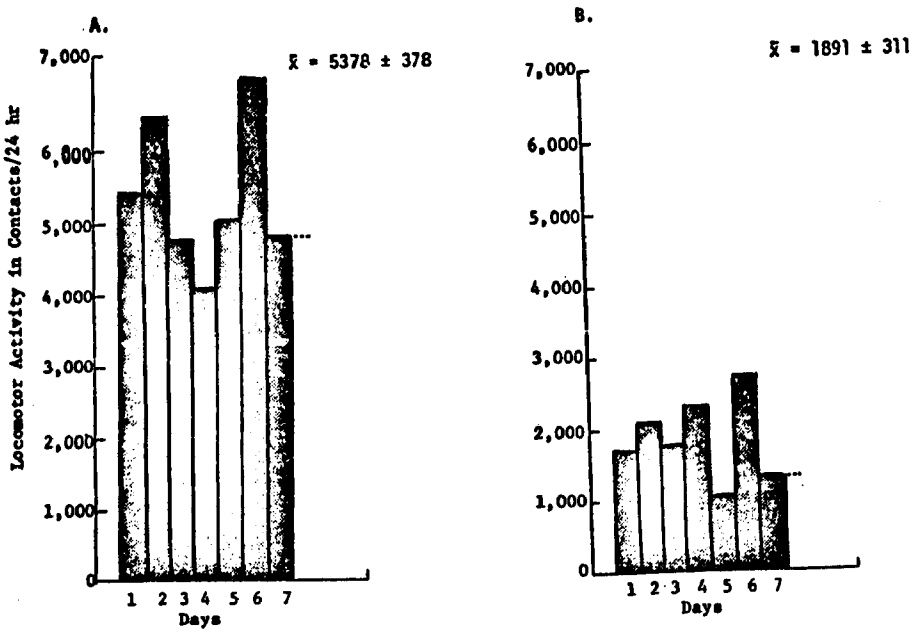


Figure 25. Consecutive daily activity totals of 2 Uca pugilator in LL, 25°C, 30 °/oo untreated control sea water in wheel actographs.

two control crabs: 5378 ± 378 contact counts/day and 1891 ± 311/day respectively for the 7-day recording period.

In a related experiment the mean daily activity rate of individual crabs kept for two weeks in either control sea water or 0.18 ppm Hg was compared (Fig. 26). These results suggest that inter-individual differences are high and would possibly mask treatment differences.

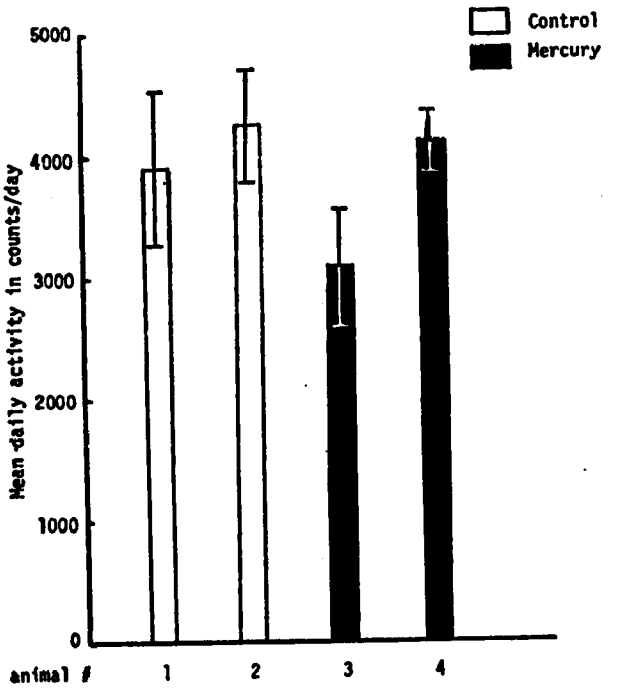


Figure 26. Mean daily totals of activity in Uca pugilator exposed for 14 days to 30 °/oo sea water containing 0.18 ppm Hg, 25°C, 12L:12D.

On the other hand, consecutive daily totals in untreated seawater seemed stable enough in each individual (Fig. 25) to serve as a control for the later Hg exposure. Therefore, a series of experiments consisting of 3 parts was initiated. Part A served as premercury control, Part B as Hg experimental, and Part C as post-Hg control. Data are presented in Figures 27 and 28 for the periodogram frequency analysis and daily activity scans for two representative crabs for the Parts A, B, C. Rhythmicity was not appreciably modified. Mean daily activity totals are shown for 5 crabs in Figure 29. A clear reduction of activity occurred. Average change of Hg-treated over control for the crabs ranged from 5% increase to 38% reduction (Fig. 29), with mean reduction for all animals of 23%.

Difficulty was encountered with some crabs (Fig. 28) following feeding and cage cleaning. The high bursts of activity would definitely interfer with an assay method. In an attempt to further refine the method several new sources of food were tried to find a way of keeping a constant food supply without fouling the living and recording compartment. "Biorell" proved most satisfactory. More feasible methods of using activity as a bioassay technique are considered in the discussion.

Electronmicroscope Study of Tissue Anatomy

Electron microscope studies of the tissues of adults treated for 42 days in 0.18 ppm Hg revealed sites of concentration and tissue damage. Mercury was found primarily in the gills, green gland, and hepatopancreas with highest concentration in the gills. The mercury caused extensive alteration of the ultrastructure of the gill filaments (Fig. 30B). Normally the filaments are characterized by tightly packed, interdigitating epithelial cells. The basal plasma membranes are thrown into folds that penetrate the cell almost to their apical surfaces. The cells contain numerous mitochondria localized within the folds (Fig. 30A). In gill tissue from crabs maintained in the sublethal concentration of mercury, the filaments showed less cytoplasmic protein, disappearance of membrane folds, and decreased number of mitochondria. Swelling and loss of the mitochondrial cristae were also observed (Fig. 30B).

Enzymatic Studies

Results of the studies on the influence of Hg on cytochrome c oxidase activity are summarized in Table 5. Enzymatic activity of gill tissue was affected primarily in tissue from warm-acclimated crabs at low temperatures. At these temperatures activity was reduced to approximately one half. Gill tissue from cold-acclimated crabs showed essentially no change in activity. In hepatopancreas tissue from both cold- and warm-acclimated crabs, however, Hg caused a marked decrease in activity.

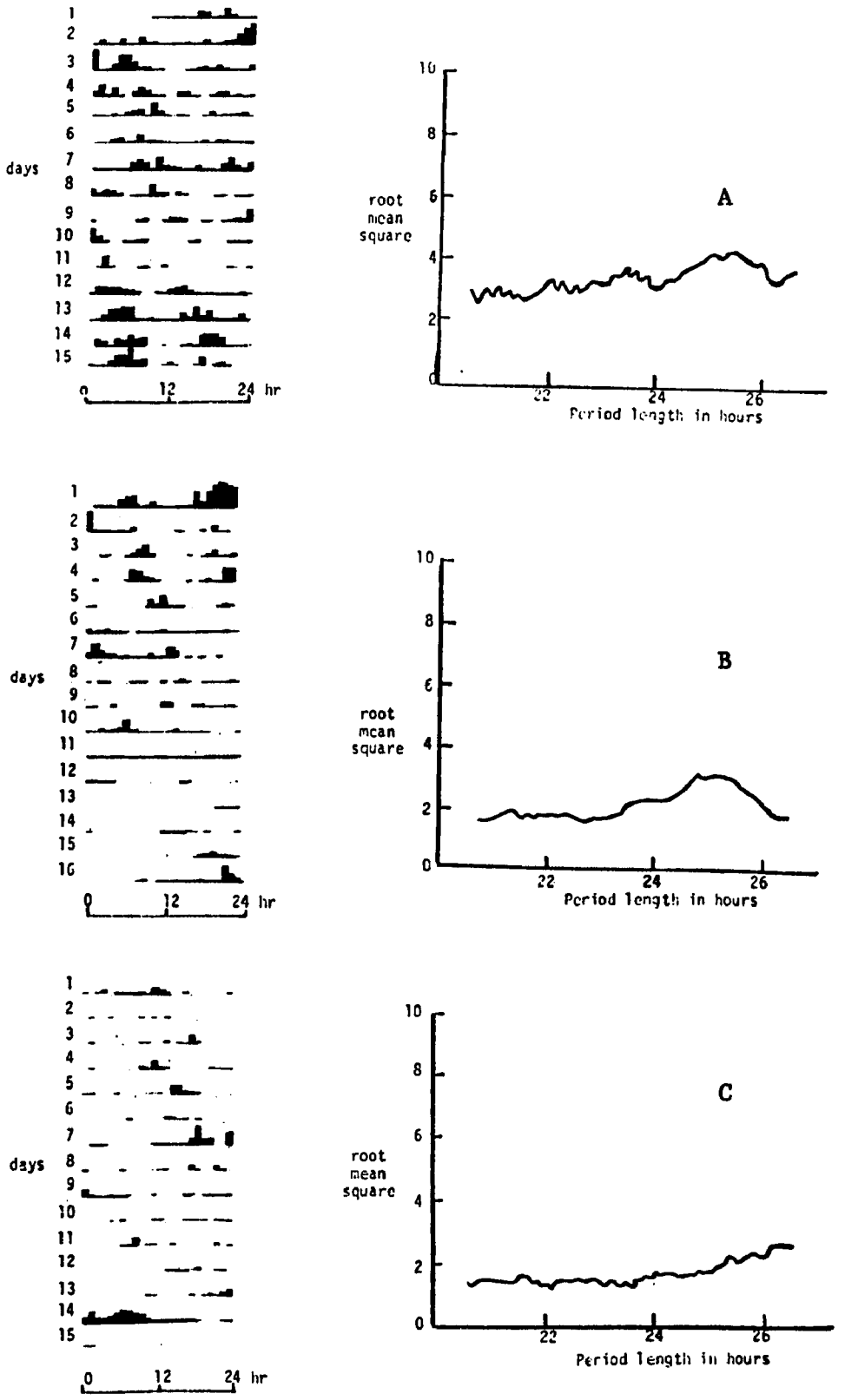


Figure 27. Activity of 1 Uca pugilator in LL, 30 °/oo seawater, 25°C in a wheel actograph. A, Control; B, 0.18 ppm; C, post-control.

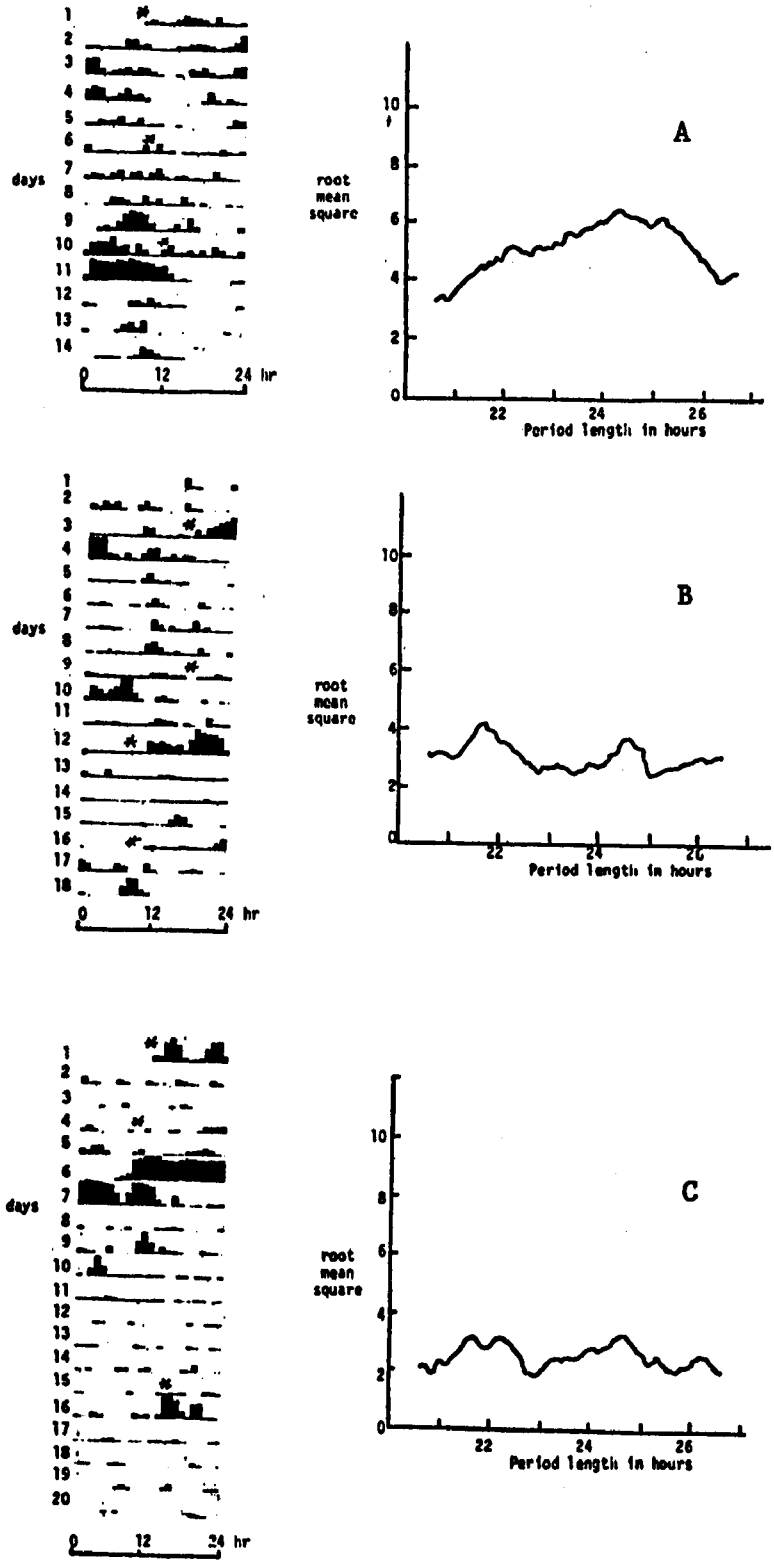


Figure 28. Activity of 1 Uca pugilator in LL, 30 °/oo seawater, 25°C in a wheel actograph. A, Control; B, 0.18 ppm; C, post-control. * indicates feeding or cage cleaning.

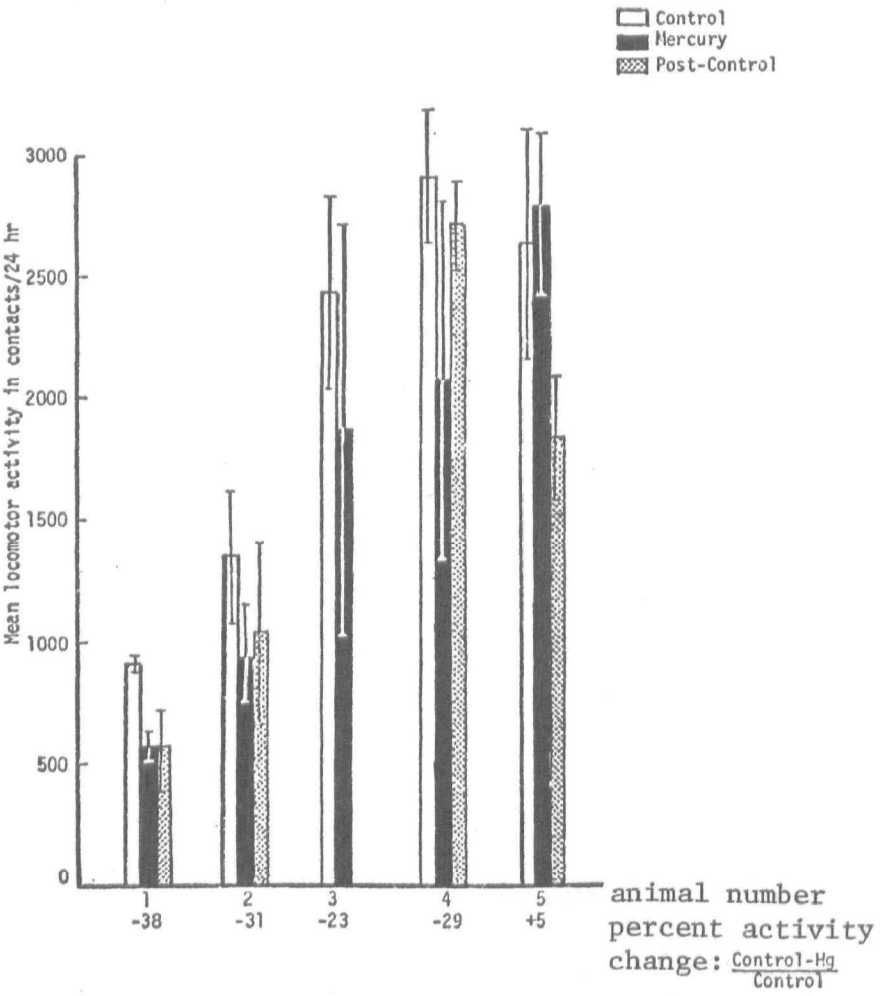
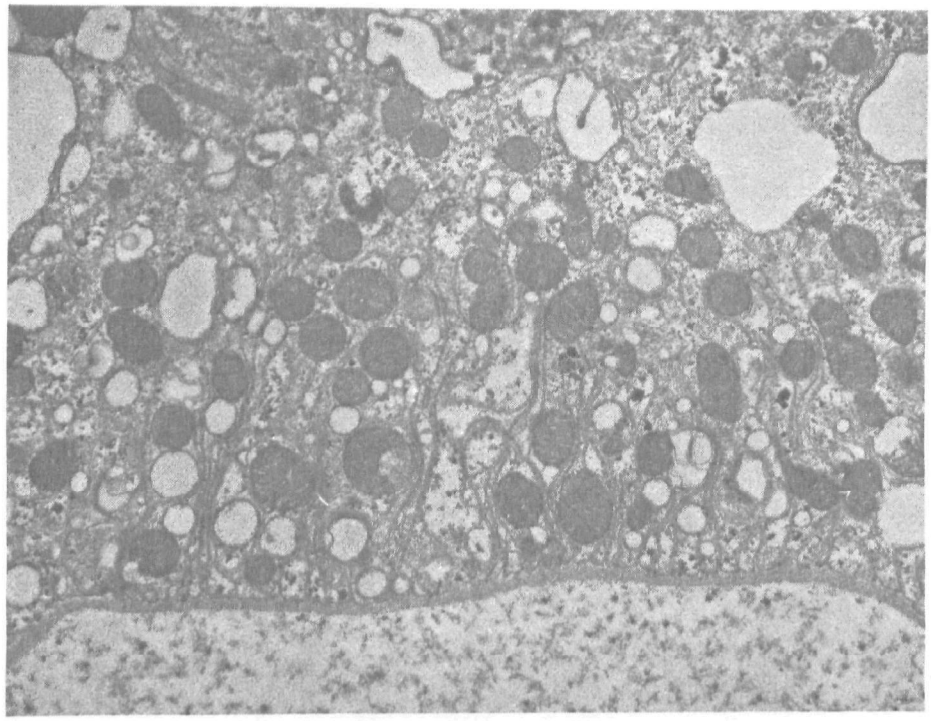
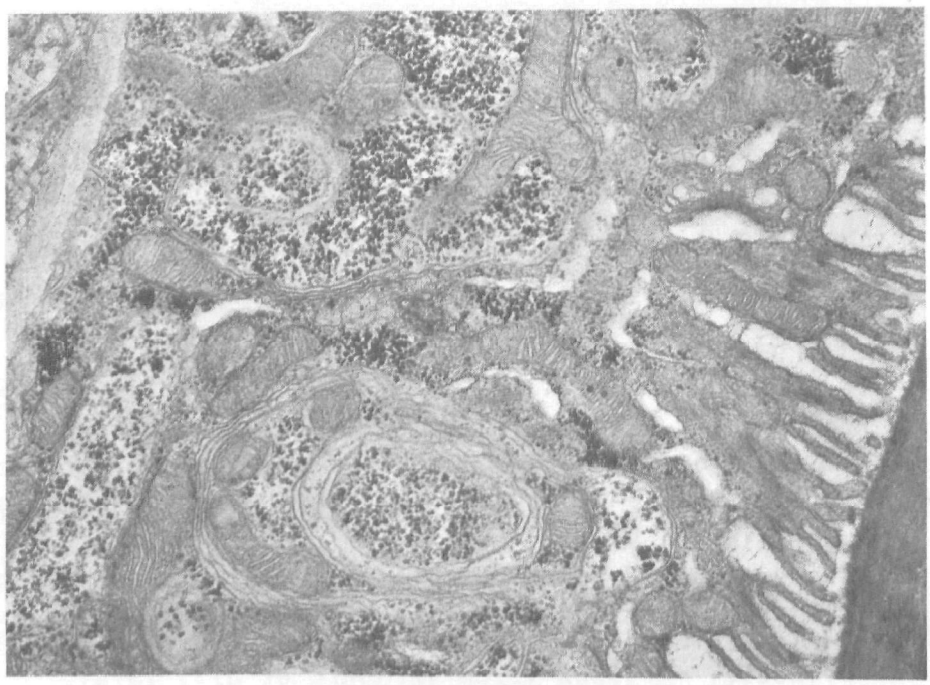


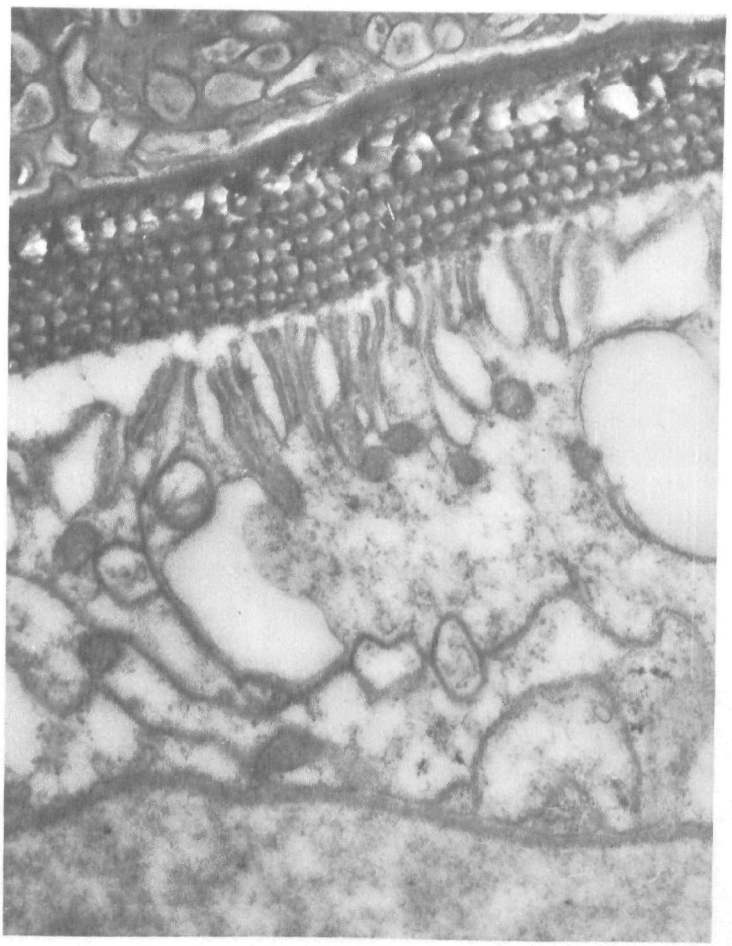
Figure 29. Locomotor activity of single fiddler crabs in LL at 25°C, first in control sea water 30 °/₀₀, followed by a treatment in sea water containing 0.18 ppm Hg, and a final post-control period in untreated sea water.





B.

Fig. 30A,B. Ultrastructure of gill tissue of control adult Uca pugilator. Normal gill filament showing interdigitating epithelial cells situated on the basal lamina. Mitochondria are localized within the folds of plasma membrane. A, 9,200X; B, 22,500X. Courtesy of N. Watabe.



C.

Figure 30C. Ultrastructure of gill tissue of adult Uca pugilator maintained in 0.18 ppm Hg for 6 weeks. Analysis of the tissues showed a concentration of approximately 17 ppm Hg. Note the loss of cytoplasmic protein. Membrane folds are not evident, and mitochondria are scarce and pycnotic. 17,500X (from Vernberg et al., 1974).

Table 5. CYTOCHROME c OXIDASE ACTIVITY IN GILL AND HEPATOPANCREAS TISSUE FROM COLD- AND WARM-ACCLIMATED CRABS EXPOSED TO 0.18 ppm Hg FOR VARYING LENGTHS OF TIME.

		mμ moles cytochrome c oxidized/mg protein/sec. Temperature °C			
Tissue	Day	15°	20°	25°	35°
Gill - W.A.	0(control)*	0.803	1.120	1.000	1.45
	3	0.600	1.040	1.130	2.04
	7	0.478	0.676	0.997	1.92
	14	0.543	0.823	1.140	2.86
	28	0.455	0.649	0.908	2.15
Gill - C.A.	0(control)*	0.831	0.996	1.756	3.081
	3	0.572	1.201	2.023	4.423
	7	0.782	1.052	1.652	3.786
	14	0.751	1.280	1.834	3.622
	28	0.703	1.067	1.890	3.488
Hepatopancreas-W.A.	0(control)*	0.558	0.651	0.934	1.330
	3	0.254	0.347	0.519	0.623
	7	0.256	0.414	0.434	0.672
	14	0.243	0.338	0.514	0.708
	28	0.301	0.322	0.436	0.612
Hepatopancreas-C.A.	0(control)*	0.631	0.938	1.180	1.420
	3	0.414	0.474	0.889	1.430
	7	0.423	0.602	0.757	2.021
	14	0.433	0.558	0.834	1.312
	28	0.316	0.448	0.615	1.334

*Not exposed to Hg.

LARVAL STUDIES

Larval studies centered on the synergistic effects of Hg and Cd combined with temperature and salinity stress. Zoeal stages I, III, V, and megalopa were selected for study. Response parameters included survival, metabolism and behavior.

Effect of Hg at Three Sublethal Concentrations Under Optimal Conditions

Viability experiments were first carried out with three concentrations of mercury (DeCoursey and Vernberg, 1972), in an optimum salinity-temperature regime (25°C, 30 °/₀₀). The highest concentration selected, 0.18 ppm Hg, was sublethal to adults exposed for a period of 6 weeks. The two lower concentrations, 9×10^{-9} M $HgCl_2$ (1.8 ppb Hg) and 9×10^{-11} M $HgCl_2$ (0.018 ppb Hg) are concentrations within the range

reported for certain polluted estuarines (Klein and Goldberg, 1970). A concentration of (9×10^{-7} M $HgCl_2$) 0.18 ppm Hg quickly proved fatal to stage I zoeae; 50% survival time was less than 24 hours (Fig. 31). The two lower concentrations of mercury also markedly affected viability, for survival time was considerably reduced in comparison to control values: 8 days in 1.8 ppb Hg or 11 days in 0.018 ppb Hg, in contrast to 18 days survival in untreated sea water.

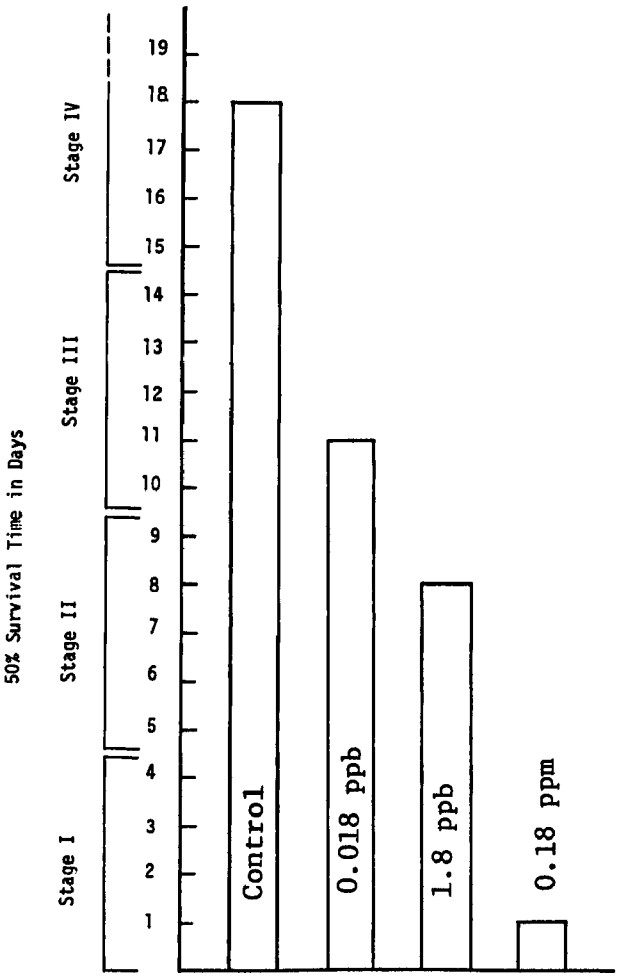


Figure 31. 50 percent survival time values for zoeae reared in untreated seawater or in three concentrations of Hg. The approximate number of days for each stage of larval development are indicated by brackets (from DeCoursey and Vernberg, 1972).

Additional viability experiments confirm the findings of the mercury-reared larvae. Experiments with 500 newly-hatched (stage I) zoeae from different hatches of eggs suggested considerable variability in survival time in 0.18 ppm Hg, ranging from 5-72 hours. When control-reared stage III or stage V zoeae were placed in 0.18 ppm Hg, they appeared even more sensitive. Only a few first and occasional third stage zoeae were able to survive for the 24-hr period. No stage V larvae were alive after 24 hours, and most died after 6 hours.

Rearing success statistics also support the above results. Survival results showed definitely reduced survival up to megalopa stage for mercury-treated larvae: 6 of 100 in 1.8 ppb Hg, 3 of 100 in 0.018 ppb Hg, but 20 of 100 in the control group.

Metabolism Studies

There was no immediate response of stage I zoeae to any of the concentrations of mercury. Oxygen uptake rates of these larvae one hour after exposure to the three experimental concentrations of $HgCl_2$ were unchanged from those of control larvae. A 6-hr exposure to 0.18 ppm Hg markedly depressed respiration rates of all stages tested (Fig. 32). The greatest decrease in metabolic rate occurred in stage V zoeae, where the rate of mercury-exposed larvae was approximately one-third that of control larvae.

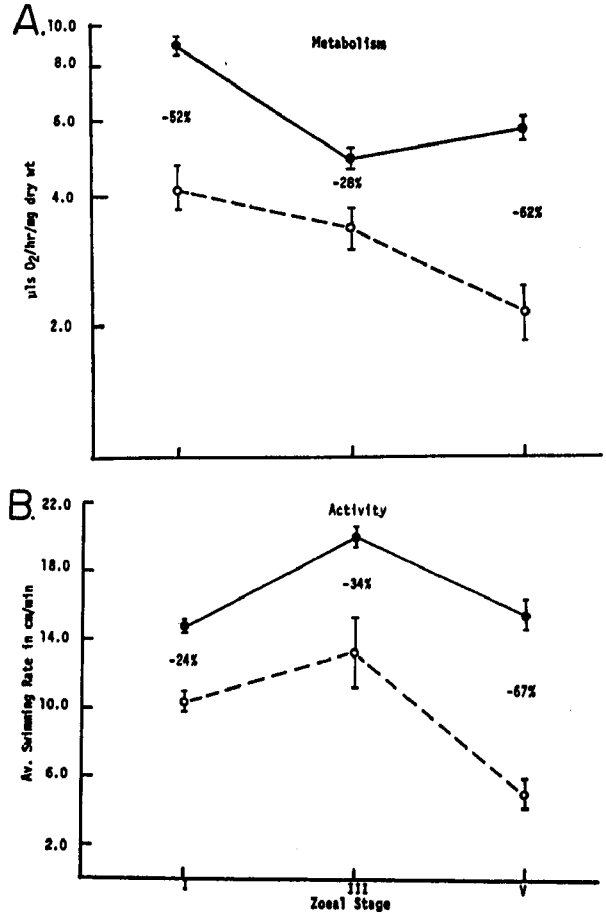


Figure 32. Percent in metabolic rates (A), and swimming activity (B) of zoeal stages I, III and V after six-hr exposure to a mercury concentration of 0.18 ppm Hg. ● = Control zoeae, o = Experimental zoeae. Vertical lines indicate ± one standard error (from DeCoursey and Vernberg, 1972).

Twenty-four hour exposure to the lowest mercury concentration used was without effect on metabolic rate in stage I zoeae, while 1.8 ppb Hg depressed rates. In stages III and V zoeae, there was a general tendency for mercury to increase metabolic rates (Fig. 33). In contrast, the oxygen uptake rates of stage V zoeae reared in mercury tended to be depressed, while the rate of stage III larvae were unchanged (Fig. 34).

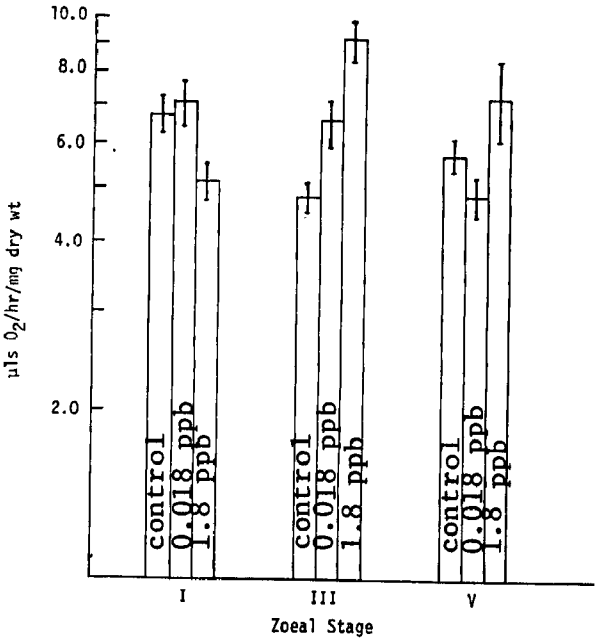


Figure 33. Acute exposure: metabolic rates of control zoeae and zoeae exposed to low concentrations of Hg for 24 hr. Vertical lines indicate ± one standard error (from DeCoursey and Vernberg, 1972).

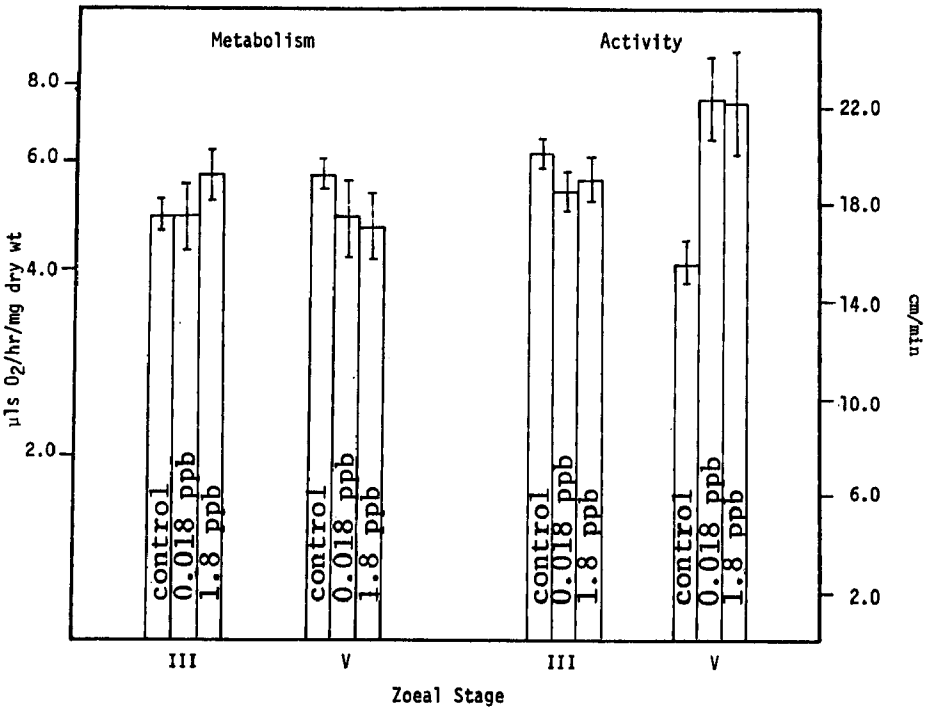


Figure 34. Chronic exposure: metabolic rates (left) and swimming activity (right) of control zoeae and zoeae reared in low concentrations of $HgCl_2$. Vertical lines indicate ± one standard error (from DeCoursey and Vernberg, 1972).

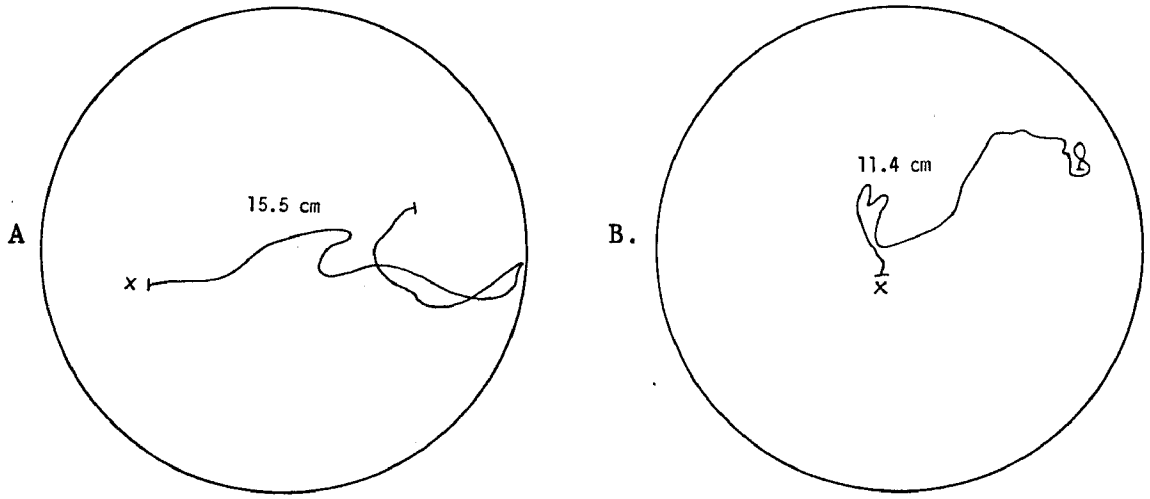
Behavior Studies

Observations on the normal swimming behavior of stage I, III, and V served as a baseline for detecting effects of the three mercury test solutions (DeCoursey and Vernberg, 1972). Using the maxillipeds, the zoeae usually swam in a fairly straight line (Fig. 35A). This type of swimming was interspersed with a variable amount of "tail lashing maneuvers," which resulted in a rapid change of direction, or often a whirling type of locomotion. Stage I zoeae usually swam in a start and stop fashion, while stage III zoeae, with a marked increase in size and complexity of the maxillipeds, were strong, steady swimmers. Stage V zoeae, which had increased greatly in weight, with little further development of the maxillipeds, were relatively slow, sluggish swimmers, often hovering close to the substrate. Such stage dependent differences are reflected quantitatively in the rate of swimming (Fig. 32B).

When zoeae were first introduced into the mercury, they often remained motionless for several seconds on the bottom of the dish, then darted erratically with considerable tail lashing for several minutes before adopting a characteristic swimming pattern. As the effects became more pronounced with time, the zoeae manifested marked swimming abnormalities such as erratic spiral swimming, swimming on their sides, or darting up from the bottom of the dish, then settling slowly to the bottom followed by disoriented twitching movements (Fig. 35B,C).

The effect of mercury solutions on normal activity was assayed at regular intervals after the start of exposure by tracking the actual swimming path in order to determine rate of activity. Six-hour exposures to 0.18 ppm Hg reduced the swimming rate of all larvae, with a greater effect on stage V larvae (Fig. 32B). Behavioral effects of 24-hr exposures are summarized in Figure 36; a concentration of 0.18 ppm Hg markedly reduced activity of all groups compared to the controls: 49% reduction for stage I, 79% for stage III, and 100% for stage V. As with 6-hr values (Fig. 32B), the older stages appear more sensitive to mercury than the newly-hatched larvae. When exposed to the two lower concentrations of mercury, the rate of swimming changed relatively litte; it was depressed to some extent for stage I, and elevated slightly for the older stages.

The chronic effects of mercury on activity are summarized in Figure 34. The highest concentration, 0.18 ppm Hg, was fatal to all zoeae, usually in less than 1 day, and therefore rearing could only be carried out in the more dilute concentrations. The data suggest that no change in activity of stage III occurred, but fifth stage swimming rate increased. This may represent a tendency for a more erratic type of swimming than in controls, as in the acute studies.



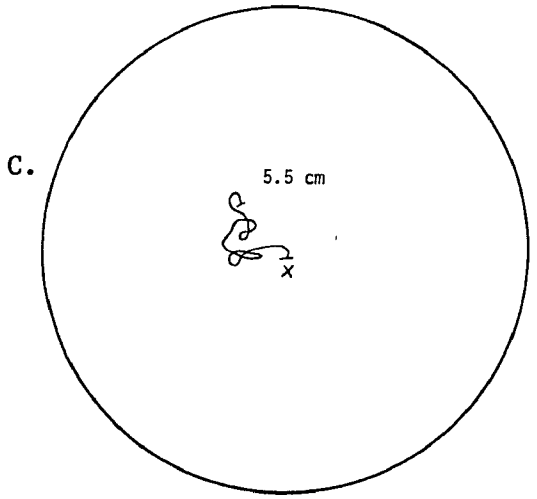


Figure 35. Effect of mercury on the swimming behavior of stage I zoeae; A. Control in 30 °/₀₀ untreated seawater; B, Experimental zoeae in 30 °/₀₀ seawater containing 0.18 ppm Hg after six-hr exposure; C. Experimental zoeae in 30 °/₀₀ seawater containing 0.18 ppm Hg after 24-hr exposure. o=outline of test chamber drawn to the scale of the track; ___=horizontal swimming track of one zoea for 60 sec.; X=starting point of test. (from DeCoursey and Vernberg, 1972).

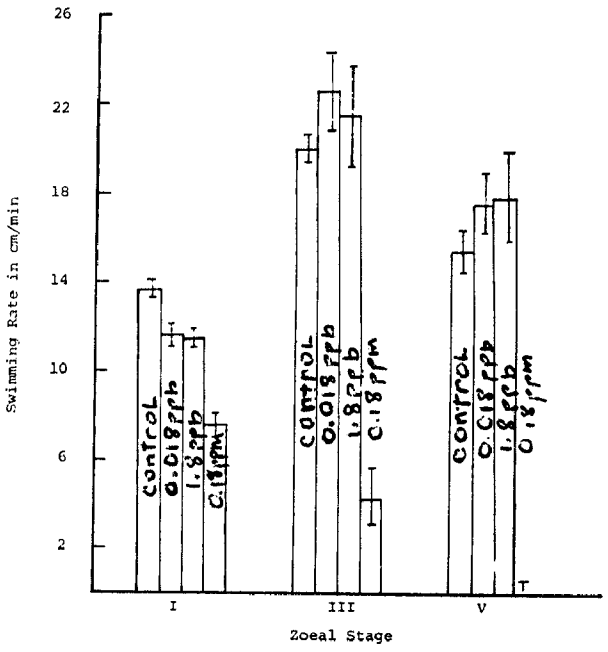


Figure 36. Acute exposure: swimming activity of control zoeae and zoeae exposed to low concentrations of Hg for 24 hr. Vertical lines indicate ± standard error (from DeCoursey and Vernberg, 1972).

Response to Hg Under Different Temperature-Salinity Regimes

Since the larvae inhabit estuaries where temperature-salinity conditions are often suboptimal, the next series of experiments considered effects of temperature-salinity stress within the range encountered in nature, with and without exposure to a low level concentration of mercury. The three parameters were survival, phototactic response, and O_2 consumption of the larvae.

After hatching, groups of control and Hg-treated larvae (1.8 ppb Hg) were reared in each of the following temperature-salinity conditions:

1. 30°C, 30 °/₀₀
2. 30°C, 20 °/₀₀
3. 20°C, 30 °/₀₀
4. 20°C, 20 °/₀₀

Viability of larvae under various test regimes is indicated by 96-hr mortality data. A total of 240 larvae were used with 30 in each of the 8 regimes, with mortality checked daily. Percent mortality data were analyzed by means of a factorial design with three factors: temperature (T), salinity (S), and mercury (Hg). Specifically, the experimental design was a 2^3 factorial with 3 replications of 100 larvae each, making a total of 24 observations. The two levels of each factor were: temperature, 20°C and 30°C; salinity, 20 °/₀₀ and 30°/₀₀; and mercury, 0 ppb and 1.8 ppb. Thus, the 24 observations may reasonably be considered as continuous responses of a function of the three factors and interactions. Since the observations are treated as percentage measurements generated by data from binomial populations, the transformation $y = \arcsin \sqrt{x}$, where x is observed percent mortality, is appropriate to stabilize variances (Mendenhall, 1968).

The 96-hr mortality studies are summarized in Figure 37. There was no difference between the controls and mercury-exposed zoeae in the 96-hr survival under conditions of high temperature (30°C) and optimum salinity (30 °/₀₀). However, control larvae maintained at high temperature and in low salinity (30°C, 20 °/₀₀) showed a 27% increase in mortality over larvae not exposed to mercury. At low temperature there was a marked increase in mortality at both optimal and low salinity with the addition of mercury. The analysis of variance for these data indicated the following factors had significant effects at the 5% level:

T, S, Hg, T x Hg, S x Hg

The interaction of T x S was not significant.

Metabolic rates were determined for a total of 90 first-stage zoeae which had been maintained for 24 hrs under the various suboptimal temperature-salinity regimes, with and without the addition of 1.8 ppb Hg. Metabolic measurements were made on 55 third-stage larvae reared in 20°C regimes with and without the addition of mercury. It was not possible to measure third-stage responses in the 30°C regimes because of the high mortality at elevated temperatures.

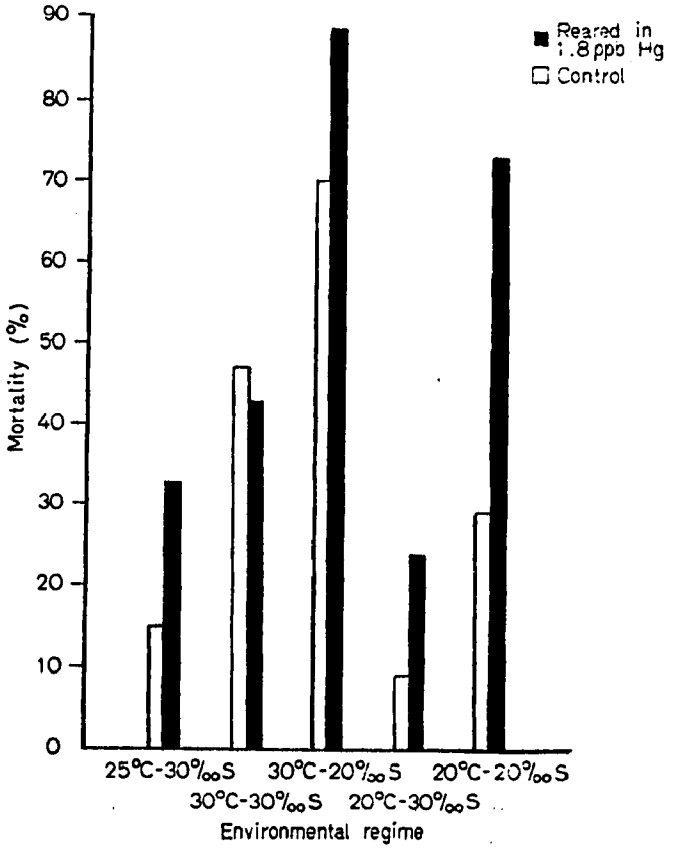


Figure 37. 96-hour survival time of U. pugilator larvae under optimal and suboptimal temperature-salinity regimes with and without the addition of Hg. Survival under optimal conditions (25°C, 30 °/₀₀) is based on data from Vernberg et al., 1973.

The metabolic rates of first-stage larvae reared under non-optimal regimes without added mercury were significantly decreased over that of larvae reared under optimal conditions (Fig. 38). The addition of mercury significantly affected the respiration rate of larvae reared under all temperature-salinity regimes, including optimal ones, except at 30°C, 30 °/₀₀. The effect seemed to be temperature dependent. At the higher temperatures, 25° and 30°C, mercury depressed metabolic rates; at 20°C, mercury enhanced metabolism in both optimal or suboptimal salinities.

Control larvae reared to the third stage under an environmental regime of 20°C, 30 °/₀₀ did not significantly differ metabolically from larvae reared under an optimal temperature-salinity regime (Fig. 39). The metabolic response of larvae from these two groups did differ, however, when the low level concentration of mercury was added to the water. The metabolic rate of larvae reared under optimal conditions increased significantly when mercury was added, while larvae reared under a suboptimal temperature regime showed a significant decrease in metabolism. The metabolic rate of larvae reared under the suboptimal temperature-salinity regime of 20°C, 20 °/₀₀ decreased to 56% of that of larvae reared under optimum conditions.

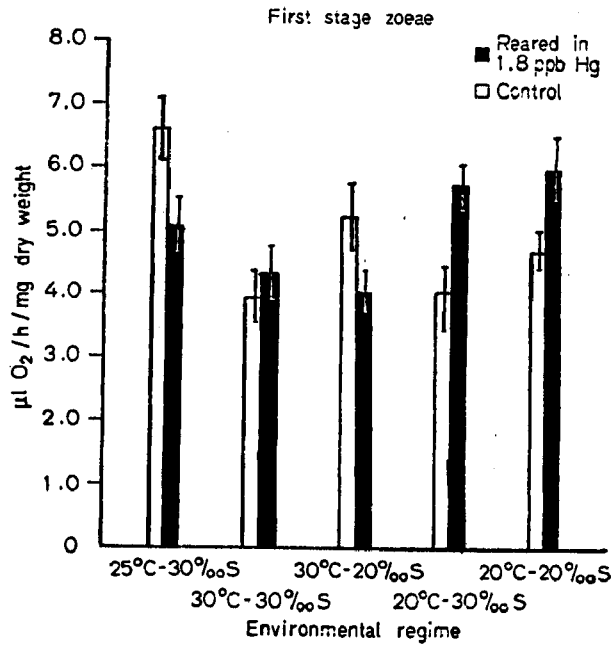


Figure 38. Metabolic rates of first-stage U. pugilator zoeae reared under optimal and suboptimal temperature regimes with and without the addition of Hg. Metabolic rates of larvae reared under optimal conditions are based on data from Vernberg et al., 1973.

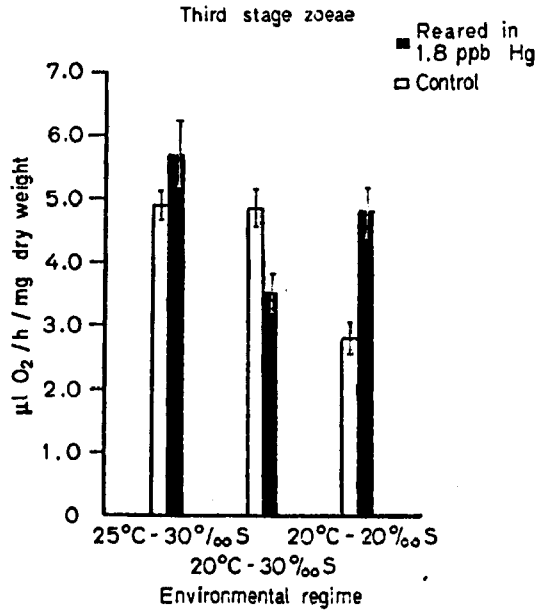


Figure 39. Metabolic rates of third-stage U. pugilator zoeae reared under optimal and suboptimal temperature regimes with and without the addition of Hg. Metabolic rates of larvae reared under optimal conditions are based on data from Vernberg et al., 1973.

The phototactic responses of zoeae were determined in a horizontal light gradient, using apparatus modified from Ryland (1960). Plexiglas® U-shaped tubes 4 cm wide x 40 cm long x 2.5 cm deep were filled with 150 cc test solution. After exposure of the larvae to the specified conditions, groups of 50 or less (stages I, III, or V) or a single larva (megalopa or crab stages) were transferred to a tube. The tubes were aligned on a base-plate divided into 10 equal segments, such that parallel rays from a narrow-beam theater lamp provided a horizontal light gradient. Intensity ranged from 1600 f.c. at the anterior end (segment 1) to 1300 f.c. at the posterior end. A black housing helped minimize light scattering. Temperature of the test chamber was maintained at 25°C.

In preliminary tests, the position of the larvae in the light gradient was noted at 10, 20, 60 minutes, and 2, 4, 6, 8, 10, 12 hours after the start of the test. Since readings did not change appreciably after 10 minutes this testing time was chosen for all later tests. The tubes were routinely reversed 180° after the test readings, and a second reading made 10 minutes later. Since reversed readings did not differ markedly from the first reading, only the initial readings were used in analyzing the responses of the larvae. Kite diagrams (Bayne, 1964) were constructed from pooled data for all animals in a specific test condition. Phototactic responses were first determined for 473 larval and early crab stages at 25°C, 30 °/₀₀ in untreated seawater. Responses were next measured for a total of 3,586 first-stage zoea which had been maintained 24 hours under the various temperature-salinity regimes with and without the addition of mercury. Tests were made for a total of 200 third-stage larvae reared in 20° regimes, under control or Hg-treated conditions. As noted above, it was not possible to measure third-stage responses in 30°C regimes due to high mortality rates at this temperature. Numbers of individuals used in each category are indicated in the figures.

The effects of suboptimal temperature-salinity-Hg regimes on phototactic response were also analyzed statistically. The basic experimental plan for stage I larvae was a 2^3 factorial experiment with the same factors (temperature, salinity, mercury) and levels as described above for the 96-hour mortality study. The same assumptions are reasonable and the response measured was the percent photopositive. All animals in the anterior 5 segments of the gradient tube were considered photopositive, and those in the posterior 5 segments photonegative. The observed percentages were again transformed by $y = \arcsin \sqrt{x}$, where x was the observed percentage. For the third-stage larvae, not enough experimental units were available for a complete analysis. However, the data were analyzed by a chi-square analysis of the 2 x 2 table of photopositive responses for two levels of temperature (20°C and 25°C at 30 °/₀₀) and the two levels of Hg (0 and 1.8 ppb). The chi-square value was also calculated at 20°C for the two levels of salinity (20 °/₀₀ and 30 °/₀₀) and the levels of Hg.

All control zoeal stages tested (I, III, and V) were markedly photopositive under optimum temperature-salinity conditions. Most megalops were also photopositive, but early crab stages appeared to be indifferent to light (Fig. 40). The phototactic response of stage I control larvae reared under the various environmental regimes shows some variability in the response (Fig. 41). The analysis of variance for these data indicate the following effects were significant:

At the 5% level: T x Hg, S x Hg
At the 10% level: Hg (and T x Hg, S x Hg)

The interaction S x T was not significant.

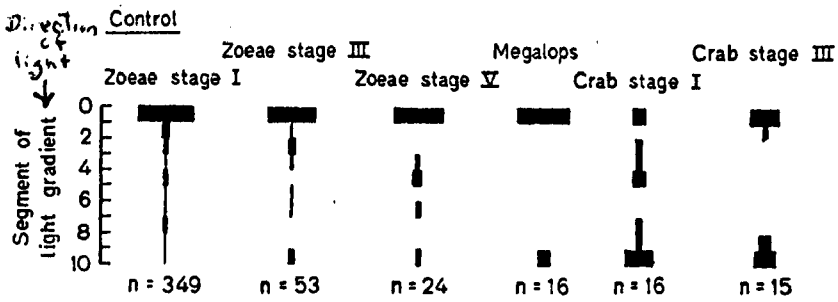


Figure 40. Positive phototactic response of U. pugilator larvae in the optimum (25°C, 30 °/oo) regime with untreated seawater (from Vernberg et al., 1973).

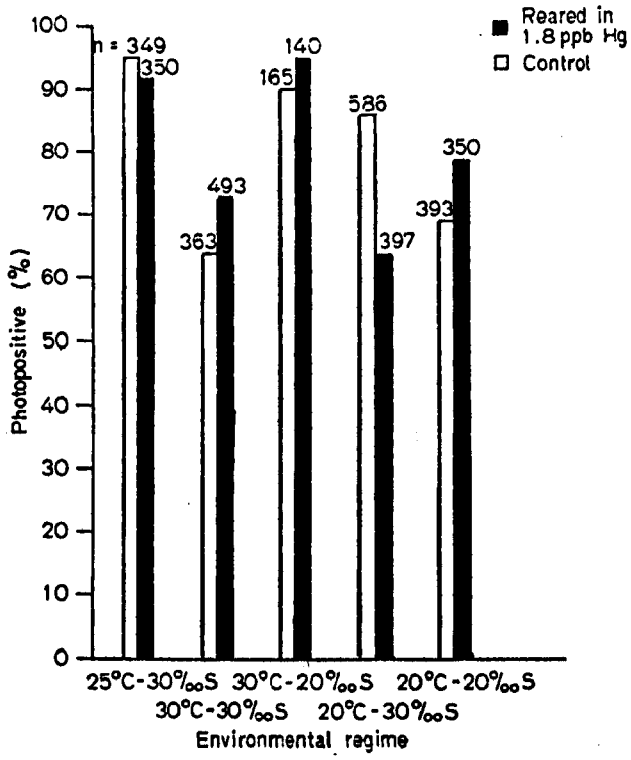


Figure 41. Phototactic response of first-stage U. pugilator zoeae reared under optimal and suboptimal regimes with and without the addition of Hg (from Vernberg et al., 1973).

Larvae reared to the third stage under suboptimal conditions showed marked changes in phototactic response. At 20°C, in a salinity of either 20 °/oo or 30 °/oo, the photopositive response of controls was sharply decreased over controls in optimal conditions (Fig. 42). Mercury-exposed larvae reared under these regimes were much more photopositive than control ones. Chi-square analysis for the two levels of temperature and the two levels of Hg (at a salinity of 30 °/oo) indicates an interaction between T and Hg at the 5% significance level. At a temperature of 20°C, however, a repetition of the experiment at the two levels of Hg (0 ppb and 1.8 ppb) and the two levels of salinity (20 °/oo and 30 °/oo) indicated no significant interaction of S with Hg.

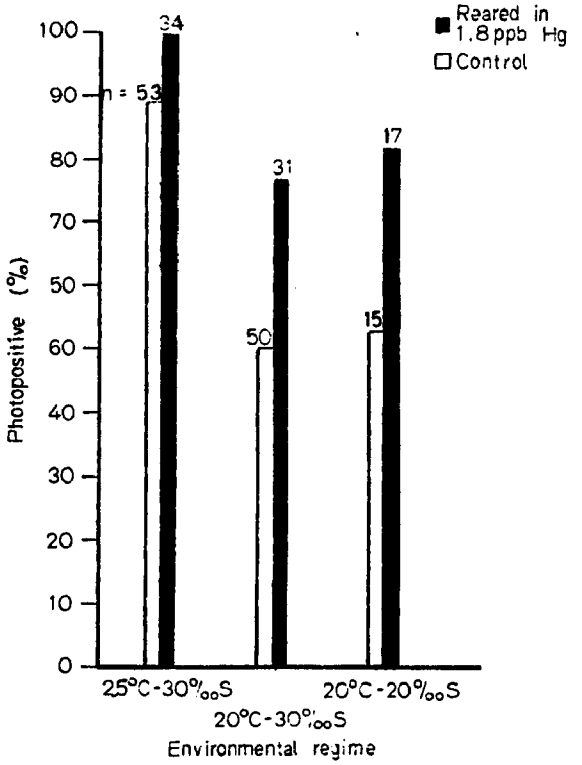


Figure 42. Phototactic response of third-stage U. pugilator zoeae with and without the addition of Hg (from Vernberg et al., 1973).

The final part of the larval studies concerned the effect of a sub-lethal dose of cadmium (1 ppb) on zoeal survival, metabolism and swimming rate.

The synergistic effects of temperature, salinity and cadmium on 96-hour mortality of zoeae was evaluated in a 5-factor temperature x 5-factor salinity matrix. One hundred zoeae were tested for each condition in the matrix. Untreated sea water was used for the control series, and the experimental series was dosed with 1 ppb Cd. The 96-hr percent mortality $\left(\frac{\text{number dead}}{\text{total}} \times 100\right)$ was calculated for each condition.

The mortality rates of cadmium-exposed zoeae were greater at 15°C, 20 °/oo; 20°C, 20 °/oo and 30 °/oo; and at 30°C, 15 °/oo and 35 °/oo.

A computer program was written for analyzing the mortality data from the 5 x 5 factor T, S matrix with and without added Cd. The results are portrayed graphically as a surface response curve series (Fig. 43).

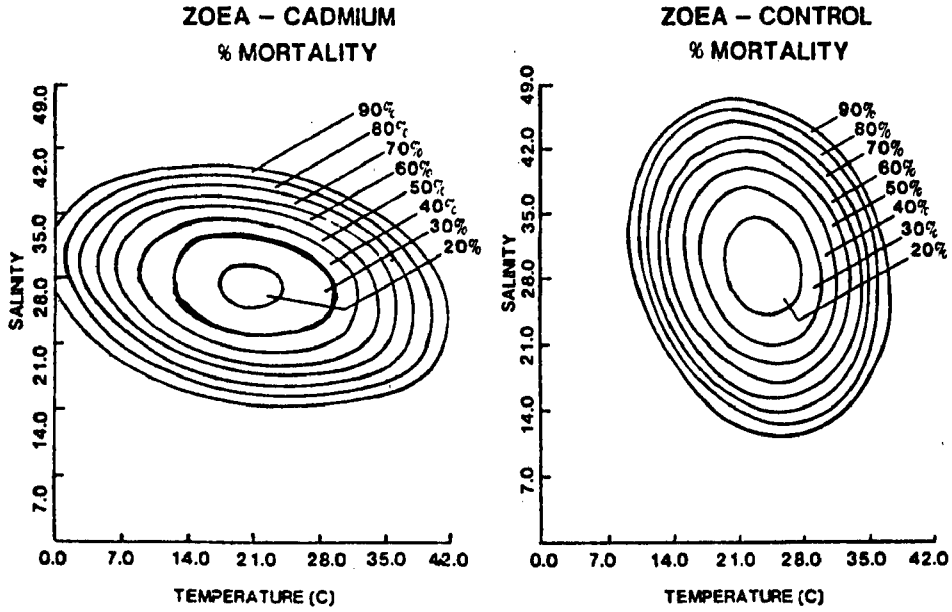


Figure 43. Surface response curves for the 5 x 5 factorial matrix of survival (S - T, with and without Cd) (from Vernberg et al., 1974).

The effects of 1 ppb cadmium upon metabolism of zoeae under optimum temperature-salinity conditions are summarized in Figure 44A. Cadmium elevates respiration of stage I and III zoeae but greatly depresses the rate in stage V zoeae.

Swimming rates of control and Cd (1 ppb) reared zoeae under optimum salinity and temperature conditions were determined for zoeal stages I, III, and V (Fig. 44B). Cadmium exposure resulted in a decrease of activity at all stages but the difference was significant only for stage I larvae (Table 6).

Table 6. EFFECT OF SUBLETHAL CADMIUM ON SWIMMING ACTIVITY OF ZOEAE.

	Control				Cd-1ppb			% Red.
Stage	Mean	S.E.	N*	Sig.	Mean	S.E.	N*	
I	23.5	1.42	18	1% level	18.2	1.62	16	22.6
III	53.1	5.8	20	n.s.	41.9	4.13	20	21.1
V	42.8	3.5	20	n.s.	37.8	5.1	18	11.6

*N is mean value/animal based on 4 determinations.

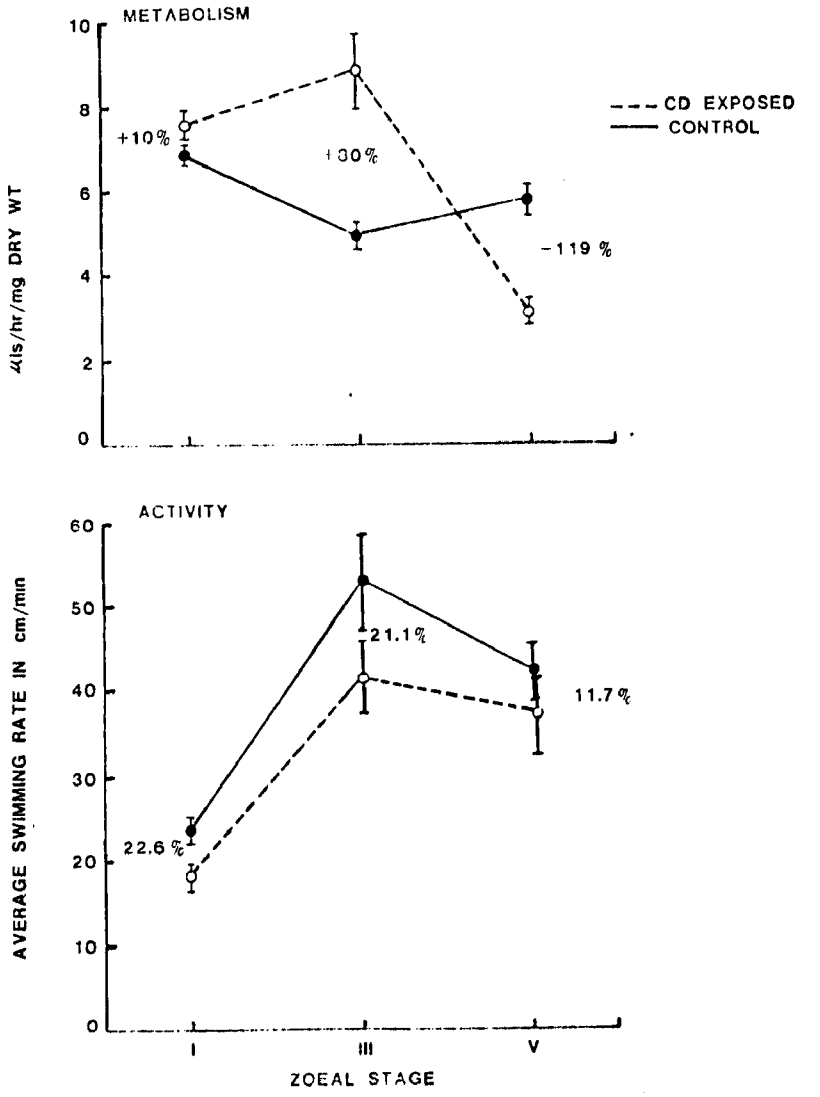


Figure 44. Percent change in metabolic rates (A), and swimming activity (B) of zoeal stages I, III, and V of Uca pugilator after rearing in 1 ppb Cd (from Vernberg et al., 1974).

Swimming rates in control larvae paralleled the response seen among controls in the mercury study with an increase in average rate in stage III,and gradual decrease to stage V as the larvae became heavier. Scale units in Figures 32 and 44B differ since the mercury studies used a tracking method of actual path and distance traveled, while Cd studies utilized a counting chamber and measured number of grid lines crossed.

SECTION VI

DISCUSSION

The sea contains trace amounts of many metals, and some are essential for normal growth in marine organisms. In higher concentrations, however, certain metals can be quite toxic, especially in combination with less than optimal environmental conditions. Adult crabs for example, can tolerate relatively high concentrations of mercury and cadmium for long periods of time when temperature and salinity are optimal, but under stressful temperature-salinity regimes, survival time is considerably shortened.

Mercury proved to be more toxic to male than to female crabs. In this study, fiddler crabs of both sexes were found to withstand mercury in combination with high temperature and low salinity better than in combination with low temperature and low salinity. Mortalities at both low and high temperatures were greater than under optimum temperature-salinity regimes (Vernberg and Vernberg, 1972a). Jones (1973) observed similar results in a study on the response of marine and estuarine isopods to Hg. He suggested that estuarine species, which are subjected to daily fluctuations of salinity and temperature, could be expected to be more adversely affected by the same concentration of Hg than open ocean animals living in a relatively stable environment.

In contrast to mercury, cadmium was most toxic at higher temperatures and low salinities, and there were no observed differences in toxicity between males and females. Other studies indicate that the influence of temperature on toxicity of pollutants is frequently unpredictable. Sprague (1970) for example, stated that "no assumptions should be made about temperature effects on toxicity." In a recent review on the effects of temperature upon the toxicity of chemical pollutants to aquatic animals, Cairns et al. (1975) have pointed out the widely varying responses of different groups of organisms to thermal-chemical stresses.

With both Cd and Hg, death of adult fiddler crabs probably is related to the accumulation of metal in the gills and the subsequent breakdown in osmoregulatory or respiratory functions. There are, however, major differences between the rate of uptake and site of accumulation of these two metals. Total mercury uptake is independent of the thermal-salinity regimes. However, at low temperatures, mercury is not translocated away from the gills to the hepatopancreas, thus leaving high mercury residues in the gill. Similarly, mercury was not translocated from the gills to the hepatopancreas under any temperature-salinity conditions in crabs that were simultaneously exposed to mercury and cadmium. Cadmium uptake, on the other hand, is strongly dependent on temperature-salinity conditions. At each temperature, crabs in low salinity water accumulated more cadmium than those exposed to high salinity. This effect is probably due to osmotic stress.

An intriguing puzzle in these studies has been the greater sensitivity of the male to mercury poisoning in comparison to the female both at high and low temperature. It cannot be explained on the basis of greater mercury uptake by tissues in male crabs under stressful environmental conditions, for these rates were essentially the same in tissues of male and female crabs (Vernberg and O'Hara, 1972). However, one clue may lie in the difference in metabolic responses of male and female crabs, both at the whole animal and the tissue level. Under conditions of thermal and salinity stress, without the addition of mercury, the metabolic rate of the female crabs tended to be more stable and less depressed than in male crabs. The addition of mercury to the already stressful conditions doubtless accentuated these differences.

One indication of why females survive Hg poisoning better than males may lie in the metabolic response of gill tissues following exposure to cold. In crabs that were cold-acclimated, there were no differences between control males and females in the metabolism of isolated tissues regardless of the temperature-salinity regimes. The same was also true for Hg-exposed male and female crabs. In warm-acclimated female crabs transferred to a low temperature-high salinity regime (5°C, 30 °/₀₀), the metabolic rate of gill tissue remained nearly constant through day 7. This was true regardless of whether or not they were exposed to Hg. In contrast, the rates of both control and Hg-treated gill tissue from males decreased sharply under this regime, with the tissue from the treated group showing an even lower rate than controls.

The basic regulatory mechanism which fails when warm-acclimated crabs are subjected to temperature-salinity extremes is unknown, but osmoregulatory failure is probably involved. In a study on osmoregulatory mechanisms in fishes, Renfro *et al.* (1974) found that $HgCl_2$ depressed ion transport. The authors suggested that part of this inhibition was due to interference with Na-K-ATPase activity; it would seem possible that a similar mechanism is involved in Hg-poisoned *U. pugilator*. The cause of the differential response between males and females to the temperature-mercury-salinity stress is unknown.

Exposure of *U. pugilator* adults to cadmium quickly altered metabolic acclimation patterns. The most striking change was the loss of compensatory metabolic temperature response to the cadmium-exposed crabs. Thus in warm-acclimated animals the metabolic response of Cd-exposed crabs remained unchanged over the temperature range 15-30°C whereas in control crabs rates increased sharply. Since the ability of temperate zone animals to adjust metabolically to temperature change is vital, loss of this metabolic ability obviously would lessen their chances for survival.

A number of difficulties were encountered in attempting to use locomotor activity of adult *Uca pugilator* as an indicator of stress. The design of the activity transducer was critical. Friction-damped whells appeared to give a reliable index of activity. Feeding of the crabs for long-term recording was a problem. Starvation definitely resulted in depressed activity levels, while intermittent feeding stimulated bursts of activity

during the time course of the experiment. A continuous supply of non-fouling food partially solved these difficulties. The high variability of activity in fiddler crabs, which was considerable, and the time and expense involved in semiautomatic recording raised serious problems for the development of an adult activity bioassay. In most cases it was possible to detect a precise frequency of rhythmic activity only by means of a periodogram computer analysis. When only daily totals of locomotor activity were used as the assay criterion, inter-individual variations proved excessive. A more feasible method of activity assay would involve use of large numbers of crabs. After maintenance in either control or experimental conditions, activity could be measured for a relatively short period in the actographs and mean values obtained for two groups.

Larval stages of U. pugilator were more sensitive to mercury by two orders of magnitude than either the adults or eggs. At a concentration of Hg sublethal to the adults and eggs, larvae only survived about 24 hours. Sensitivity of the larvae to acute mercury exposure increased as the larvae developed. A few larvae were able to live and grow in dilute mercury solutions with only slight changes in activity or metabolism. However, viability tests showed that the normal mortality rate among developing control zoeae was greatly accelerated in mercury-stressed larvae. The small proportion of larvae to reach stage V in 1.8 ppb Hg or 0.018 ppb Hg doubtless represents highly resistant individuals. Such results suggest that marine crustacean larvae may be considerably more susceptible to mercury pollution than previously suspected. Differences in tolerance to mercuric acetate have been found in life cycle stages of the fish Oryzias latipes where embryos were more sensitive than larvae, and these in turn were more sensitive than adults; the least sensitive was the egg stage (Akiyama, 1970).

Despite the fact that there are wide fluctuations in temperature and salinity in the estuary, numerous studies have shown that most temperate zone species of crustacean larvae develop over a rather limited temperature-salinity range (Costlow and Bookhout, 1971; Costlow et al., 1962, 1966; F.V.Vernberg and W.B.Vernberg, 1976). Our survival data on larvae of U. pugilator reared under suboptimal regimes demonstrated that developing larvae are particularly sensitive to warm water and low salinity. Mortality at high temperature (30°) was greatly increased over 25° (optimum) values, and only a few U. pugilator larvae underwent development to the crab stage under low salinity regimes regardless of the temperatures. At low temperatures, mercury sharply increased mortality rates. However, at high temperatures, control mortalities were so great without mercury that the added stress of mercury reduced survival only slightly.

Physiological responses of larvae also reflect the stress of suboptimal conditions. The immediate effect of reduced temperature (20°C) with optimum salinity was to depress the metabolic rate of the zoeae, although the animals did acclimate with time to the lower temperature. This acclimation is evidenced by the fact that the metabolic rate of third stage larvae reared at 20°C was the same as the rate in those larvae reared at 25°C. In a combination of low temperature and low salinity,

however, the zoeae did not show metabolic acclimation. U. pugilator larvae do not tolerate low salinity waters as well as other species of Uca (F.J.Vernberg and W.B.Vernberg, 1976). Since U. pugilator is restricted to sandy substrate habitats where there is rapid mixing of water with the more saline waters of the incoming tide, they are not normally exposed to low salinity waters. The lack of observed metabolic acclimation at low salinity confirms these ecological observations. The marked alteration of respiration rates in larvae exposed to mercury could appreciably affect the ability of the larvae to compete in the estuarine environment. At 20°C, 20 °/oo, for example, metabolic rates of both stage I and stage III zoeae were lower than those of zoeae reared under optimal conditions. Similar responses to decreased temperature have been noted for other organisms, and are generally considered to be adaptative responses (Vernberg and Vernberg, 1972b). Mercury effectively minimized this adaptive response.

The locomotor behavior of larval U. pugilator also reflected heavy metal toxicity. With the 3 concentrations of mercury employed, swimming activity was modified in direct proportion to the concentration and duration of exposure. Modification of locomotor activity could decrease the ability to avoid predation or to capture food.

As with many intertidal zone animals, the behavior of the larval stages is modified by their response to light. Many species are photopositive throughout larval life. In newly-hatched larvae, this response brings them into the phytoplankton-rich waters where they grow and develop. In older stages, positive phototactic responses insure movement to the surface and eventually into intertidal areas suitable for metamorphosis and later development (Thorson, 1964). The response for stage I Uca larvae was strongly photopositive regardless of the salinity or temperature regime. Other workers have found that temperature and reduced salinity can modify the response to light. These modifications are thought to have adaptive value for the larvae. In the eyed-veliger stage of Mytilus edulis, for example, the larvae concentrate toward the light at temperatures between 7° and 15°C, but when temperatures are raised to 20°C, the larvae are no longer photopositive (Bayne, 1964). Thorson (1964) has suggested that the lack of photopositive response at high temperatures would tend to remove the larvae to deeper, cooler water where they are metabolically better adapted. The phototactic response of stage I zoeae was not significantly modified by the addition of mercury at any of the environmental regimes tested. However, in third-stage larvae reared at 20°, the photopositive response of the control group was considerably reduced compared to the mercury treated group. Salinity apparently was not a critical factor since the phototactic response was essentially the same in larvae reared at either 30 °/oo or 20 °/oo.

Although cadmium did increase mortality of zoeae over that of control larvae, it was not as toxic as mercury. Under optimum conditions, the same number of cadmium-exposed zoeae survived to the megalops stage as controls; it was only under suboptimal conditions that mortalities increased.

The metabolic rates of zoeae reared in cadmium were more affected than were those of zoeae reared in mercury. The metabolic rates of mercury-reared zoeae were essentially the same as those of control zoeae (Fig. 36). In cadmium-reared larvae, however, both stage III and stage V zoeae were markedly different from that of control larvae (Fig. 47). Such results suggest again that pollutants can modify normal physiological function, thereby reducing chances for survival in nature, but the mode of action of a heavy metal contaminant may well vary with the metal involved.

SECTION VII

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16. ABSTRACT

Studies have been carried out on the synergistic effects of sublethal concentrations of mercury (Hg) and/or cadmium (Cd) in conjunction with temperature and salinity stress on larval and adult fiddler crabs, Uca pugilator. Six biological parameters of the adult organism were monitored including survival, tissue uptake, metabolism, behavior, microscopic anatomy, and enzymatic activity,using metal concentrations of 0.18 ppm Hg and 1.0 ppm Cd. Studies with larval stages (zoeal stages I, III, V and megalops) considered survival, metabolism and behavior under conditions of 1.8 ppb Hg and 1.0 ppb Cd. The effect of mercury or cadmium on Uca pugilator depands upon a number of factors, including stage of the life cycle, sex, thermal history, and environmental conditions. Data presented here suggest that the mode of action of the two metals is not the same.

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