THE SYNERGISTIC EFFECTS OF TEMPERATURE, SALINITY, AND MERCURY ON SURVIVAL AND METABOLISM OF THE ADULT FIDDLER CRAB, *UCA PUGILATOR*[1]

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ABSTRACT

Gill tissues of fiddler crab, *Uca pugilator*, were the major site of mercury concentration; lesser amounts accumulated in the hepatopancreas and green gland. Very small amounts were found in the carapace and muscle tissue. No significant differences in the amount of mercury in tissues of males and females were found.

A concentration of mercury sublethal to fiddler crabs under optimum conditions of temperature and salinity greatly reduced survival times when crabs were placed under conditions of temperature and salinity stress. Males were more susceptible to the synergistic effects of mercury in combination with environmental stress than were females.

Metabolic rates of male and female fiddler crabs were affected by prolonged exposure to mercury both under optimum environmental conditions and under temperature and salinity stress. Metabolic rates of males were more adversely affected than those of females.

Estuaries are an extremely important part of the marine environment. Yet often an estuary becomes so grossly polluted that much of the biota is destroyed before it is recognized that the quality of water affects the biology of such an area. Part of the problem is the subtleness of the effects of sublethal concentrations of man-introduced pollutants. In low concentration the pollutant may have no observable effect on a given population of animals if environmental conditions remain at an optimum. However, when another environmental parameter becomes stressful, it may synergistically interact with the sublethal concentration of pollutant and the organism dies. Many estuaries are polluted, and since one of the chief characteristics of estuaries is the rather extreme environmental fluctuations that occur throughout the year, knowledge of synergistic interaction on estuarine animals is important in the preservation of estuarine ecosystems.

This study was undertaken to determine the effect of a sublethal concentration of mercury on the metabolism of adult male and female fiddler crabs, *Uca pugilator* (Bosc), maintained under optimum and stressful conditions of temperature and salinity, and the synergistic effects on survival of this species with sublethal concentration of mercury in combination with salinity and thermal stress. This species was selected because it is one of the more abundant and ecologically important species in an estuarine ecosystem.

MATERIALS AND METHODS

Crabs used in this study were collected in the Georgetown, S.C., area during the fall and winter months. After collection the animals were brought into the laboratory where they were maintained in plastic boxes containing a thin layer of seawater having a salinity of approximately 30‰. All crabs were kept in constant temperature boxes at 25°C and on a 12-hr light-dark photoperiod for at least 2 weeks. Crabs were fed on Clark's fish pellets every third

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day; the water was changed after each feeding. Preliminary studies were undertaken to determine the amount of $HgCl_2$, an inorganic mercury compound, that could be added to the water without killing the crabs. A concentration of 9×10^{-7} M $HgCl_2$ was found to be sublethal for crabs which were kept under optimal conditions of temperature and salinity. Under these conditions crabs survived for a 2-month period with only slight mortality. The experiment was terminated at this point. The initial concentration of mercury, 9×10^{-7} M $HgCl_2$ was 0.18 ppm Hg (or 0.18 mg/liter seawater).

Tissues of crabs were analyzed for mercury following exposure to 0.18 ppm mercury in 30‰ seawater at a temperature of 25°C for 1, 3, 7, 14, and 28 days. Tissues of crabs maintained under the same conditions but without added mercury were also analyzed. Tissues were removed from 10 crabs for each assay and frozen immediately. The concentration of mercury in each tissue was then determined on a Perkin-Elmer Mercury Analyzer System-50.[3] The techniques were based on the Environmental Protection Agency method developed by the Analytical Quality Control Laboratory, using dilute nitric acid to digest the samples. Determinations were made by South Carolina State Board of Health personnel. Five tissues were assayed: gill, hepatopancreas, green gland, abdominal muscle, and carapace. None of the tissues were kept frozen for more than 1 week. Tissues from 20 males and females (two determinations each) were assayed for each of the five experimental time exposures. Since the amount of mercury proved to be essentially the same in tissues of both males and females, all data were pooled.

To determine the synergistic effects of the normally sublethal concentrations of mercury and stressful environmental factors, crabs acclimated to 25°C, 30‰ seawater were placed in seawater with a salinity of 5‰ containing 0.18 ppm mercury or in 5‰ seawater without mercury and maintained at either 5°C or 35°C. At each experimental temperature, four groups of animals were used. Thus at 5°C, 5‰, one group of 30 males and a second group of 30 females were used as controls; in the experimental group 30 males and 30 females were maintained under the same conditions except the water contained 0.18 ppm mercury. The same procedure was followed at 35°C and in a salinity of 5‰. Survival of both experimental and control crabs was followed for 28 days or until 50% of any one group had died. The temperatures of 5°C and 35°C were selected since they represent low and high temperature extremes which fiddler crabs experience seasonally in South Carolina marshes. A salinity of 5‰ is also encountered by them in the field.

Oxygen consumption of control and experimental animals was determined by means of a Gilson respirometer using respiration flasks with a volume of approximately 140 cc. Base-line oxygen consumption measurements were made on 10 males and 10 females in untreated seawater (30‰) at 25°C. These same crabs were then maintained under the same conditions but with 0.18 ppm Hg added to the water, and metabolic determinations made on days 1, 3, 7, 14, 21, and 28. Only medium-sized crabs in the intermolt stage were used to avoid any variation due to molting or metabolic size relationships.

Oxygen consumption rates were also determined on crabs exposed to mercury in combination with temperature and salinity stress. Metabolic measurements were made on crabs maintained in 5‰ seawater at 5°C (control crabs) and crabs kept in 5‰ seawater at 5°C with 0.18 ppm Hg added to the water (experimental crabs). Oxygen consumption rates were then determined after 1 and 3 days exposure for experimental crabs and 1, 3, and 7 days for control crabs. These conditions proved too stressful for most of the crabs to survive longer periods of time. The same experimental procedures were followed for crabs kept in 5‰ at 35°C with and without added mercury. Since these conditions were less stressful than the combination of low salinity and low temperature, it was possible to measure the metabolic rate of these crabs on days 1, 3, 7, 14, and 21 for experimental animals and to day 28 for control crabs. All results are expressed as μliters of oxygen consumed per hour per gram live weight.

[3] Reference to trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

RESULTS

MERCURY UPTAKE BY TISSUE

Mercury was not detected in the untreated seawater, although there were detectable traces of mercury found in the Clark's fish pellets fed to the crabs. The hepatopancreas of the control animals (animals collected in the same region as experimental animals and maintained in seawater without mercury addition) had approximately 0.03 ppm mercury, but no mercury was found in any of the other tissues. Within the first 24 hr after exposure to 30‰ seawater at 25°C containing an initial concentration of 0.18 ppm mercury, however, gill tissue contained 1.73 ppm mercury; the amount of mercury in this tissue increased steadily with continued exposure (Figure 1). Of the five tissues assayed for mercury content, gill tissue was found to have the highest concentration. Mercury also accumulated in the hepatopancreas and green gland although much less rapidly and at a lower concentration level (Figure 1). Lower 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.

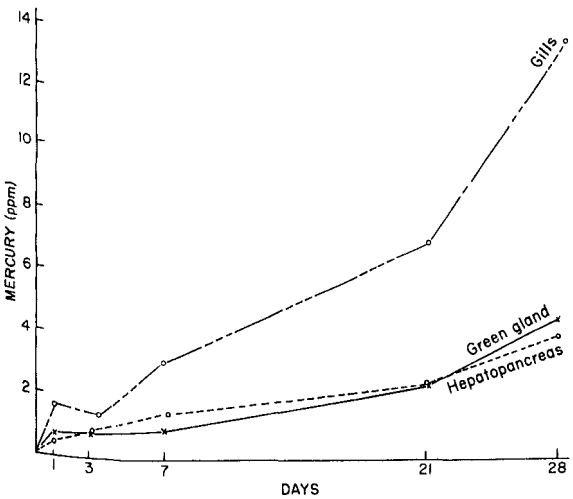

FIGURE 1.—Mercury in tissues of *Uca pugilator* after exposure of the crabs to 9×10^{-7} M $HgCl_2$ (0.18 ppm Hg) in 30‰ seawater at 25°C for varying lengths of time.

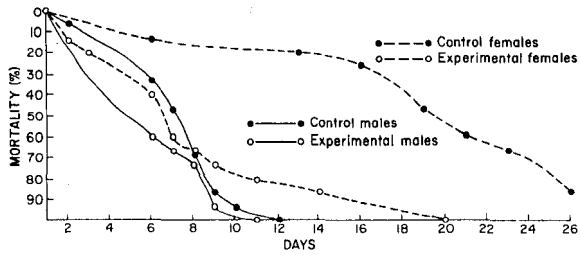

FIGURE 2.—Mortality of *Uca pugilator* in 5‰ seawater at 5° C with and without the addition of 9×10^{-7} M $HgCl_2$ (0.18 ppm Hg).

LETHAL LEVELS

Preliminary studies established that under optimum conditions of temperature (25°C) and salinity (30‰) the crabs could survive for prolonged periods of time (at least 2 months) in seawater having an initial concentration of 9×10^{-7} M $HgCl_2$ (0.18 ppm mercury). Under temperature and salinity stress, however, this concentration of mercury significantly shortened survival time. For example, under conditions of low temperature (5°C) and low salinity (5‰), 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 survived 21 days but 50% of the males were dead within 7 days. Under the same temperature and salinity conditions with the addition of 0.18 ppm mercury, males survived 6 days, but 50% of the females died by day 8 (Figure 2). Under conditions of low salinity (5‰) and high temperature (35°C), conditions very 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 (Figure 3). With the addition of 0.18 ppm mercury, however, survival times of both males and females are reduced. Under conditions where 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% of the females survived to day 26 (Figure 3).

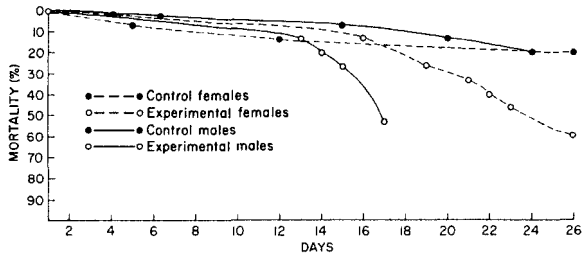

FIGURE 3.—Mortality of *Uca pugilator* in 5‰ seawater at 35°C with and without the addition of 9×10^{-7} M $HgCl_2$ (0.18 ppm Hg).

METABOLIC EFFECTS

Although a low level concentration of mercury was not lethal to the crabs under optimum environmental conditions, metabolic rates of these crabs were affected, especially those of males. Initially, metabolic rates were established for both males and females at 25°C in 30‰ seawater, and the rates for males and females were essentially the same (Figure 4). After the base-line rate was determined, the same animals were then maintained at 25°C in 30‰ seawater with the addition of mercury, and metabolism of the crabs was determined periodically for 28 days. The metabolic rate of the males remained essentially unchanged through day 3. By day 7, however, the metabolic rate had dropped to 32% of that of untreated crabs; by day 21 the rate had decreased by 48%; and by day 28 the rate was 20% lower than the base-line value. In the female, oxygen uptake values also decreased by day 7, but by day 14 the metabolic rate returned to the base-line rate and remained essentially at this level through day 28. Although initially the same, the rate of oxygen uptake of males was significantly lower than that of the females after 21 days in this sublethal concentration of mercury, and the metabolic rate of the males had not returned to the same level as it was before the crabs were placed in mercury by the end of the 28 day experimental period (Figure 4). Both males and females, however, continued to survive for another month under the same mercury regime as before without any significant increase in mortality.

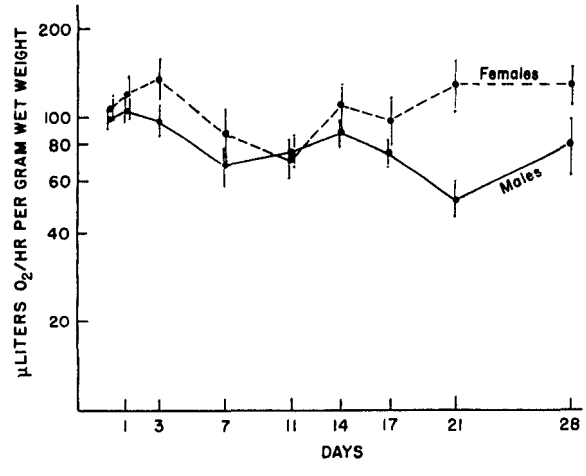

FIGURE 4.—Oxygen uptake rates of male and female *Uca pugilator* maintained in 30‰ seawater containing 9×10^{-7} M $HgCl_2$ (0.18 ppm) at 25°C. The base-line rate is represented by the first set of data points on the left. The vertical bar through each mean value is the standard error.

Under conditions of low temperature (5°C) and salinity (5‰) stress, not only did females survive much longer than males, but also the females were better able to maintain a steadier rate of oxygen uptake (Figure 5). 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 was not significantly different from that of the female experimental or male and female control crabs, after a 1-day exposure to mercury, but by day 3 the rate dropped markedly (Figure 5).

Oxygen uptake rates of female control crabs maintained in low-salinity water (5‰) 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 (Figure 6). The metabolic rates of experimental 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 time period (Figure 6).

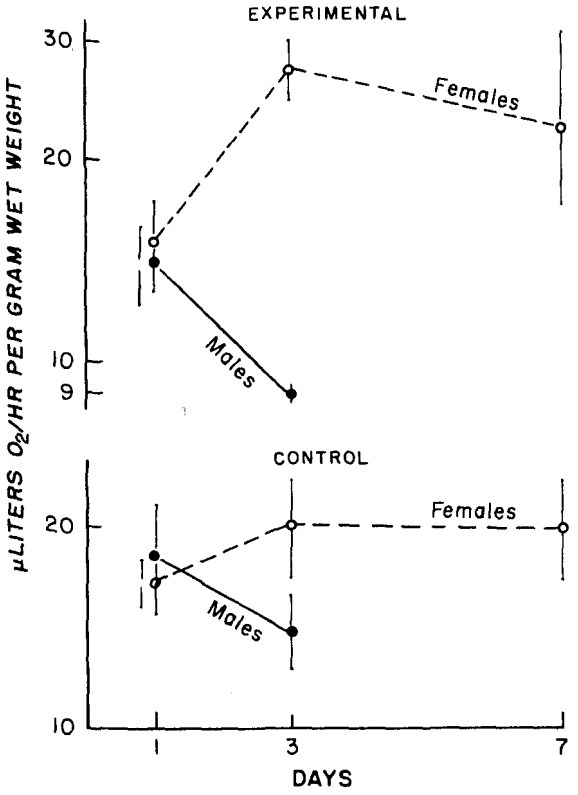

FIGURE 5.—Oxygen uptake rates of male and female *Uca pugilator* maintained at 5° in 5‰ seawater with and without the addition of 9×10^{-7} M $HgCl_2$ or 0.18 ppm. The vertical bar through each mean value is the standard error.

DISCUSSION

Fiddler crabs are capable of rapidly removing mercury from their surrounding aqueous media and retaining it in their tissues. However, not all tissues concentrate mercury to the same degree. The rapid accumulation and large concentration of mercury in gill tissue of fiddler crabs and the lesser but significant amounts of mercury found in the hepatopancreas and green gland are similar to results obtained in experiments involving other heavy metals. Bryan (1966), for example, found the highest concentration of zinc in the gills and hepatopancreas. He related these concentrations to the fact that excess zinc can be stored in the hepatopancreas in the crab *Carcinus maenus* and concentrated and excreted across the gills.

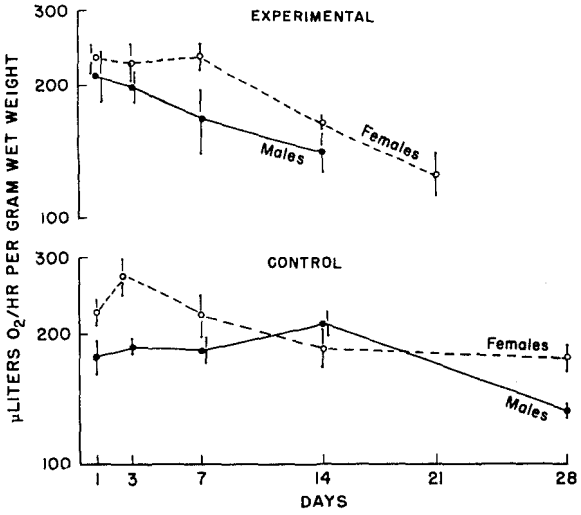

FIGURE 6.—Oxygen uptake rates of male and female *Uca pugilator* maintained at 35°C in 5‰ seawater with and without the addition of 9×10^{-7} M $HgCl_2$ or 0.18 ppm. The vertical bar through each mean value is the standard error.

Uca pugilator can adapt quickly to a wide range of adverse environmental fluctuations (Vernberg and Vernberg, 1970). The sudden changes in temperature and salinity that do occur usually do not persist for prolonged periods of time, and conditions usually ameliorate within a week or two. Results presented in this paper indicate that the crabs can withstand low salinity and high temperature better than low salinity coupled with low temperature, findings consistent with the earlier generality proposed by Panikkar (1940). Further, Lockwood (1962) stated that since ionic regulation is thermally influenced, organisms survive dilute medium more successfully when the rate of ion uptake as compared with ion loss increases faster with increasing temperature. Under both sets of conditions, however, the added stress of concentrations of mercury that are sublethal under optimum conditions adversely affected survival rates under stressful conditions and more markedly in males than in females. Bryan (1971) has speculated that the increased lethality of a heavy metal under stressful conditions is in some way related to changing rates of absorption. Our data are another example of the principle

that multiple environmental factors, each at a sublethal level, interact synergistically to cause death. Earlier papers, especially the classic paper of McLeese (1956), emphasized the lethal role of "normal" environmental factors, whereas we have demonstrated the importance of pollutants as part of the "normal" environment of many species.

Under optimal conditions of temperature and salinity mercury generally decreased metabolic rates of the males; effect on metabolic rates of females was much less pronounced. This differential effect of mercury on the metabolism of males and females is difficult to understand. On an interspecific basis, differences between resistance of larvae of *Artemia salina* and *Elminius modestus* to mercury have been related to differences between rates of uptake rather than of tissue resistance (Corner and Rigler, 1958). However, since the amount of mercury in tissues of both male and female fiddler crabs was essentially the same, these differences would not appear to be related to differences in uptake of the mercury. 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 the rate of male crabs. The addition of mercury to the already stressful conditions accentuated these differences.

Our results indicate, then, that a concentration of mercury that is sublethal under optimum conditions of temperature and salinity, may greatly reduce the ability of the population to survive under normally stressful conditions of temperature and salinity flux.

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