

Physiological and avoidance responses of juvenile mud crab *Scylla serrata* to mercury

¹Harold M. Monteclaro, ¹Ricardo P. Babaran, ¹Roman C. Sanares,
²Emilia T. Qunitio

¹ College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Iloilo, Philippines; ² Southeast Asian Fisheries Development Center–Aquaculture Department, Iloilo, Philippines. Corresponding author: H. M. Monteclaro, hmmonteclaro@up.edu.ph

Abstract. The physiological and avoidance responses of juvenile mud crab *Scylla serrata* to mercury was evaluated by determining mortality using a renewal-type acute toxicity test and assessing crabs' ability to avoid toxic concentrations. The 96-h LC₅₀ of mercury to juvenile mud crab was computed to be 0.04 mg L⁻¹. When transferred to clean waters, crabs that survived exposure to concentrations lower than 0.04 mg L⁻¹ had better chances of surviving than those that were exposed to higher mercury concentrations. Avoidance of juvenile mud crabs to mercury was determined using a fluvarium, which provided the crabs a choice between untreated and Hg-treated waters. Results showed that juvenile crabs were not able to avoid waters that contain 0.1 mg L⁻¹ mercury, a concentration that was more than twice the 96-h LC₅₀ value. Juveniles previously pre-exposed in 1/50th of the 96-h LC₅₀ value had a higher avoidance threshold and were not able to avoid waters with 1 mg L⁻¹ mercury. Results suggest that juvenile mud crab is unable to avoid waters containing lethal levels of mercury and this may have potential impacts on crab biomass, distribution, growth, and development.

Key Words: survival, avoidance, crustacean, mercury.

Introduction. Human activities such as mining, industrialization, and waste disposals have elevated the natural level of metals in the aquatic environment thereby threatening the ecology of aquatic organisms (Hart & Fuller 1979; Camargo & Alonso 2006; Lottermoser 2010). Among trace metals, mercury is particularly notable because of the considerable danger it presents to humans and animal populations (WHO 1989). Mercury can be released into the aquatic environment through several mechanisms such as the weathering of mercury mines (Gray et al 2000) and ore processing using the amalgamation process (Gustin et al 2003). The amalgamation process of extracting gold from rock is mostly practiced by small-scale miners in the Philippines, Suriname, Brazil and other countries (Appleton et al 1999; Gray et al 2002; Buot et al 2014). During rains, water effluents from point sources enter streams and rivers causing elevated levels of mercury in the receiving water bodies (Appleton et al 1999; Buot et al 2014). A major concern is the potential effect of mercury on riverine and estuarine organisms and their prey (Ernawati 2014). Organisms that may be affected directly by mercury in tropical areas include commercially important mud crabs *Scylla serrata* (Forsskål 1775). Knowledge of the physiological and behavioural responses of mud crab to mercury will help fishery managers determine the impact of mercury pollution.

This study determined the lethal concentration of mercury to juvenile mud crabs and its post-exposure effects. While toxicity of mercury to adult *S. serrata* has been reported (Nagabhushanam et al 1986; Krishnaja et al 1987), more studies are needed to fully understand the effect of mercury on smaller sized crabs. Very few studies were conducted to determine the preference–avoidance behaviour of crustaceans to heavy metals. With the increasing threat of pollution in the aquatic environment, information on the physiological and behavioural responses of organisms is helpful in mitigating effects on the organisms. This study determined if mud crab will avoid mercury plumes if given

an opportunity, and whether prior conditions to which mud crab has been accustomed tend to alter the preference–avoidance response.

Material and Method. A total of 1,620 juvenile mud crab *S. serrata* obtained from the mud crab hatchery of the Southeast Asian Fisheries Development Center – Aquaculture Department (SEAFDEC-AQD) in Iloilo, Philippines were used throughout the experiment. Tests were conducted between November 2006 and January 2007.

Acute toxicity test. To determine the toxicity of mercury to juvenile mud crabs, standard toxicity procedures from APHA et al (1995) were followed. Twenty crabs, which were starved one day prior to and during the exposure, were placed in glass aquarium holding 20 L test water with analytical grade mercuric chloride, HgCl_2 (Ajax Chemicals, 9 Short Street, Auburn, N.S.W. 2144 Australia). Based from the results of a range-finding test, the following mercury concentrations were used: 0 (control), 0.0100, 0.0325, 0.0550, 0.0775 and 0.1000 mg L^{-1} . Test solutions were prepared daily by the appropriate dilution of stock toxicants in pre-aerated seawater.

Each crab was placed in small perforated plastic containers to prevent cannibalism and facilitate handling. Crabs were exposed in triplicate containers for each mercury concentration. A renewal toxicity test was employed for this study. There was no aeration during the experiment. The number of dead crabs in each container was counted 24, 48, 72 and 96 h after the beginning of the test. The LC_{50} , which is the lethal concentration at which 50% of the crabs died, was computed after Reed & Muench (1938).

Post-exposure survival test. The methods of APHA et al (1995) and Shealy & Sandifer (1975) were employed for this test. After the end of the acute toxicity test, all survivors from the test concentrations with 0.0100, 0.0325 and 0.0550 mg L^{-1} Hg were washed with seawater and transferred to clean, mercury-free seawater. Surviving crabs from the control groups during the LC_{50} test served as the control group for this test. The crabs were reared and fed daily for 3 weeks in mercury-free water to determine whether crabs exhibit delayed mortality after exposure to mercury. The number of dead individuals including moulting and occurrence of morphological abnormalities, if any, in each group were recorded daily.

Avoidance test. Avoidance behavior was tested in a fluvium modified from Kroon & Housefield (2003). The fluvium (Figure 1) was constructed of fiberglass (inner dimensions: 90 cm length x 40 cm width x 25 cm depth). The separator (60 cm length) starts at the end of the upstream end and placed exactly in the middle of the fluvium. It was fed from the two feeder tanks: one for untreated water and one for mercury-treated water, which were freshly prepared during each trial. Water from each feeder tank entered the fluvium at a rate of 2 L min^{-1} into each of the two independent lateral halves. The flow rate was determined from the dye test to establish a uniform flow of treated and untreated water into the two lateral halves. From here, the water continuously flowed into the outflow pipes, which established water height at 2 cm. In control runs, untreated water was added to both lateral halves of the fluvium. In treatment runs, untreated water was added to one lateral half, whereas a mercury-treated solution was added to the supply line of the other half. Freshly-prepared mercury solutions with the following concentrations were used: 0.001, 0.01, 0.1, 1, 10 and 100 mg L^{-1} . The treated and untreated channels were altered randomly to negate any bias the crab may have for either side.

The juvenile mud crabs used in the test were from three groups based on pre-exposure: unexposed, exposed at low mercury concentration (1/250th of the 96-h LC_{50}) for 24 h, and exposed at high mercury concentration (1/50th of the 96-h LC_{50}) for 24 h. Methods modified from Olsen & Hoglund (1985) and Kroon & Housefield (2003) were employed. Twenty crabs were placed in the holding area, which was a perforated stainless steel cylinder lowered into position 10 min before the toxicant was administered. The cylinder was raised 2 min after the toxicant was introduced into the aquarium, i.e., the time when mercury was expected to reach the rest area based from

the dye test, and crabs were allowed equal access to the arms with treated and untreated water. The positions of the crab in the test area were recorded every 30 sec with a digital camera without flash to minimize disturbance. For each frame, the position of each juvenile (i.e., position of the snout) was determined visually, and the number of crabs in each lateral half of the test area was counted. The test ended after 20 min, thus there were a total of 40 images.

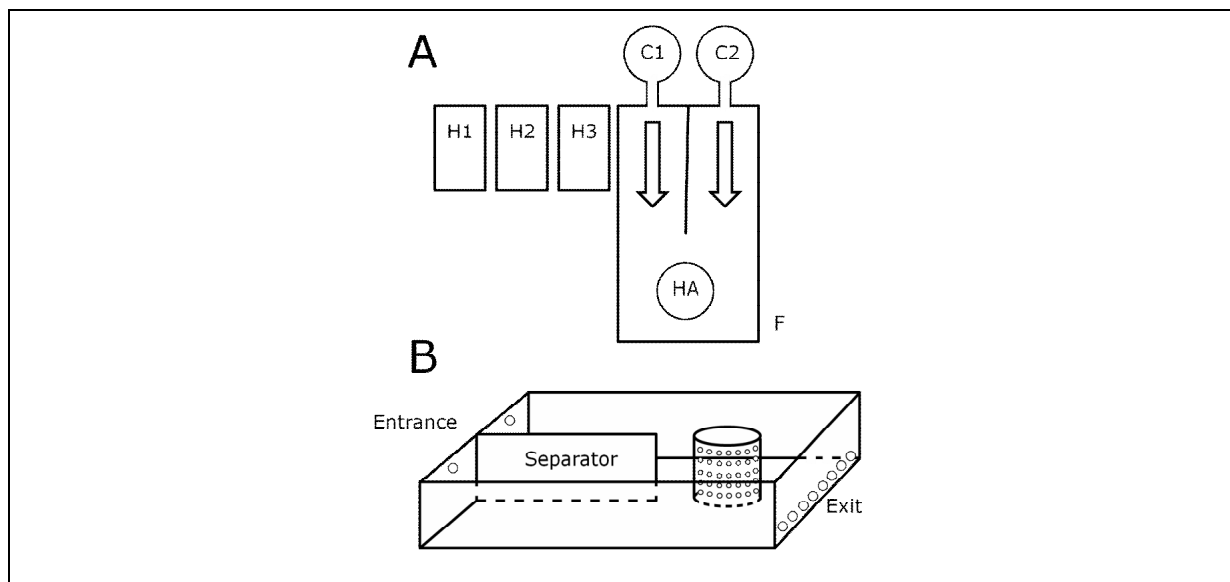


Figure 1. A. General lay-out of the preference-avoidance test area. Abbreviations: C = container with untreated or mercury-treated water; F = fluvium; HA = holding area for crabs prior selection of the left or right chamber; H = holding tanks for unexposed and mercury-exposed crabs. B. Detailed outline of the fluvium (not in scale) used in the preference-avoidance study.

The total number of crabs in each lateral half over the 40 images was calculated. Subsequently, the mean percentage of crabs in each lateral half of the test area was determined from all the 40 frames combined. This provided the mean proportion of crabs in the experimental half of the test area over a 20-min run. This mean proportion was considered the behavioural response of the crabs in that particular run and was considered as a single replicate in the data analysis. Each test concentration had three replicates.

Crabs were used only once. During the test, the set-up was enclosed by curtains to prevent possible crab response to human movements. After each experiment, the fluvium was washed appropriately with 5% HNO_3 , rinsed and washed with detergent, and again rinsed.

Statistical analyses. Data were processed using SigmaPlot 11.2. All data sets were tested for normality. In the post-exposure survival test, survival rates were tested for significant difference using the one-way ANOVA. In the avoidance test, proportions for an indifferent reaction (control tests) were performed using the paired t-test. In treatment runs, comparisons of proportions were performed using ANOVA. If significant differences were indicated, individual groups were compared using the Duncan's Multiple Range Test.

Results and Discussion

Toxicity tests. While no crab died in the control group, mortality of juvenile mud crabs generally increased with increasing mercury concentration and exposure period. Table 1 shows the toxicity of mercury to juvenile mud crabs at different exposure periods. The 96-h LC_{50} was computed at $0.04113 \text{ mg L}^{-1}$, a concentration which is recorded in water bodies near mercury and gold mines (Appleton et al 2006; Maramba et al 2006). Although it is difficult to compare results of toxicity studies because of differences in test conditions, the computed LC_{50} value in juvenile mud crabs is lower than most of the LC_{50}

values reported in juveniles and postlarvae of other marine crustaceans exposed in mercury (Connor 1972; Shealy & Sandifer 1975; Green et al 1976; Glickstein 1978; Mariño-Balsa et al 2000). Juvenile mud crabs are more susceptible to poisoning than adults; adults are 16 times more tolerant to mercury than juveniles (Nagabhushanam et al 1986; Krishnaja et al 1987).

Table 1
Computed lethal concentrations for juvenile mud crabs *Scylla serrata* exposed to mercury at different exposure times

| Time (h) | Mercury concentration (mg L ⁻¹) | | | | |
|----------|---|------------------|------------------|-------------------------------|--------------------------------|
| | LC ₀ ^a | LC ₂₅ | LC ₅₀ | LC ₇₅ ^b | LC ₁₀₀ ^c |
| 24 | 0.0100 | 0.07687 | 0.09049 | - | - |
| 48 | 0.0100 | 0.04071 | 0.05396 | 0.06675 | - |
| 72 | - | 0.03407 | 0.04711 | 0.05852 | - |
| 96 | - | 0.03028 | 0.04113 | 0.05218 | 0.07550 |

^a 0.0100 mg L⁻¹ was the lowest concentration used; ^b Mortality was less than 75% after 24h; ^c Mortality was less than 100% after 24, 48 and 72 h.

Post-exposure survival. When transferred to clean waters, the survival rates of juvenile crabs previously exposed in different mercury concentrations were significantly different (ANOVA, $p < 0.01$). The survival of juvenile mud crabs previously exposed to 0.0550 mg L⁻¹ was significantly lower than the survival of crabs previously exposed to 0.0100 mg L⁻¹ and in control groups (Table 2). Results suggest that juveniles exposed to water with mercury content more than the 96-h LC₅₀ value will not survive, even after the mercury concentration in surrounding waters have decreased, possibly due to gill and hepatopancreas damages (Krishnaja et al 1987). Mortality of crabs previously exposed in 0.0550 mg L⁻¹ occurred within 4 days after transfer. Survival seemed certain when the juvenile outlives the first 4 days after exposure.

Table 2
Survival and moulting rates of juvenile mud crab *Scylla serrata* after transfer from mercury-laden water to clean seawater

| Mercury conc. (mg L ⁻¹) in previous exposure* | Mean % survival after 21 d** | Mean % of molted crabs |
|--|---------------------------------|------------------------|
| 0 | 96.67 ^a | 86.67 |
| 0.0100 | 96.67 ^a | 96.67 |
| 0.0325 | 83.33 ^{ab} | 100 |
| 0.0550 | 46.67 ^b | 100 |

* n = 30 per treatment; ** Means with the same letter notations have no significant difference (ANOVA, $p < 0.01$)

All, except five crabs, moulted during the post-exposure test. Rate of moulting seemed faster in crabs previously exposed to the higher mercury concentrations, but moulting rates were not statistically compared because of difference in pre-moult stages at the start of the experiment. Two crabs, one each from 0.0325 and 0.0550 mg L⁻¹, died after moulting.

Avoidance tests. In control runs, when juvenile mud crabs were released at the start of the test, they spread throughout the fluvarium and were seen to establish their own territory. Some individuals travelled from one end to another end of the fluvarium. Others moved from the left half to the right half of the fluvarium, and vice versa. At the end of the 20 min control test, the juvenile crabs were distributed throughout the entire fluvarium. Table 3 shows the mean proportions of juvenile mud crabs that were recorded in the left and right halves of the fluvarium during the 20 min control test. The mean proportion of crabs in both lateral halves had no significant different (paired t-test, $p >$

0.05). These results indicate that crabs showed no bias in choosing between the left and right chambers.

Table 3

Mean proportions \pm standard deviation of juvenile mud crabs recorded in the left and right halves of the fluvium during control tests

| <i>Fluvium chamber</i> | <i>Unexposed*</i> | <i>Pre-exposed LC₅₀/250*</i> | <i>Pre-exposed LC₅₀/50*</i> |
|------------------------|-------------------|---|--|
| Left | 0.49 \pm 0.04 | 0.47 \pm 0.02 | 0.46 \pm 0.03 |
| Right | 0.51 \pm 0.04 | 0.53 \pm 0.02 | 0.54 \pm 0.03 |

*n = 3 replicates per treatment.

Avoidance response of unexposed crabs. Mean proportions of crabs that were recorded in mercury-treated waters decreased as mercury concentration increased. The mean proportions of crabs that stayed in waters containing different mercury concentrations were significantly different (ANOVA, $p < 0.01$) (Table 4). The mean proportions of unexposed crabs recorded in waters containing 0.001, 0.01 and 0.1 mg L⁻¹ were not significantly different from the hypothetical 50% proportion of no avoidance (Duncan's Multiple Range Test, $p < 0.01$). In contrast, mean proportion of crabs recorded in waters that contained 1, 10 and 100 mg L⁻¹ were significantly different from the hypothetical value of no avoidance (Duncan's Multiple Range Test, $p < 0.01$). Results suggest that unexposed crabs could not discriminate waters that contain 0.1 mg L⁻¹ mercury or less. Non-avoidance to 0.1 mg L⁻¹ mercury has dire consequences to juvenile mud crabs whose 96-h LC₅₀ is estimated at 0.04 0.1 mg L⁻¹.

As seen in Table 1, 0.1 mg L⁻¹ mercury is nearly similar to the 24-h LC₅₀. The lack of avoidance appears to render mercury especially hazardous to juvenile mud crabs. This has probable impacts on crab biomass and distribution because of mortality and potential alteration of crab movements. It may also lead to probable impacts on crab's well-being.

Table 4

Mean proportions \pm standard deviation of the mean of juvenile mud crabs found in Hg-treated half of the fluvium

| <i>Hg concentration (mg L⁻¹)</i> | <i>Unexposed*</i> | <i>Pre-exposed LC₅₀/250*</i> | <i>Pre-exposed LC₅₀/50*</i> |
|---|------------------------------|---|--|
| 0.001 | 0.55 \pm 0.06 | 0.55 \pm 0.06 | 0.50 \pm 0.05 |
| 0.01 | 0.51 \pm 0.01 | 0.48 \pm 0.01 | 0.50 \pm 0.03 |
| 0.1 | 0.48 \pm 0.04 | 0.42 \pm 0.05 | 0.41 \pm 0.02 |
| 1 | 0.36 \pm 0.03 ^a | 0.33 \pm 0.04 ^a | 0.38 \pm 0.03 |
| 10 | 0.26 \pm 0.07 ^a | 0.25 \pm 0.08 ^a | 0.28 \pm 0.07 ^a |
| 100 | 0.26 \pm 0.03 ^a | 0.19 \pm 0.02 ^a | 0.22 \pm 0.06 ^a |

^a Significantly different from the 50% hypothetical value of no avoidance (DMRT, $p < 0.05$); * n = 3 replicates per treatment.

Given an opportunity, juvenile mud crabs would avoid plumes of mercury. However, this avoidance threshold is higher compared to other reported avoidance levels for other heavy metals like copper, nickel and cadmium (Folmar 1976; Black & Birge 1980; Korver & Sprague 1989; Svecovicus 1999). Studies examining single concentrations of mercuric chloride also confirmed avoidance at high concentrations (Jones 1947; Summerfelt & Lewis 1967; Scherer & Nowak 1973; Kamchen & Hara 1980). In contrast, Black & Birge (1980) demonstrated preference to low levels of mercury, i.e., 0.0002 mg L⁻¹, by rainbow trout (*Oncorhynchus mykiss*).

Avoidance response of pre-exposed crabs. When previously exposed to low mercury concentration, the avoidance response of crabs was similar to unexposed individuals. The crabs exhibited avoidance to waters containing 100, 10 and 1 mg L⁻¹ mercury but not to mercury concentrations 0.1 mg L⁻¹ and lower (Duncan's Multiple Range Test, $p < 0.01$)

(Table 4). At a high concentration pre-exposure, the threshold level of juvenile mud crabs altered. The mean proportions of crabs in waters containing 1, 0.1, 0.001 and 0.0001 mg L⁻¹ mercury were not significantly different with the hypothetical value of no avoidance but not to waters containing 10 and 100 mg L⁻¹ mercury (Duncan's Multiple Range Test, $p < 0.01$). This shows that the crabs did not avoid waters containing 1 mg L⁻¹ mercury or less. Results suggest that exposure of juvenile mud crabs to high mercury concentrations elevated the avoidance threshold to the pollutant.

The avoidance threshold of an animal to a toxicant was reported to increase or decrease after previous exposure depending on the type of species and pollutant tested. For example, rainbow trout previously exposed to potassium dichromate solutions were found to prefer concentrations matching their pre-exposure level (Anestis & Neufeld 1986). Pre-exposed rainbow trout demonstrated preference response to copper of maximal intensity (Svecevicus 1999). Similarly, previous exposure of fathead minnows (*Pimephales promelas*) resulted to preference to elevated metal conditions (Hartwell et al 1987). In lake whitefish (*Coregonus clupeaformis*), its preference-avoidance behaviour was altered after exposure to cadmium (McNicol & Scherer 1993).

It is not clear why previous exposure to mercury would lead to higher avoidance threshold. Because mercury is known to destroy the olfactory sites of an organism (Hara et al 1976; Sutterlin & Sutterlin 1971), it is possible that the crab's olfaction had been damaged which resulted to reduced ability to detect mercury ions.

Conclusions. This study determined the physiological and avoidance responses of juvenile mud crab *S. serrata* to mercury. The 96-h LC₅₀ of mercury to juvenile mud crab was computed to be 0.04 mg L⁻¹. The fate of an exposed crab depends on the level of exposure. Individuals exposed at concentrations higher than the LC₅₀ showed a higher probability of mortality. Although the juvenile crabs can detect the presence of mercury, they do not avoid lethal mercury concentrations. The crabs could not avoid waters containing 0.1 mg L⁻¹ or less. In addition, the avoidance response of crabs to mercury is influenced by the level of pre-exposure to the toxicant. Pre-exposure of crabs in high mercury concentrations elevated the avoidance threshold. A 24-h exposure to 1/50th of the 96-h LC₅₀ resulted to juvenile crabs that could not avoid waters containing 1 mg L⁻¹. These results showed that juvenile mud crabs are unable to avoid waters containing lethal levels of mercury which may have potential impacts on crab biomass, distribution, growth, and development.

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Authors:

Harold M. Monteclaro, Institute of Marine Fisheries and Oceanology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, 5023 Iloilo, Philippines, e-mail: hmmonteclaro@up.edu.ph
 Ricardo P. Babaran, Institute of Marine Fisheries and Oceanology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, 5023 Iloilo, Philippines, e-mail: rpbabaran@yahoo.com
 Roman C. Sanares, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, 5023 Iloilo, Philippines, e-mail: rcsanares2002@yahoo.com
 Emilia T. Qunitio, Southeast Asian Fisheries Development Center–Aquaculture Department, Tigbauan, Iloilo, Philippines, e-mail: etqunit@seafdec.org.ph

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