Heavy Metal Levels in Mud Crabs (Scylla spp.) from East Bataan Coast

Chona Camille E. Vince Cruz*, Gliceria Ramos and Ma. Carmen Ablan-Lagman
[1] *Biology Department, COS, De La Salle University*
**Corresponding Author: chona.vincecruz@dlsu.edu.ph*

Abstract: Heavy metal levels (Pb and Cu) on adult mud crabs (*Scylla* spp.) from the East Bataan Coast were determined. Muscle tissue from each crab was processed through nitric acid digestion, and analysed using atomic absorption spectroscopy. The average concentrations in the samples were 3.37 x 10^{-3} mg/L, and 1.01 mg/L – both within WHO acceptable limits. These were contrasted with the levels found in sediments from catch and grow out sites (17.9 mg/L, 14.5 mg/L, and 37.8 mg/L, 31.3 mg/L), and were found to be significantly lower in concentration. It takes 3-8 months before adult mud crabs can be harvested from grow out ponds, and the data suggests that the organisms, even when constantly exposed to high levels of Pb and Cu do not bio-accumulate toxic levels of these heavy metals. Mud crabs constitute a major economic natural resource in the Philippines, and in the province of Bataan. Commanding high prices due to the quality of its meat, it is also a preferred culture product due to its impressive size, rapid growth rate, and high flesh content. Mud crab culture in Bataan is primarily driven by grow-out of captured juveniles in pen or pond cultures sourced from nearby bodies of water – making them vulnerable to prolonged exposure to pollutants. The East Bataan Coast shares its waters with the polluted Manila Bay, giving rise to the concern of the quality of seafood acquired from the area.

Key Words: Mud crabs, heavy metals, ecotoxicology, food safety, bioaccumulation

1. Introduction

Mud crabs (*Scylla* spp) are high value resources that are abundant in the Philippines. Yearly productions in the country has reached more than 16,000 tons in 2012 (Dela Cruz 2013) and with recent improvements in mud crab rearing technology it is predicted to go higher. Mud crabs are preferred due to high quality meat, and rapid growth rate with some species reaching carapace widths of 240 mm from juvenile sizes of 4-6 mm in just 6-8 months (Joel and Sanjeevaraj 1983; Overton and Macintosh 2002).

Mud crab culture in the country is dependent on the grow-out of bought or captured juveniles in ponds or pens, with water sourced from adjacent brackish bodies of water (Gaillard 2010). The burrowing nature of the organism gives it constant contact with pollutants that build up on surface sediments. There has been rising concern on the consumption of fish and seafood due to reports on heavy metal bioaccumulation in the tissue of these organisms (Verbeke et al 2005). Levels of heavy metals in marine ecosystems have been rising at alarming rates due to continuous deposits from industrial activity (David 2003; Ali and Fishar 2005). The prolonged exposure of fish with these substances has made some species a source of mercury, arsenic, cadmium, and lead exposure to humans (Khansari et al 2005; Castro-Gonzalez and Mendrez-Almenta 2008). The shared waters of Manila Bay, Bacoor Bay in Cavite, and the East Bataan Coast, where mud crab farms are located, have high levels of lead and copper (Prudente et al 1994).

There is little known about bioaccumulation of heavy metals in mud crabs. Previous studies have looked at the effect of prolonged contact with sub-lethal levels of mercury, lead and cadmium which resulted to tissue necrosis in the gills and hepatopancreas (Krishnaja et al 1987). In closely related species, morphological deformities and aberrant sexual morphology have been observed as developmental effects of heavy metal exposure (Weis et al 1992; Groenendijk et al 1998; Rodriguez et al 2007; Ford et al 2004).

In this study, tissue from mud crabs captured and grown in seawater fed fish ponds along the East Bataan Coast were tested for concentrations of lead and copper. These were compared with concentrations in sediments from catch and grow-out sites of the organism. Morphological aberrations that may have resulted to heavy metal exposure were also noted.

2. METHODOLOGY

2.1 Sampling

Fifty (50) adult mud crabs, with carapace sizes 100 mm and above, were acquired from a grow-out farm located along the East Bataan Coast in Orani, Bataan (14.8000°N 120.5333°E). Samples were kept at 4°C in transport and were immediately frozen in separate containers.

Surface sediments, about 0-10 cm deep of the substrate, were gathered from catch and grow out sites. The three replicates per site, at 50 g each, were stored in sterilized plastic containers and kept at 4°C during transport (Jung 2001).

2.2 Morphological assessment of mud crab samples

Mud crab samples were assessed morphologically by looking at obvious deformities attributed to heavy metal exposure, such as asymmetry (Groenendijk et al 1998), ambiguous sexual features (Rodriguez et al 2007; Ford et al 2004), and tissue necrosis in the gills and hepatopancreas (Krishnaja et al 1987).

2.3 Tissue preparation and digestion

Twenty five grams of abdominal muscle was acquired by pooling tissue samples from multiple crabs. Initial morphological assessment showed a subset of the samples exhibited sexual morphological ambiguity so samples were grouped into 2 pools: those with known genders and those with ambiguous genders. Three replicates for each group was generated.

The tissues were oven-dried at 50°C for 48 hours and then pulverized. Digestion was done in two rounds using 1% HNO_3 (1st round: 2 mL; 2nd round: 10 mL). Breakdown of tissue and evaporation of solvent was facilitated by heating of samples at 60°C. Hydrogen peroxide was added to de-pigment the samples, and the products were re-dissolved in 25 mL distilled deionized water after complete evaporation of solvents (Jumawan et al 2010).

2.4 Soil processing and digestion

Soil samples were ground and 2 g from each replicate underwent a 3-step digestion using 10 mL of concentrated hydrochloric/nitric acid (1:1), 10 ml of 3:1 hydrochloric/nitric acid, and 10 ml of 5% nitric acid. Digestion and evaporation of acids for each digestion step was done by heating at 40°C. Final products were re-dissolved in 50 mL of 1% nitric acid and filtered (Jung 2001).

2.5 Determination of heavy metal levels and analysis

Concentrations of copper (Spectrometry wavelength λ = 327.4 nm) and lead (λ = 405.8 nm) using a slit size of 0.2 nm were determined using atomic absorption spectrometry (Avila-Perez et al 1999). The standard curve was prepared using standard concentrations of 0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, 0.7 mg/L, 1.0 mg/L, 3.0 mg/L, 5.0 mg/L, 7.0 mg/L, and 10.0 mg/L.

Heavy metal concentrations are reported as means and expressed as mg/L. A t-test was done comparing the heavy metal levels between the pooled samples of known and ambiguous genders, and between catch and grow-out sites.

3. RESULTS AND DISCUSSION

3.1 Observed morphological ambiguities

Asymmetry was not observed in any of the samples. Examination of gills and viscera showed no signs of necrosis (Fig. 1) but of the 50 samples, 9 had ambiguous sexual morphology, based on the shape and pigmentation of the abdominal flap (Fig. 2).

Fig 1. Viscera of mud crab showing no abnormal growths or necrosis.

Fig 2. Abdominal flap shapes of mud crabs from Orani, Bataan. (A) A female has it wider and darker in color. (B) Those with ambiguous features have a narrower U-shape with lighter color, similar to features found in immature females except these samples have carapace widths (>110 mm) exceeding published values of sub-adult females for all *Scylla* species. (C) The male's is narrow and white.

3.2 Heavy metal levels in mud crab tissue

Levels of Cu and Pb in both pool types were well within the permissible limits as set by the World Health Organization (WHO) (Gyampo et al 2013) (Table 1). No significant concentration difference was found between samples of known gender and those with ambiguous morphologies.

Table 1. Cu and Pb concentrations in tissue samples of mud crabs (*Scylla* spp) from Orani, Bataan

Heavy metal levels (mg/L)	Known Genders	Ambiguous Genders	Average	WHO Limits (Gyampo et al 2013)
Cu	9.94×10^{-1}	1.05	1.01	2.00
Pb	2.85×10^{-3}	3.89×10^{-3}	3.37×10^{-3}	1.00×10^{-2}

3.3 Heavy metal levels in sediments from catch and grow-out sites

Heavy metal levels in catch sites were significantly higher than those from grow-out sites. The levels found in the catch site were within previously published values of heavy metal contamination of Manila Bay (Hosono et al 2010) (Table 2).

Table 2. Cu and Pb levels in sediments from catch and grow-out sites of mud crabs (*Scylla* spp) from Orani, Bataan.

Heavy metal levels (mg/L)	Catch site	Grow-out site	Manila Bay (Hosono et al 2010)
Cu	37.8	31.3	37-39
Pb	17.9	14.5	16-19

3.4 Implications of data

Catch sites in Orani, Bataan along the East Bataan Coast shares the same polluted state as Manila Bay. Crablets transfer to coastal sites from their offshore birthing zones once they reach a size of

4mm after their final molt from the larval phase (Overton and Macintosh 2002). Captured juveniles for seeding grow-out ponds have carapace widths ranging from 40-50 mm (Gaillard 2010) and development to this size typically takes 7-19 days. Developing crabs in Orani are most exposed to heavy metals during this time period. Morphology of the adults showed that the level of exposure during this time period has no obvious developmental effects on symmetry, and if ever there was any histological damage on the gills and the hepatopancreas they are no longer detectable.

Crustaceans exposed to polluted environments have been observed to exhibit better regulation of blood osmotic concentrations at lower salinities and increase Na/K-ATPase levels in gills (Harris and Santos 2000). This, together with a higher release of ammonia suggests increased activity in ion channel pumps that may be aiding in the secretion of absorbed heavy metals. Another mechanism in heavy metal sequestration involves the action of metallothioneins. The action and production of *S. serrata* hepatopancreas metallothionein has been studied and was seen to be efficient in binding to and aiding heavy metal detoxification for cadmium, zinc and lead (Olafson, Kearns and Kim 1979; Viarengo and Nott 1993) but not for copper (Olafson, Kearns and Kim 1979). This could account for the absence of significant bioaccumulation in the tissue of the samples for Pb, and the large amounts of Cu in the tissues of the sample. Copper, though, is an essential heavy metal that plays specific roles in the growth and development of animals. Copper is an essential component of haemolymph (Van Aardt and Erdman 2004) and is essential in eye development and maintenance of cytochrome activity (Gupta and Mathur 1983).

Though grow-out sites have significantly lower levels of heavy metals, the use of the same polluted water from the Bay replenishes heavy metal levels in the system. Regular draining and drying, and the occasional dredging of the ponds in between farming seasons may be helping to prevent the build-up of contaminants. Juveniles and sub-adults are grown in the ponds for 3-9 months before being sold in markets (Gaillard 2010; Fortes 1999). Extensive exposure to heavy metals has been known to affect molting (Weis 1992) and interfere with hormones of the endocrine system (Ford et al 2004). Although no obvious morphological aberrations were found in carapace structure and features of the samples, almost 20% of the samples exhibited ambiguity in gender. The shape and pigmentation of abdominal flaps are the simplest means to distinguish sex for mud crabs (Fig. 2) and the observed aberrations resemble features found in immature females (Islam et al 2010).

First maturity sizes in *Scylla* are reported at 90mm when functional sexual organs have developed (Joel and Sanjeevaraj 1983; Overton and Macintosh 2002) and maturity has been known to occur faster in tropical areas because of warmer waters (Brown 1993; Wildman 1974). The average carapace width of the samples with ambiguous genders was 112.5 mm, with the smallest at 105.7 mm – all beyond the expected carapace sizes at full maturity. Copper interferes with the actions of methyl farnesoate and the gonad inhibiting hormone, both critical in proper ovarian development, in crustaceans (Rodriguez et al 2007). This could cause delays in sexual maturation of the organism, accounting for the aberrant reproductive morphology in relation to its size. Intersex and demasculinization have also been observed in crustaceans captured in industrially polluted areas; while the cause is still unclear interference of the endocrine system, potentially by heavy metals, is postulated (Yang et al 2008; Ford et al 2004). In this study, no observations were made on the state of vitellogenesis or the internal sexual organs. The heavy metal levels in the mud crab tissue were not significantly different from the male/female pools and those with indeterminate genders. This does not discount the possibility that the exposure in catch and grow out sites have affected the development of the organisms.

4. CONCLUSIONS

Heavy metal levels in the edible muscle of the mud crabs (*Scylla* spp) that were captured and grown in ponds found along the polluted East Bataan Coast are within acceptable limits of human consumption. Constant exposure to polluted water and sediments for more than 6-9 months of growth and development did not produce classical symptoms of developmental defects in crustaceans caused by heavy metal exposure, such as asymmetry, carapace deformation, or tissue necrosis. Twenty percent of the samples, however, showed aberrant sexual morphology but its correlation to heavy metal exposure is unclear.

Laboratory studies on prolonged exposure of mud crabs to higher concentrations at sublethal levels of Cu and Pb can be done to check if there is a threshold at which *Scylla* can efficiently flush out the substances from its system. Controlled experimentation can also be done to check if delays in sexual maturity, or any forms of sexual deformities occur when these organisms are exposed to Cu and Pb at juvenile and adult stages. Bioaccumulation of other trace metals like arsenic, cadmium, and mercury can also be determined.

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