Heavy Metal Concentration in Horseshoe Crab (*Carcinoscorpius rotundicauda and Tachypleus gigas*) Eggs from Malaysian Coastline

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Abstract The level of trace elements (Cu, Zn, Fe, Ni, Pb, and Cd) was measured in eggs of horseshoe crabs, *Carcinoscorpius rotundicauda*, and *Tachypleus gigas,* from Malaysia. The concentrations (μg/g wet weight) of these elements in *C. rotundicauda* eggs ranged from 18.84 to 65.44 for Cu, 34.65 to 104.08 for Zn, 4.497 to 75.95 for Fe, 1.88 to 11.17 for Ni, 0.52 to 3.64 for Cd, and non-detectable for Pb. The level of these elements in *T. gigas* eggs was from 30.54 to 120.32 for Cu, 46.34 to 88.96 for Zn, 21.88 to 88.96 for Fe, 4.71 to 7.82 for Ni, 0.02 to 4.11 for Cd, and 10.00 to 25.84 for Pb. *C. rotundicauda* eggs showed significantly higher amounts of trace elements except for Ni and Cd. The heavy metals analyzed were higher than the range of permissible limit for human consumption.

1 Introduction

The discoveries on the contamination of horseshoe crabs by chemical pollutants emerged from 2003 in an investigation of species distribution in Peninsular Malaysia. There are only four living species of horseshoe crab, *Limulus polyphemus*, *Tachypleus tridentatus*, *T. gigas*, and *Carcinoscorpius rotundicauda* (Sekiguchi, 1988). *T. gigas* and *C. rotundicauda* can be found in Malaysia, where they spawn throughout the year. Adult horseshoe crabs migrate from the offshore continental shelf to spawn on intertidal sandy (*T. gigas*) and mud-sandy beaches and mangrove area (*C. rotundicauda*) at every full and new moon (Hajeb et al.*,* 2005a). Horseshoe crabs inhabit shallow marine waters, generally on sandy bottoms where they move about or burrow just beneath the surface, preying on other animals.

The sensitivity of horseshoe crab embryos toward chemical pollution and impacts on their developments has been showed by few studies (Botton, 2000;

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Botton et al., 1998; Itow et al., 1998a). Itow et al. (1998b) showed that tributyltin (TBT), Hg, Cd, Cr, and Zn inhibited the regeneration of walking legs in horseshoe crabs. Botton (2000) provided evidence of negative impacts of long exposure to Hg and Cd on embryonic and larval development of American horseshoe crab. However, in comparison to early developmental stages of other arthropods, horseshoe crab embryos, and trilobite larvae showed a high tolerance to Hg and Cd (Connor, 1972; Kraus et al., 1988; Bat et al., 1998). Botton et al. (1998) reported greater tolerance of larvae to Cu and Zn than embryos.

Rapid economic growth in Malaysia has resulted in increasing production and usage of toxic chemicals such as trace metals (Agusa et al., 2005). The Strait of Malacca (Fig. 1) is one of the most important habitats for *Tachypleus gigas* and *Carcinoscorpius rotundicauda* (Christianus et al., 2004). The Strait of Malacca is subjected to a great variety of pollutants due to its strategic location as a major international shipping lane and the concentration of agriculture, industry and urbanization on the west coast of Peninsular Malaysia (Abdullah et al., 1999).

Fig. 1 Map showing sampling locations of horseshoe crabs, *T. gigas,* and *C. rotundicauda* in the Straits of Malacca, Malaysia. (PD = Port Dickson; JB = Johor Bahru)

Levels of heavy meals in coastal Malaysia have been reported by Law and Singh (1991), Ismail et al. (1995), and Yap et al. (2002, 2004). Moreover, the Strait of Malacca is one of the most vulnerable areas to contamination by oil spills (Eng et al., 1989). Harmful substances released by human activities will be accumulated in marine organisms through the food web. Consequently, there may be human health risks caused by consumption of contaminated seafood. Horseshoe crab eggs are consumed by local fishermen and coastal residents in Malaysia. However, no studies have been conducted on contaminant loads in horseshoe crab eggs, or the potential risk to human health in this country.

To investigate the present contamination levels, patterns of accumulation, and possible toxic effects of pollution, the levels of trace metals (Cu, Zn, Fe, Ni, Pb, and Cd) were measured in eggs of horseshoe crabs from the coast of Malaysia. This investigation can act as a beginning for future research on horseshoe crabs in order to conserve them and to prevent them from being lost in this country. It is important to establish whether heavy metals contaminants are contributing to changes in horseshoe crab populations.

2 Materials and Methods

Samples of female horseshoe crabs collected from two sites along the Strait of Malacca during full moon (Fig. 1). Samples were transferred to the laboratory; prosomal length and body weight were measured for each. Horseshoe crab eggs were extracted and kept in –20°C until using for analysis.

All samples were digested in concentrated HNO_3 in the hot block digester in low temperature (40°C) for 1 hour and high temperature (135°C) for at least 3 hours. Digested samples were then cooled and subsequently diluted in deionized water into 40 ml. After filtration, samples were analyzed for Cd, Cu, Pb, Fe, Ni, and Zn using a Perkin–Elmer Model 4100 air–acetylene flame atomic absorption spectrophotometer.

To avoid contamination, all the glassware used was soaked in detergent solution overnight, then rinsed, and soaked in 10% (v/v) HNO_3 overnight. To check for contamination, procedural blanks were analyzed in every ten samples. Quality control samples, made from standard solutions of Cd, Cu, Pb, Fe, Ni, and Zn, were analyzed in every ten samples to check for the metal recoveries. The percent recoveries were 99% for Cd, 97.5% for Cu, 96% for Pb, 98.3% for Fe, 92.8% for Ni, and 99% for Zn. Detection limits were 1 ng/g for Cu and Fe, 5 ng/g for Cd, 7 ng/g for Zn, and 8 ng/g for Ni and Pb.

The description of samples and sampling locations are presented in Table 1. The Pearson correlation coefficient was used to measure the strength of the

Table 1 Description of horseshoe crab samples and sampling locations

Species	Number of samples	Location	Habitat	Human activity in location	Prosomal width (cm) mean (range)	Female body weight (g) mean (range)
T. gigas	11	Port Dickson (PD)	Sandy beach	Fishing boat traffic, Recreation	19.87 (19.10–21.50)	600 (520–740)
	16	Johor Bahru (JB)	Sand-muddy beach	Fishing boat traffic, urban waste release	20.81 (19.40–22.60)	731.43 (570–910)
C. rotundicauda	–	Port Dickson (PD)	–	–	NF	NF
	14	Johor Bahru (JB)	Mangrove	Industrial and urban waste release	12.39 (10.60–13.50)	158.75 (120–200)

NF, not found.

association between trace element concentration and prosomal width. Differences among trace element concentrations were tested by ANOVA. A *P* value of less than 0.05 was considered to indicate statistical significance. T-tests were conducted to compare the data obtained from two collection sites. All statistical analysis was done using MINITAB Statistical Software (Release 14).

3 Results

The levels of trace metals in the eggs of *T. gigas* and *C. rotundicauda* are presented in Table 2. In general, Zn concentration was the highest followed by Fe and Cu. *T. gigas* showed significantly higher levels of Cu, Zn, and Fe than *C. rotundicauda* collected from Johor Bahru (JB). Pb was not detected in the egg samples of either horseshoe crab species from JB. In terms of Cd, content, there was no significant difference between the two species, while *C. rotundicauda* had higher amounts of Ni compared to *T. gigas* ($P < 0.005$). There were higher levels of Cu, Zn, Fe, Ni, Pb, and Cd in *T. gigas* samples from JB than PD ($P <$ 0.005). Comparison between two sampling locations demonstrated higher level of four elements, Cu, Zn, Fe, and Cd, in *T. gigas* samples from JB site ($P < 0.005$).

There was a size difference between adult female *T. gigas* from two sites; samples from PD were larger and heavier ($P < 0.005$) (Table 1). There were significant positive correlations between prosomal width and levels of Cu, Zn, Fe, and Ni for *C. rotundicauda* (Fig. 2). Conversely, heavy metal levels in *T. gigas egg* samples were not significantly correlated with the size of the female.

4 Discussion

T. gigas egg samples from PD site showed higher levels of Ni and Pb than JB, but the reverse trend was seen for Cu, Zn, and Fe (Table 2). The concentrations of metals in *C. rotundicauda* eggs from JB were generally similar to T. gigas from the same site (*C. rotundicauda* was not found at PD). Comparing the current data with Kannan et al. (1995) on concentration of heavy metal in *T. tridentatus* in Japan, Malaysian horseshoe crabs showed higher levels of all the metals analyzed. These two species of horseshoe crab also showed higher levels of Pb and Cd than reported by Burger (1997) and Burger et al. (2002) in American horseshoe crab eggs. The high correlation for Cd and Pb in both species can be explained by their bioaccumulation by age (Burger et al., 2002).

Higher levels of Cu, Zn, Fe, and Cd in *T. gigas* eggs from JB and Ni and Pb from PD sites can suggest that there are some sources of these metals in these areas. These contaminants possibly arose from industrial, agricultural, and shipping activities in the Strait of Malacca (Agusa et al., 2005; Yap et al., 2004). Horseshoe crab eggs showed higher levels of trace elements than other

Table 2 Heavy metal concentration (µg/g wet wt.) in horseshoe crab egg samples from the Straits of Malacca

Species	Location	*N*	Cu mean (range)	Zn mean (range)	Fe mean (range)	Ni mean (range)	Pb mean (range)	Cd mean (range)
T. gigas	Port Dickson PD	11	57.27 (44.83–70.02)	62.94 (53.08–71.62)	44.35 (32.45–61.68)	5.93 (4.71–7.82)	18.26 (10.00–25.84)	0.29 (0.02–0.52)
	Johor Bahru (JB)	16	78.45 (30.54–120.32)	100.21 (46.34–150.53)	60.26 (21.88–88.96)	1.02 (0.05–4.8)	ND	3.59 (1.38–4.11)
C. rotundicauda	Johor Bahru (JB)	14	36.79 (18.84–65.44)	63.33 (34.65–104.08)	36.42 (4.49–75.95)	4.47 (1.88–11.17)	ND	2.28 (0.52–3.64)

ND, non-detectable.

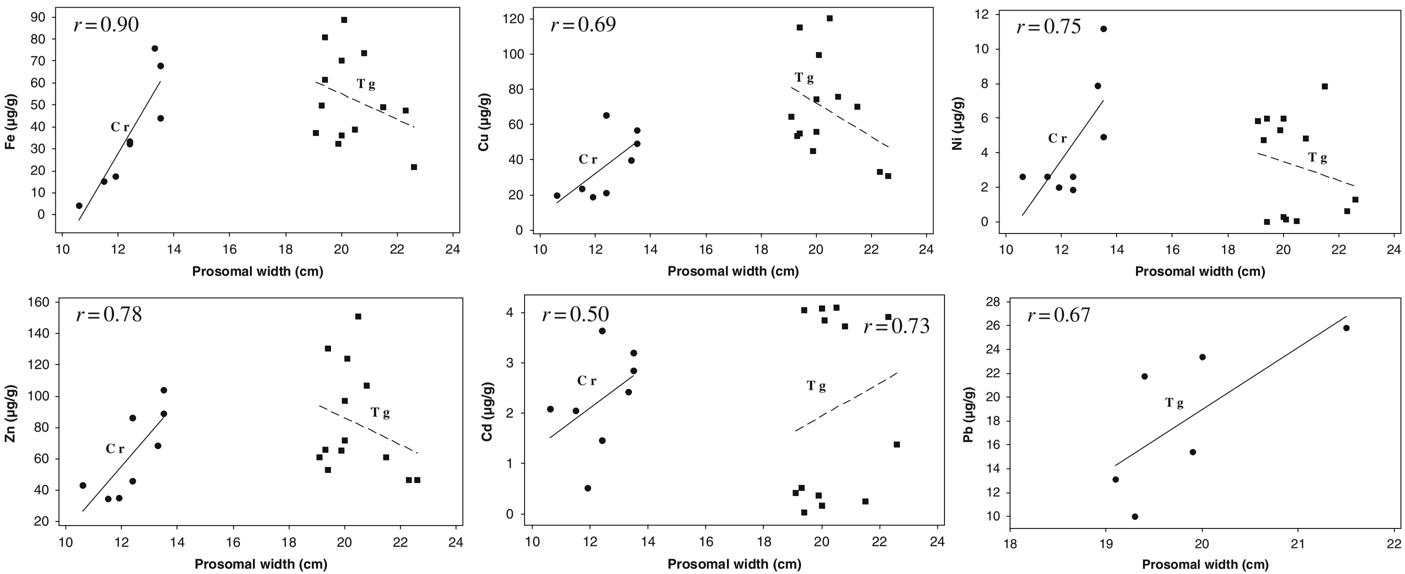

Fig. 2 Correlation between metal levels (µg/g) and prosomal width (cm) in horseshoe crab samples (circles: *C. rotundicauda;* squares: *T. gigas*)

Table 3 Comparison of heavy metal concentrations (µg/g) with other biota in the Straits of Malacca

Biota	Weight base	Cu	Zn	Pb	Cd	Reference
Green liped mussle (*Perna viridis*)	Wet	1.00–3.00	10.8–30.0	0.50–5.90	0.10–1.80	Ismail (1993)
Fish	Wet	0.51–1.05	5.50	0.10–0.29	–	Law and Singh (1991)
Fish	Wet	–	2.30–6.48	0.21–32.00	0.03–0.05	Babji et al. (1979)
Fiddler crab (*Uca annulipes*)	Wet	8.02–19.91	10.63–19.10	3.65–9.41	1.56–2.54	Ismail et al. (1991)
Hermit crab (*Clibanarius sp*)	Wet	22.34–86.00	23.55–42.87	4.02–12.67	1.43–2.33	Ismail et al. (1991)
Mollusks	Wet	6.00–15.00	18.00–47.00	7.00–17.00	0.10–2.50	Ismail and Ramli. (1997)
Sediments	Wet	4.00–670.00	4.00–550.00	3.40–46.50	0.10–2.10	Ismail and Ramli. (1997)
Prawn	Wet	12.80–159.00	5.00–16.00	0.06–5.90	009–0.80	Ismail et al. (1995)
C. rotundicauda	Wet	18.84–70.02	34.65–150.53	ND	0.52–4.11	Current study
T. gigas	Wet	44.83–70.02	53.08–71.62	10.00–25.84	0.02–0.52	Current study

ND, non-detectable.

marine biota in this area of Malaysia (Table 3). High levels of heavy metals in the eggs of horseshoe crabs could impair development (Burger, 1997; Itow et al., 1998a). Based on Zhou and Morton (2004), horseshoe crabs are benthic predators, feeding mainly on bivalve and mussels. Data from Yap et al. (2004) showed lower levels of Cu, Cd, Pb, and Zn in sediments and green-lipped mussel, *Perna viridis,* from the Strait of Malacca compared to the metal content of horseshoe crab. This may be explained by accumulation of these metals in horseshoe crab body and its sequestration from the female during egg formation. The positive correlations between metal concentrations in the eggs and female body size (especially in *C. rotundicauda*, Fig. 2) can be partially related to the longer exposure of the larger horseshoe crab to the polluted areas, assuming that size and age are correlated (Hajeb et al., 2005b).

The ability of horseshoe crab embryos and larvae to survive in the presence of heavy metals implies the potential for these minerals to be passed on to shorebirds and other predators (Botton, 2000). Levels of contaminants in eggs of horseshoe crabs are also of interest because they are being consumed by fishermen and some local people as a delicacy. The level of these metals is considered to be high when compared to permissible limits set by Malaysian Food Regulation (1985) for Cu (30.0 mg/kg ww), Cd (1.00 mg/kg ww), Zn (100 mg/kg ww), and Pb (2.00 mg/kg ww). However, the consumption of these eggs is not that high to be consider as serious health risk to the population.

In conclusion, the level of heavy metals in the eggs of horseshoe crabs is of interest as a bioindicator of pollutant levels in the Strait of Malacca and an indicator of potential problems for developing horseshoe crabs. On a world-wide basis, reduction of horseshoe crab populations has been attributed to over harvesting (Burger, 1986; Botton, 2000; Botton and Loveland, 2001), the use of eggs as food (Kungsuwan et al., 1987), and inorganic and organic contaminants (Burger et al., 2002; Burger, 1997; Kannan et al., 1995). This study showed that two horseshoe crab species may be affected by heavy metal pollutants in the Strait of Malacca.

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