Sains Malaysiana 41(9)(2012): 1063–1069

A Comparative Study of Condition Indices and Heavy Metals in *Perna viridis* Populations at Sebatu and Muar, Peninsular Malaysia

(Kajian Perbandingan bagi Indeks Kondisi dan Logam Berat dalam Populasi *Perna viridis* di Sebatu dan Muar, Semenanjung Malaysia)

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ABSTRACT

This study compared some allometric parameters (shell length, shell width, shell height, total dry weight of soft tissues, condition index and heavy metals (Cd, Cu, Pb and Zn) in the different soft tissues of Perna viridis *collected from Sebatu and Muar estuary. It was found that the total dry weight of soft tissues and condition index of mussels collected from Sebatu were significantly (p<0.05) higher than those in Muar. The significantly (p<0.05) higher concentrations of Cu in most soft tissues and some of Cd indicated a higher bioavailability of Cu and Cd at Muar than Sebatu. In addition, the significantly (p<0.05) higher levels of Cu, Cd, Zn and Pb in surface sediments collected from Muar supported the observable anthropogenic impacts at Muar than Sebatu and hence, higher metal contamination at Muar than Sebatu. The higher condition index value in mussels recorded in Sebatu than in Muar was believed to be a result of higher metal contamination at Muar estuary.*

Keywords: Condition index; heavy metals; Perna viridis

ABSTRAK

Kajian ini membandingkan sesetengah parameter alometrik (panjang cangkerang, lebar cangkerang, tinggi cangkerang, jumlah berat tisu kering, indeks kondisi dan logam berat (Cd, Cu, Pb dan Zn) di dalam bahagian tisu kering yang berlainan bagi Perna viridis *yang disampel dari Sebatu dan Kuala Muar. Kajian menunjukkan bahawa jumlah berat tisu kering dan indeks kondisi bagi kupang yang disampel dari Sebatu adalah lebih tinggi secara signifikan (p<0.05) berbanding dengan Muar. Kepekatan Cu yang lebih tinggi secara signifikan (p<0.05) di dalam kebanyakan tisu lembut dan sesetengah bagi Cd menunjukkan ketersediaan bagi Cu dan Cd adalah lebih tinggi di Muar dari Sebatu. Tambahan pula, kepekatan bagi Cu, Cd, Zn dan Pb yang lebih tinggi secara signifikan (p<0.05) di sedimen permukaan yang disampel dari Muar menyokong impak antropogenik di Muar dari Sebatu. Oleh itu, pencemaran logam berat di Muar adalah lebih tinggi dari Sebatu. Indeks kondisi di kupang yang direkod di Sebatu dari Muar adalah dipercayai disebabkan oleh pencemaran logam yang lebih tinggi di Kuala Muar.*

Kata kunci: Indeks kondisi; logam berat; Perna viridis

INTRODUCTION

In the present study, allometric parameters (shell length, shell width, shell height, total dry soft tissues, condition index (CI)) and heavy metals (Cd, Cu, Pb and Zn) were determined from Sebatu and Muar estuary. These two sites were chosen because Sebatu was a highly spatfall coastal waters for green-lipped mussel *Perna viridis* in Malaysia with artificial hanging substrates as a commercial aquacultural method (Al-Barwani et al. 2004, 2005, 2007) while Muar estuary is a bottom dwelling natural bed mussels, and Sebatu is a known pristine site with no observable human activities in the surrounding (Yap et al. 2005a) while Muar estuary is a relatively human activity site potentially receiving domestic wastes, and impacts from boating activities and some observable land-based activities. Previous study on heavy metals in the total soft tissues of green-lipped mussel *Perna viridis* from Sebatu and Muar were reported by Yap et al. (2005a, 2005b) and Yap et al. (2003a), respectively.

However, a more detailed discussion on the water parameters and alometric of *P. viridis* has not been done. Hence, the objectives of the present study were to compare some *in-situ* water parameters, CI values and heavy metals of *P. viridis* collected from Sebatu and Muar estuary. This study focused on CI of *P. viridis* because it is a simple index that reflects changes in the nutrient state of mussel such as stored energy reserves and the animal's metabolic response to environmental stress (Widdows 1985a, 1985b; Yap et al. 2002). The index is also an indirect estimate of the health condition of the organism when it is under environmental stress (Yap et al. 2002).

MATERIALS AND METHODS

Mussels *P. viridis* were collected from Sebatu (Malacca) and Muar estuary (Johore) (Figure 1), on 11 September 2004 and 15 September 2004, respectively. Surface bottom sediments (0-5 cm) near the mussel habitats were also collected at the same time. Besides, some physico-chemical parameters including depths, water temperature, dissolved oxygen, pH, water salinity and conductivity were also measured *in-situ* by using YSI 556 MPS (Multi-Probe System), during sampling periods in both sites. All the samples were stored in a cool box compartment after collection in the field. In the laboratory, 30 individuals were measured for their shell lengths (maximum anterior-posterior), shell widths (lateral dimension) and shell heights (dorsal-ventral) with a vernier caliper to an accuracy of 0.01 cm. For the determination of total dry soft tissue weight, the total wet soft tissues of *P. viridis* were carefully removed by deshelling the mussel with a stainless steel knife. The dry weight of the total soft tissue of the mussel was determined after drying the total soft tissue individually for at least 72 h at 105°C to constant dry weights. Surface sediments were also dried with the above similar method.

The total dry soft tissues were then digested in concentrated HNO_3 (AnalaR grade, 69%) while dried 63 μm sieved sediments were digested in a combination of concentrated HNO_3 (AnalaR grade; BDH 69%) and $HClO_4$ (AnalaR grade; BDH 60%) in the ratio of 4:1. They were placed in a hot-block digester first at low temperature for 1 h and then were fully digested at high temperature (140°C) for at least 3 h. The digested samples were then diluted to a certain volume with double distilled water (DDW). After filtration, the prepared samples were determined for Cd, Cu, Pb and Zn by an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in μg/g dry weight. To avoid possible contamination, all glassware and equipment used were acid-washed and the accuracy of the analysis was checked against the blanks and the standard addition testing procedure. Procedural blanks and quality control samples made from the standard solutions for Cd, Cu, Pb and Zn were analyzed every five samples in order to check for sample accuracy. Percentages of recoveries for heavy metal analyses were acceptable between 80% and 110%.

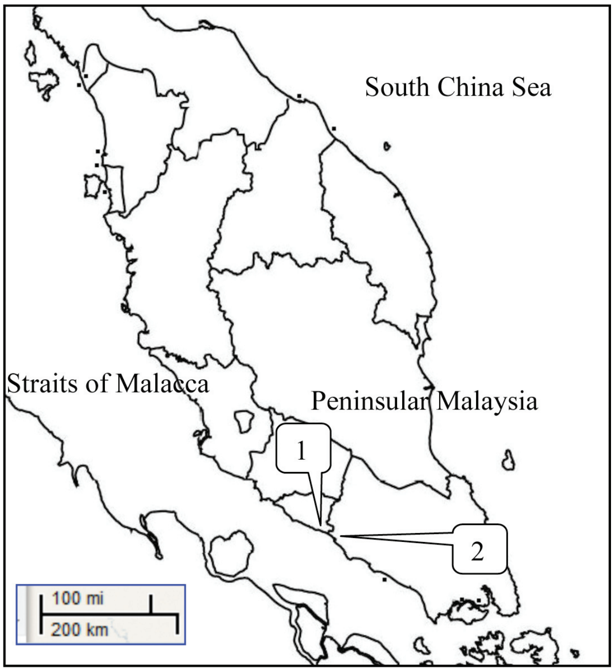

FIGURE 1. Map showing the sampling sites for *Perna viridis* and surface sediments in (1) Sebatu and (2) Muar estuary

Condition index (CI) was calculated base on the quotient of the total soft tissue dry weight for each mussel and the shell volume for each mussel, multiplied with a constant, namely 1000 (Roberts et al. 1986; Yap et al. 2003b). This index was calculated as shown below:

$$\text{CI (g/cm}^3) = \frac{\text{total soft tissue dry weight (g)}}{\text{shell volume}} \times 1000.$$

To estimate the proportion in which metal occurs in mussel and in the associated sediment, bivalve sediment accumulation factors (BSAFs) were calculated for the four metals in the different tissues of mussels, according to a formula as described by Szefer et al. (1999) below:

$$\text{BSAF} = \frac{\text{mean metal concentration in mussel tissue}}{\text{mean metal concentratiton in associated sediment}}.$$

Based on the BSAF, the different tissues of the mussels can be classified into 1) macroconcentrator (BSAF > 2), 2) microconcentrator (1 < BSAF < 2) and 3) deconcentrators (BSAF < 1), as proposed by Dallinger (1993).

The relationships between total soft tissues and other shell allometric parameters were statistically analyzed by correlation analysis and multiple linear stepwise regression analysis (all the three statistical analyses were multivariate analysis), by using STATISTICA 99 edition (Yap et al. 2010a). These data was transformed by log10 (mean +1) in order to reduce the variance (Zar 1996). T-test were also performed by using the similar statistical software.

RESULTS AND DISCUSSION

The overall statistics of allometric parameters in mussels collected from Sebatu and Muar are presented in Table 1. It was found that the total dried soft tissues (TDST) in Sebatu population was higher than in Muar population regardless of the higher shell lengths and shell widths from Muar population. The higher TDST resulted in significantly (p<0.05) higher CI at Sebatu than in Muar population. The CI values from the Muar population (8.96-22.73 g/cm^3) was almost within those (10.15-20.92 g/cm^3) reported from Peninsular Malaysia (Yap et al. 2003b) but those from Sebatu population was higher (15.9-35.40 g/cm^3). Previously, Yap et al. (2002) reported that mussels collected from contaminated and uncontaminated sites in the field exhibited that the CI values correlated negatively with Cd (r = -0.62, p<0.01) and Pb (r = -0.70, p<0.01) in which their results was confirmed by a laboratory study. This supported the finding from the present study in which higher metal concentrations in the soft tissues of mussels from Muar estuary had a lower CI value when compared with Sebatu population. The present findings also showed that the CI values are negatively correlated with Cd (r = -0.31, p<0.05), Pb (r = -0.30, p<0.05), Cu (r = -0.49, p<0.05) and Zn (r = -0.53, p<0.05). The high CI value from Sebatu population was due to gonadal condition of the mussels before spawning (Al-Barwani et al. 2005).

Table 2 shows the temperature, DO, pH, salinity and conductivity of sampling sites at Sebatu and Muar. Significant differences (p<0.05) for salinity and conductivity between the two sites are found while there are no significant (p>0.05) differences for temperature, DO and pH. The higher salinity at Sebatu sampling site could be due to its being away from the river Sebatu estuary while Muar sampling site is close to the river estuary and the mixing with freshwater is therefore higher. This resulted in a lower salinity and conductivity at Muar sampling site.

From correlation coefficients in Table 3, one similarity found in these two sites was that CI was

TABLE 1. Overall values (mean ± standard error) of allometric parameters in mussels collected from Sebatu and Muar. TSW and TDST are presented in g; shell length, shell height and shell width are presented in cm; CI is presented in g/cm^3 (N= 30)

Allometric parameters	Muar	Sebatu	t-test
TSW	9.95 ± 0.26	10.25 ± 0.25	p>0.05; ns
TDST	0.99 ± 0.05	1.62 ± 0.06	p<0.05
Shell length	7.83 ± 0.05	7.55 ± 0.04	p>0.05; ns
Shell height	3.21 ± 0.03	3.25 ± 0.03	p>0.05; ns
Shell width	2.44 ± 0.03	2.21 ± 0.02	p>0.05; ns
Condition index	16.15 ± 0.71	29.56 ± 0.77	p<0.05

Note: TSW= total shell weight; TDST= total dried soft tissues.
ns= not significant.

TABLE 2. Dates of sampling and some physico-chemical parameters (mean ± standard error) of water samples recorded *in-situ* during sampling periods from Sebatu and Muar

	Date of sampling	11 Sep 2004 (11:40am)	15 Sep 2004 (10:50am)	T-test
		Sebatu	Muar	
1	Temperature (°C)	30.43 ± 0.25	29.88 ±0.10	p>0.05; ns
2.	Salinity (ppt)	30.83 ±0.52	27.90 ±0.00	p<0.05
3.	Conductivity(μS/cm)	46673 ±764	42910 ± 20.82	p<0.05
4.	Dissolved oxygen (mg/L)	5.75 ±0.03	6.30 ±0.15	p>0.05; ns
5.	pH	7.73 ±0.01	8.03 ± 0.01	p>0.05; ns

Note: ns= not significant.

TABLE 3. Correlation coefficients between allometric parameters in mussels collected from Sebatu and Muar estuary (N= 30, based on log10(X+1))

Sebatu	TSW	TDST	Length	Height	Width	CI
TSW	1.00	0.75*	0.66*	0.76*	0.73*	0.40*
TDST		1.00	0.56*	0.48*	0.67*	0.88*
Length			1.00	0.48*	0.45*	0.27^{ns}
Height				1.00	0.39*	0.09^{ns}
Width					1.00	0.40
CI						1.00
Muar	**TSW**	**TDST**	**Length**	**Height**	**Width**	**CI**
TSW	1.00	0.32^{ns}	0.75*	0.60*	0.73*	-0.06^{ns}
TDST		1.00	0.57*	0.39*	0.36^{ns}	0.89*
Length			1.00	0.62*	0.62*	0.23^{ns}
Height				1.00	0.54*	0.02^{ns}
Width					1.00	-0.07^{ns}
CI						1.00

Note: TSW= total shell weight; TDST= total dried soft tissues; CI= condition index.
Values with *= p< 0.05; ^{ns}= not significant at p>0.05.

significantly (p<0.05) correlated with TDST and this result is expected when the formula to generate CI was referred to. A difference was found based on the number of significant correlation coefficients, namely 13 out of 15 pairwises in Sebatu but only 8 out of 15 in pairwises in Muar. This difference indicated the higher gonadal condition at Sebatu than Muar. Based on MLSRA in Table 4, for both populations from Sebatu and Muar, it is found that CI was significantly (p<0.05) influenced by TDST, shell height, shell width and shell length while total shell weight was removed.

The concentrations (μg/g dry weight) of heavy metals in mussel *Perna viridis* and surface sediments in Sebatu and Muar are given in Table 5. Based on surface sediments, the levels of Cd, Cu, Pb and Zn were all significantly (p< 0.05) higher at Muar than Sebatu, indicating higher metal contamination at Muar estuary. Based on the different soft tissues of mussels, it was found that some soft tissues of mussels from Muar had higher levels of metals than Sebatu population, indicating higher metal bioavailability to biomonitor *P. viridis* from Muar. However, some tissues did not show such a pattern.

The BASF values in the different tissues of *P. viridis* are given in Table 6. In general, for all the four metals, the BSAF values in Muar are lower than those in Sebatu. For Cu, all the BSAF values the different tissues of Muar population are less than 1 (deconcentrators) while those values in Sebatu are higher than 1 but below 2 (microconcentrators) except for remainder and byssus in which both tissues are higher than 2 (macroconcentrators). For Cd, all BSAF values in Muar population are below 1 except for mantle and remainder (1< BSAF< 2; microconcentrators) while all BSAF values in Sebatu are more than 2 (macroconcentrators) except for gonad (1< BSAF< 2; microconcentrator). For Zn, all BSAF values in Muar population are below 1 (deconcentrators) while those in Sebatu are between 1 and 2 (microconcentrators) except for mantle, remainder and byssus (> 2; macroconcentrators). For Pb, all the BSAF values in both populations are below 1 (deconcentrators) although values in Sebatu are higher. The higher BSAF values found in Sebatu than Muar can be considered as higher metal bioavailabilities in Sebatu than in Muar (Yap et al. 2010b; Yap & Edward 2010). However, this phenomenon does not indicate higher metal concentrations

TABLE 4. Influential dependent variables on condition index (CI) of *Perna viridis* by using multiple linear stepwise regression analysis (based on log10[mean+1]) N= 30

Sebatu	CI = 3.35 + TDST (1.35)- Height (0.33)- width (0.26)- length (0.18). (R = 0.995, p<0.0001)
Muar	CI = 2.97 + TDST (1.13) – Width (0.29) – Height (0.21) – Length (0.11). (R = 0.997, p<0.0001)

Note: Condition index (CI) as a dependent variable while dependent variables included in this study were total shell weight, shell length, shell height, shell width and total dried soft tissues (TDST).

TABLE 5. Concentrations (mean ± standard error, μg/g dry weight) of heavy metals in the different soft tissues of *Perna viridis* and surface sediments collected from Sebatu and Muar estuary

	Muar	Sebatu		Muar	Sebatu	
Tissues	Cu		t-test	Cd		t-test
Mussels						
Gonad	12.9 ± 0.13	9.32 ± 0.28	p< 0.05	1.28 ± 0.29	0.94 ± 0.29	p< 0.05
Mantle	12.3 ± 0.05	7.53 ± 0.29	p< 0.05	4.66 ± 0.06	2.69 ± 0.42	p< 0.05
Remainder	16.9 ± 0.48	18.9 ± 0.47	p> 0.05	4.58 ± 0.08	3.75 ± 0.15	p< 0.05
Byssus	20.5 ± 0.66	19.5 ± 0.30	p> 0.05	1.07 ± 0.06	3.09 ± 0.27	p< 0.05
Gill	14.2 ± 0.38	10.05 ± 0.31	p< 0.05	1.73 ± 0.09	1.92 ± 0.23	p> 0.05
Foot	11.5 ± 0.72	7.39 ± 0.15	p< 0.05	0.87 ± 0.17	1.73 ± 0.30	p< 0.05
Sediment	42.2	6.66	p< 0.05	2.51	0.64	p< 0.05
	Zn		t-test	Pb		t-test
Mussels						
Gonad	119 ± 0.20	116 ± 0.43	p> 0.05	10.2 ± 0.76	14.8 ± 0.65	p< 0.05
Mantle	145 ± 3.71	142 ± 1.18	p> 0.05	5.65 ± 0.22	16.2 ± 0.44	p< 0.05
Remainder	138 ± 2.59	143 ± 0.90	p> 0.05	12.5 ± 0.18	9.49 ± 0.43	p< 0.05
Byssus	149 ± 1.70	144 ± 0.53	p> 0.05	18.5 ± 0.54	16.8 ± 0.58	p> 0.05
Gill	131 ± 1.70	74.2 ± 0.76	p< 0.05	15.7 ± 0.55	9.26 ± 0.27	p< 0.05
Foot	105 ± 1.52	109 ± 1.35	p> 0.05	11.98 ± 0.45	8.72 ± 0.40	p< 0.05
Sediment	162	63.2	p< 0.05	64.5	32.2	p< 0.05

Note: Values in bold indicated significantly (p< 0.05) higher metal levels in Muar than Sebatu.

TABLE 6. BSAFs in the different tissues of *Perna viridis* collected from Sebatu and Muar estuary

	Muar	Sebatu	Muar	Sebatu
Tissues	Cu BSAF	Cu BSAF	Cd BSAF	Cd BSAF
Gonad	0.31	1.40	0.51	1.47
Mantle	0.29	1.13	1.86	4.20
Remainder	0.40	2.84	1.82	5.86
Byssus	0.49	2.93	0.43	4.83
Gill	0.34	1.51	0.69	3.00
Foot	0.27	1.11	0.35	2.70
Tissues	Zn BSAF	Zn BSAF	Pb BSAF	Pb BSAF
Gonad	0.73	1.84	0.16	0.46
Mantle	0.90	2.25	0.09	0.50
Remainder	0.85	2.26	0.19	0.29
Byssus	0.92	2.28	0.29	0.52
Gill	0.81	1.17	0.24	0.29
Foot	0.65	1.72	0.19	0.27

in the different tissues of Sebatu population when compared to Muar. Therefore, the BSAF values calculated from the present study can only be used to estimate metal accumulation capacity and classifications in the different tissues in both populations, as proposed by Dallinger (1993). The higher bioavailability of metals to Sebatu mussel population could be explained by the significantly ($p<0.05$) higher salinity at Sebatu sampling site than Muar which could potentially affect metal availability and changes in salinity may affect several physiological processes that influence the accumulation of trace metals by bivalves (Mo & Neilson 1993). Earlier, Yap et al. (2005c) reported that the difference of metal concentrations between Nenasi and Kuala Pontian populations could be due to the significant ($p<0.05$) differences in salinity and conductivity.

If metal contamination at Muar population was the main reason, the decreased CI value at Muar mussels following metal exposure could be explained by two factors. First of all, a mechanical response and a metabolic requirement for the stressed mussels at Muar could cause a lower CI value in the stressed mussels. The physiological partial valve closure in the mussels had certainly reduced the filtration rate which resulted in decreased amount of algae filtered by the mussel (Akberali & Trueman 1985). Owing to the mussels might have to use more food than that filtered in order to maintain the normal metabolic activities, the stored glycogen, carbohydrate and protein of *P. viridis* might be utilized in order to maintain those activities. Secondly, induction of metallothionein could be the reason why there were significantly ($p<0.001$) higher levels of metals were measured in the soft tissue of Muar population than Sebatu. This correlated with the metals being bound to metallothionein and therefore they could be stored in detoxified forms (Viarengo et al. 1985). Stored energy would be utilized to meet this metabolic requirement for detoxification processes. Subsequently, the decline of stored energy was likely to be measured as a lower CI value. Therefore, the lower CI value of mussels collected from contaminated waters indicated that they had reduced amount of mussel soft tissues (Yap et al. 2002).

The lower CI values in mussels collected from metal-contaminated were also supported by several related studies in other bivalve species. For example, Lares and Orians (1997) reported significant ($p<0.001$) negative relationships between the CI values and Cd (r = -0.62) and Pb (r = -0.79) in *M. californianus* collected from the field while Cotter et al. (1982) reported that the CI value of *M. edulis* was lower in mussels exposed to higher Zn concentrations than those of the controls, under laboratory conditions. Earlier, Wilson & McMahon (1981) also found that CI values in populations of *M. edulis* and *Littorina rudis* decreased when the level of Cu increased while Page et al. (1984) reported that concentrations of Cu and Pb in the tissues of *M. edulis* collected from the field negatively correlated with CI values. Nicholson (1999) reported that *P. viridis* from contaminated waters in Hong Kong had significantly lower CI values than mussels collected from a clean site. Lucas and Beninger (1985) proposed the CI of mussels collected from the field could provide an immediate assessment of the long-term nutrient reserves and energy status of the mussel in relation to heavy metal pollution. According to Rebelo et al. (2005), the CI variations observed on the temporal and spatial scale were likely to have been caused by availability of organic matter and spawning, rather than spionid infestation or metal body burdens. They also found that the CI of *Crassostrea rhizophorae* is negatively correlated with Cd levels in the oyster tissue. Leung and Furness (2001) found that gastropod *Nucella lapillus* exposed to 0.05 ppm Cd had a significant low level of CI when compared with the control. On the other hand, Amiard et al. (2004) reported that the CI variations in mussels *Mytilus edulis* (filter-feeders), periwinkles *Littorina littorea* (grazing-feeders) and dogwhelks *Nucella lapillus* (carnivora, bivalve predators) were linked to sexual ripening in mussels rather than contamination by Ni and Va.

CONCLUSION

The higher CI values of mussels at Sebatu than in Muar estuary could be plausibly due to higher metal contamination and more human activities in the surroundings at Muar estuary since the levels of temperature, DO, pH, salinity and conductivity were relatively similar between the two sampling sites. This would create ecological stress on the Muar population in which the mussels need energy to cope with the stress and thus reduced CI value. Further study by mussel transplantation between the two sites is highly recommended in order to understand the CI variation better.

ACKNOWLEDGEMENTS

The authors wish to thank Research University Grant Scheme (RUGS), (Pusat Kos: 9316800), provided by Universiti Putra Malaysia.

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Received: 8 December 2011
Accepted: 2 April 2012