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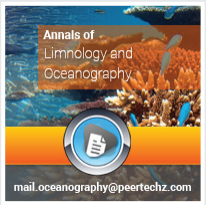

LIFE SCIENCES GROUP

ANNALS OF
Limnology and Oceanography

ISSN: 2641-3078 DOI: https://dx.doi.org/10.17352/alo

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Dates: Received: 12 December, 2016; **Accepted:** 28 December, 2016; **Published:** 30 December, 2016

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Keywords: Dender/sex effects; Metal accumulation; Mussels; Foot

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Research Article

The Use of Foot of the Green-Lipped Mussel is *Perna Viridis* as an Alternative Method to Reduce the Gender Effect on the Bioaccumulation of Cu and Zn in the Mussel

Abstract

In this study, the green-lipped mussels *Perna viridis* were collected from a high activity sampling at Senibong in the Straits of Johore and two relatively clean sites with fish aquacultural activity at Bagan Tiang (Perak) and Sg. Semerak (Kelantan). The mussels were dissected by gender into byssus, crystalline style, foot, gill, gonad, mantle and muscle. By gender, these pooled and dissected soft tissues and total soft tissues were determined for Cu and Zn. The variabilities of both metals between male and female were investigated by using coefficient of variation (CV). It is found that the low CV values among all the dissected soft tissues was foot, indicating a more accuracy in the biomonitoring of Cu and Zn using *P. viridis*'s foot. This suggests that foot of *P. viridis* can be used as an alternative method to reduce the gender effect on the bioaccumulation of Cu and Zn.

Introduction

In biomonitoring studies of heavy metals using marine mussels, the use of soft tissues without mentioning the ratios of male and female in a mussel population is always reported. For instance, Kljakovic´-Gašpic´ et al., Benali et al. [1,2] determined the trace metals in *Mytilus galloprovincialis* from eastern Adriatic and Algerian west coast, respectively, without differentiating the male and female individuals during analysis. If the number of female individuals analyzed is significantly higher than the male, the comparison of metal concentrations among the different populations would become low in accuracy. Furthermore, the ratios of male and female in the mussel populations collected are unknown during sampling until they are shucked from the shells and gender is usually revealed (although not accurately) from the color of the gonadal tissues. It has been widely reported in the literature [3-5], that gender is one of the intrinsic factors that could potentially cause variability of metal bioaccumulation in the body tissues of marine mussels including the green-lipped mussel *Perna viridis* [6].

In the study of metal concentrations of bivalves, it is very often that both genders are not separated and they are pooled and later analyzed for heavy metals. Regardless of the ratio of gender in the pooled samples, the results obtained will be used to compare different geographical mussel populations. Thus, these data used for comparison would become somewhat not convincing and needs further investigations. According to Yap et al. [7], in *P. viridis*, the background concentrations of metals in the females were generally higher than those in the males but the differences are not significant (P> 0.05).

Even if the mussels are separated and analyzed for metals by gender, the workload for the whole procedures are time consuming and complicated in terms of metal data obtained. In this study, coefficient of variation (CV) was used to determine the metal variability between male and female of *P. viridis*. It is hypothesized that selection of a particular tissue in *P. viridis* based on the lowest variability of metal concentrations between male and female can reduce the gender effect on the biomonitoring data. This would eventually increase the validity of biomonitoring data regardless of what genders of mussels are analyzed. Therefore, the objective of this study was to select a particular dissected soft tissue with the lowest CV and to propose its use as an alternative method to reduce the gender effect on the bioaccumulation of Cu and Zn.

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Materials and Methods

Mussel samples were collected from Senibong, Bagan Tiang and Sungai Semerak (Figure 1). The reason why these sites were selected in this study because Senibong is an busy shipping site and potentially receiving industrial and domestic in the Straits of Johore while Bagan Tiang and Sg. Semerak are relatively unpolluted sites in Perak and Kelantan, respectively, with no observable human activities in the surroundings except for fish aquaculture (Table 1). All collected samples were kept in a cool box compartment until transportation to laboratory. In the laboratory, all the mussels from each site were separated into males and females based on the colour of the gonadal tissues (Figure 2). The gonadal contents after differentiation through visual inspection were further scrutinized by microscopic examination [6]. About 15 individual of one gender from one population were then dissected into different soft tissues namely muscle, crystalline style (CS), foot, mantle, gills, gonad and byssus. The size of mussels was selected from similar shell lengths between male and female individuals from each sampling site.

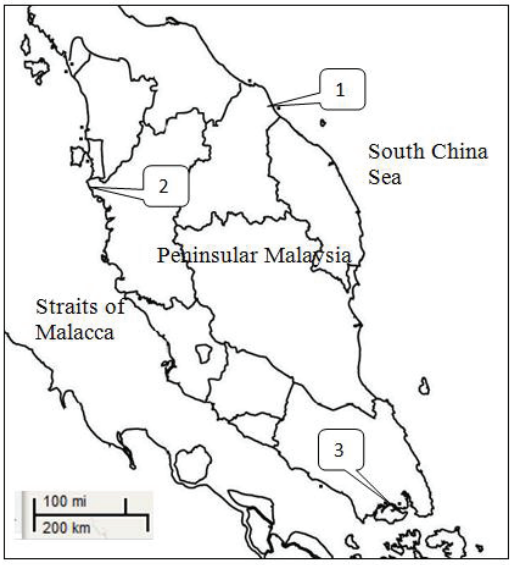

Figure 1: Map showing the sampling sites for *Perna viridis* in Peninsular Malaysia (1 = Sg. Semerak; 2= Bagan Tiang; 3= Senibong).

Table 1: Sampling locations, sampling dates, shell lengths of Perna viridis and descriptions of sampling sites.

Sampling sites	GPS	Sampling dates	Shell lengths (cm)	Site description
Sungai Semerak, Kelantan	N 05°51.560′; E 102°30.270′	13 May 2008	1) 6.22 ± 0.24 (5.57-6.87 2) 6.65 ± 0.18 (6.20-7.13)	A jetty and fishing village
Bagan Tiang, Perak.	N 05°08.517′; E 100°22.459′	19 April 2005	1) 8.51 ± 0.27 (6.49-10.22) 2) 8.76 ± 0.26 (7.04-10.57)	An offshore, fishes and mussel aquacultural area
Senibong, Johore.	N 01°28.001′; E 103°43.618′	3 May 2008	1) 7.37 ± 0.16 (6.90-7.82) 2) 6.97 ± 0.25 (6.31-7.64)	A busy shipping lane. Potentailly receiving Industrial effluents

Note: Under shell lengths- 1= Male; 2= Female.

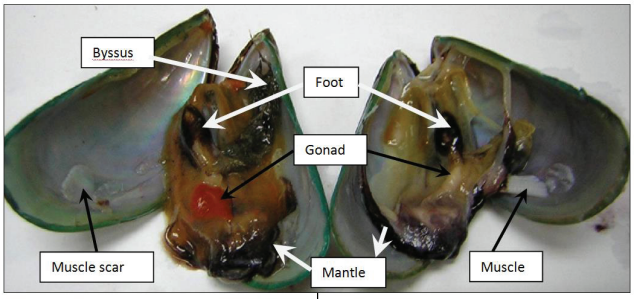

Figure 2: Picture showing the different soft parts, including foot, of female (left; gonadal tissues in orange colour) and male (right; gonadal tissues in creamy colour) individuals of *Perna viridis*.

By gender, three replicates of each pooled dissected soft tissues and the total soft tissue of mussels were dried in 105°C until constant dry weights for at least 72 hours.

In detail, each individual of dried tissue of mussels was digested in concentrated HNO_3 (AnalaR grade, BDH 69%). They were placed in a hot-block digester first at low temperature for 1 hour and then they were fully digested at high temperature (140°C) for at least 3 hours. The digested samples were then diluted to a certain volume with double distilled water. After filtration, the prepared samples were determined for Cu and Zn by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model Analyst 800. The data were presented in µg/g of sample dry weight. To avoid possible contamination, all glassware and equipment used were acid-washed and procedural blanks were analyzed once for every five samples. Quality control samples made from standard solutions of Cu and Zn were analyzed in every five samples to check for the metal recoveries. The metal recoveries were being satisfactory (90-110%). The T-test between any two variables was conducted by using the STATISTICA software package.

Coefficient of variation

The coefficient of variation (CV) value is a well-known unitless index of 'relative' variation [8]. It is useful to relate the arithmetic means and the standard deviation together. The CV value is most useful in comparing the variability of several different samples, each with different arithmetic means. This is because a higher variability is usually expected when the standard deviation increases, and the CV value is a measure that accounts for the variability [9,10]. In each tissue, the CV value was calculated from the untransformed data as:-

$$CV\ (\%) = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Results and Discussion

Results of T-tests for shell lengths between male and female mussels are given in Tables 2 and 3. Apparently, the differences of shell lengths between male and female mussels in all the

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three sampling sites are not significant (P> 0.05). Therefore, the mussel body size may not contribute significantly to the variation of metal accumulation in the mussels.

The CV values of Zn and Cu between male and female in the different soft tissues of *P. viridis* collected from three sites are presented in Tables 2 and 3, respectively. For Zn, the lowest CV values among all the soft tissues investigated are consistently found in foot of *P. viridis* in Sg. Semerak (2.0%), Bagan Tiang (8.0%) and Senibong (4.4%) populations. For Cu, the lowest CV values are found in Sg. Semerak population (1.3%). In Bagan Tiang population, there are three soft tissues namely CS, foot and gill are recorded to have CV values below 5% while in Senibong population, foot and gills are found to be among the lowest CV found (6.6-6.7%).

Among foot, CS and gills, the mussel's foot is a more practical tissue to reduce the gender variations of Cu and Zn

Table 2: Comparison of shell lengths, and Zn concentrations (µg/g dry weight) with their coefficients of variations (CVs) in the different tissues, between male and female of *Perna viridis*. (N= 15).

	Sites	Tissues	Female	Male	SD	mean	CV
1.	Semerak	Shell length	6.65^{a}	6.22^{a}	-	-	-
		Byssus	31.6^{a}	27.0^{a}	3.20	29.3	11.0
		CS	**38.0**^{a}	**35.0**^{a}	**2.10**	**36.5**	**5.80**
		Foot	61.2^{a}	62.9^{a}	1.20	62.0	**2.00**
		Gill	182^{b}	119^{a}	44.3	150	29.4
		Gonad	**132**^{a}	**127**^{a}	**4.00**	**129**	**3.10**
		Mantle	117^{a}	181^{b}	45.2	149	30.3
		Muscle	120^{b}	72.3^{a}	34.0	96.4	35.3
		TST	73.5^{a}	60.9^{a}	8.90	67.2	13.2
2.	B. Tiang	Shell length	8.76^{a}	8.51^{a}	-	-	-
		Byssus	122^{b}	85.3^{a}	26.3	104	25.3
		CS	24.8^{a}	41.2^{b}	11.6	33.0	35.1
		Foot	51.3^{a}	57.4^{a}	4.30	54.3	**8.00**
		Gills	76.9^{a}	127^{b}	35.4	102	34.8
		Gonad	52.3^{a}	62.2^{a}	7.00	57.3	12.2
		Mantle	**69.4**^{a}	**61.1**^{a}	**5.80**	**65.2**	**8.90**
		Muscle	65.4^{b}	45.2^{a}	14.3	55.3	25.9
		TST	NA	NA	-	-	-
3.	Senibong	Shell length	6.97^{a}	7.37^{a}	-	-	-
		Byssus	58.8^{b}	24.1^{a}	24.5	41.5	59.2
		CS	71.1^{a}	61.8^{a}	6.60	66.4	9.90
		Foot	88.2^{a}	82.8^{a}	3.80	85.5	**4.40**
		Gill	192^{b}	152^{a}	28.6	172	16.6
		Gonad	123^{a}	101^{a}	15.6	112	14.0
		Mantle	133^{b}	99.5^{a}	23.8	116	20.5
		Muscle	138^{b}	105^{a}	23.5	121	19.4
		TST	**107**^{a}	**97.8**^{a}	**6.60**	**103**	**6.50**

Note: TST= total soft tissues. Values in bold are those tissues with low CV values. NA= data not available. Similar (a, a) and different (a, b) letters in superscript indicate the difference between male and female of any parameters are not significant (P> 0.05) and significant (P< 0.05), respectively.

Table 3: Comparison of shell lengths, and Cu concentrations (µg/g dry weight) with their coefficients of variations (CVs) in the different tissues, between male and female of *Perna viridis*. (N= 15).

	Sites	Tissues	Female	Male	SD	mean	CV
1.	Semerak	Shell length	6.65^{a}	6.22^{a}	-	-	-
		Byssus	24.5^{b}	15.8^{a}	6.10	20.1	30.4
		CS	37.2^{a}	32.7^{a}	3.20	35.0	9.10
		Foot	6.59^{a}	6.71^{a}	0.10	6.70	**1.30**
		Gill	15.9^{b}	73.5^{a}	40.7	44.8	90.9
		Gonad	36.4^{b}	93.8^{a}	40.6	65.1	62.3
		Mantle	19.7^{a}	18.2^{a}	1.10	18.9	5.60
		Muscle	11.7^{a}	12.8^{a}	0.80	12.3	6.70
		TST	5.71^{a}	5.12^{a}	0.40	5.40	7.70
2.	B. Tiang	Shell length	8.76^{a}	8.51^{a}	-	-	-
		Byssus	22.2^{a}	34.4^{b}	8.60	28.3	30.4
		CS	**39.9**^{a}	**38.9**^{a}	**0.70**	**39.4**	**1.90**
		Foot	**6.93**^{a}	**6.47**^{a}	**0.30**	**6.70**	**4.80**
		Gills	**7.44**^{a}	**7.59**^{a}	**0.10**	**7.50**	**1.40**
		Gonad	7.03^{a}	9.33^{b}	1.60	8.20	19.9
		Mantle	7.17^{a}	7.88^{a}	0.50	7.50	6.70
		Muscle	8.46^{a}	7.04^{a}	1.00	7.70	13.0
		TST	NA	NA	-	-	-
3.	Senibong	Shell length	6.97^{a}	7.37^{a}	-	-	-
		Byssus	62.2^{b}	10.9^{a}	36.3	36.6	99.1
		CS	47.9^{b}	35.3^{a}	8.90	41.6	21.3
		Foot	**10.3**^{a}	**11.3**^{a}	**0.70**	**10.8**	**6.70**
		Gill	**13.9**^{a}	**12.7**^{a}	**0.90**	**13.3**	**6.60**
		Gonad	11.7^{b}	7.45^{a}	3.00	9.6	31.4
		Mantle	22.2^{b}	14.9^{a}	5.20	18.5	27.9
		Muscle	10.3^{a}	8.34^{a}	1.40	9.30	14.7
		TST	17.7^{a}	15.0^{a}	1.90	16.3	11.5

Note: TST= total soft tissues. Values in bold are those tissues with low CV values. NA= data not available. Similar (a, a) and different (a, b) letters in superscript indicate the difference between male and female of any parameters are not significant (P> 0.05) and significant (P< 0.05), respectively.

because gills are more related to ambient dissolved metals in the seawater (hence, more variable since it is the first organ to connect with the metal entry) [6], while is more difficult to be dissected when compared to foot. For gonad and mantle, both tissues are highly influenced by spawning condition of the mussels [4]. Therefore, the variation of metal concentrations in these tissues is high and therefore the accuracy of metal data based on these tissues is low.

In term of practicality, foot is much more feasible and easily dissectable since it is located in the outside of the mussel body (Figure 2) when compared to CS and gills in which CS and gills are harder to dissect out of the mussel body. In addition, the function of foot is protrusion to outside the mussel shell in search of suitable substratum in time of unfavorable conditions occur. Thus, foot is important in making new byssal threads in a new substratum. The metal levels found in the foot are believed to have been fully assimilated. According to a review by

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Fraser et al. [11], exposure effects can be affected by the gender of mussels. Sokolowski et al. [12], reported that significant differences in trace metal concentrations between genders in *Perna perna* might have resulted from more intense formation of reproductive tissues and metal accumulation in sexual products of females during the prespawning and spawning periods. Therefore, the present finding is useful to reduce the errors of Cu and Zn accumulation due to gender effects.

Conclusion

In conclusion, the advantages of the present findings are a) the mussel foot is an easily dissectable soft part of mussels which warrant a simple application, b) the use of CV is relatively simple and easy to apply for the determination of metal variability between male and female of mussels, c) less time in biomonitoring of Cu and Zn, and finally d) higher precision in the use of foot as bimonitoring organ of Cu and Zn since low CV are found between male and female of *P. viridis*. Therefore, compared to many reported studies using total soft tissues, the use of mussel foot can reduce the gender effect on the bioaccumulation of Cu and Zn. Besides, a higher precision of Cu and Zn interpretation regardless of what gender of mussels are collected from the tropical coastal waters, can be obtained. However, further investigations under controlled laboratory conditions are still necessary to validate our claim.

Acknowledgement

The author wants to acknowledge the partial financial support provided through Fundamental Research Grant Scheme (FRGS) Phase 1/2016 [Vote no. 5524953] by Ministry of Higher Education Malaysia.

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