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THE LENGTH OF THE CRYSTALLINE STYLE OF *Perna Viridis* IN RELATION TO SHELL LENGTH, SHELL WIDTH AND SHELL HEIGHT: DATA FOR FUTURE REFERENCE

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Abstract: The crystalline style (CS) is a gelatinous rod-like body that contains starch-digesting enzymes in the hemolymph of the bivalves. The lengths of the CS, shell lengths, shell widths and shell heights of the green-lipped mussel *Perna viridis*, collected from seven sampling sites in southern coastal waters of Peninsular Malaysia, were measured. The lengths of CS in relation to shell length, shell width and shell height were 72-73%, 211-218% and 154-157%, respectively. The correlation analysis indicated that the length of CS is positively and significantly ($P< 0.001$) correlated to the shell length ($R= 0.81$), shell width ($R= 0.82$) and shell height ($R= 0.64$). The percentages of the CS length to the shell parameters could be potentially used to identify the different mussel species since different mussel species have a specific CS length to the shell length. The present findings can serve as an important reference for comparative purpose with other bivalve species.

KEYWORDS: crystalline style length, shell size, *Perna viridis*

Introduction

The crystalline style (CS) is a gelatinous rod-like body that contains starch-digesting enzymes such as carbohydrases, in the hemolymph of the scallop, *Placepecten magellanicus* (Wojtowicz, 1972) and amylase in the clam *Mya arenaria* (Rodrick, 1979). These enzymes and the mixing of the stomach contents by the rotation of the CS against the gastric shield facilitate the breakdown of food particles.

The bivalve sizes or morphological characteristics are dependent upon environmental conditions, such as food concentration and water temperature (Boidron-Metairon, 1988; Strathamann *et al.*, 1992).

On the other hand, the length of the CS and the shell parameters could be influenced by the food availabilities during digestion (Rodrick, 1979). According to some literature (Wojtowicz, 1972; Rodrick, 1979), the CS dissolves at its anterior end, releasing enzymes into the stomach when food was available. Therefore, it was assumed that fluctuation of enzyme content in the CS was in accordance with food availability. This could influence the length of the CS in the short term. In ecotoxicological studies, the use of the CS of the green-lipped mussel *Perna viridis* as a biomonitoring organ for Cu (Yap *et al.*, 2006a) and Ni (Yap *et al.*, 2006b) bioavailabilities in coastal waters of Peninsular Malaysia was proposed.

This preliminary study focussed on the crystalline style (CS) length of *P. viridis* (Family: Mytilidae) in order to discuss its potential uses. The objectives of this study were to determine the relationships and percentages of the length of the CS in relation to the shell length, the shell width and the shell height of *P. viridis* samples collected from seven geographical sites from the southern part of Peninsular Malaysia.

Materials and Methods

Sampling, storage and sample preparation

Green-lipped mussels *P. viridis* were collected from seven geographical populations (Figure 1) (six sites in the Straits of Johore and one from Senggarang population bought from a mussel roadside stall). In the laboratory, these samples were determined for the shell lengths (distance between the umbo (anterior) and the posterior shell margin), shell widths, shell heights (distance between the ventral and dorsal shell margins), total CS wet weight and CS length. The CS was carefully pulled from the mussel's digestive tract in order to get the complete CS length. All of the parameters were measured by using a vernier calliper with an accuracy of 0.05 cm, except for the total CS wet weight, which was measured by using an electronic balance with an accuracy of 0.001g. Identification of the sexes of the mussels was determined according to the colours of the gonadal tissues (Yap *et al.*, 2006c). Males were distinguishable by the milky-white gonads while the females had bright orange/brick-red coloured gonads (Rajagopal, 1991).

Statistical Analysis

The data obtained were statistically analysed by using the Statistical Analysis System (SAS) for Windows, Release 6.12 software. The Spearman's product moment correlation coefficient on the $\log_{10}$ (mean + 1) transformed data (Zar, 1996) was applied to determine the strength and significance levels of the relationships. Below are the formulae used to determine the percentages (%) of the length of the CS in relation to the shell length, the shell width and the shell height:-

$$\text{CSLength}= \frac{\text{CSLength}}{\text{SLength}} \times 100$$

$$\text{CSWidth}= \frac{\text{CSLength}}{\text{SWidth}} \times 100$$

$$\text{CSHeight}= \frac{\text{CSLength}}{\text{SHeight}} \times 100$$

Results and Discussion

Table 1 shows the allometric parameters in male and female individuals of *P. viridis* from seven sampling sites. When all the shell parameters and CS lengths or CS weights were compared among all the seven sites, there was no specific trend of difference found. Especially when the metal-polluted sites at Senibong and Kg. Masai (both located at the eastern part of Johore Causeway) were compared to the three relatively-unpolluted sites located at the western part of Johore Causeway (Yap *et al.*, 2006d), there was no specific differences found for CS lengths or CS weights. This might indicate the food availabilities along the Johore Straits were relatively similar.

Combining all the sampling sites, the ranges of the shell lengths in the females (Table 2) and males (Table 3) were 48.3 to 97.4 mm and 44.4 to 83.0 mm, respectively. As for the shell widths, they ranged from 17.3 to 33.0 mm for females and 14.4 to 32.1 mm for males, while for the shell heights they ranged from 23.0 to 40.5 mm for females and 22.5 to 38.3 mm for males Statistical analysis showed that these lengths, widths and heights were not significantly ($P > 0.05$) different between males and females. The lengths of the CS ranged from 30.0 to 75.0 mm for both male and female individuals while the weights of the CS ranged from 0.01 to 0.07 g for both male and female individuals.

The percentages of the length of CS in relation to the shell length, the shell width and the shell height from the seven mussel populations are also shown in Tables 2-3. In general, in the female individuals, the length of the CS in relation to the shell length, width and height are 72.3%, 219% and 157%, respectively (Table 2). On the other hand, in the male individuals, the length of the CS in relation to the shell length, shell width and shell height were 73.0%, 211% and 155%, respectively (Table 3).

There is limited information to compare with the present findings in the literature. The CS of the freshwater mussel, *Parreysia corrugata* was reported as being 30-35 mm in the CS length (Lomte and Jadhav, 1980), when compared to *P. viridis* (30.0-75.0 mm for both male and female individuals). The percentage of the length of the CS and the shell length of *P. corrugata* ranged from 72.3-73.0%, compared to *P. viridis* (72.3% for female and 73.0% for male) from the present study which is very close. Alyakrinskaya (2001) reported that the CS lengths of the studied molluscs were rather long. The shortest style, whose CS length did not reach half (< 50%) of the shell length, was recorded in *Macoma baltica*. This species was followed by *Mytilus edulis* and *Mytilus galloprovincialis* (~50%), *Mya arenaria* and *Donax julianae* (~60%), and *Dreissena polymorpha* and *Anodonta complanata* (~65%). In the two latter species, the longest style length could reach 80%. The longest CS length (up to 87%) was recorded in *Unio tumidus* (Alyakrinskaya, 2001). The length of the CS of *P. viridis,* which ranged at 72.3-73.0% of the shell length as found in this study, was longer than the similar mussel family (Mytilidae) namely *M. edulis* and *M. galloprovincialis* (~50%) (Alyakrinskaya, 2001). Therefore, the percentage of the CS length to the shell length could be used to identify the different mussel species since different mussel species had a specific CS length to shell length.

From Table 4, it can be observed that the correlation coefficients between the lengths of CS-shell parameters were almost similar in the females and males. When the males and females were combined, the correlation coefficients were the highest between the CS length- shell width (R= 0.82, P< 0.001), followed by CS length-shell length (R= 0.81, P< 0.001) and CS length- shell height (R= 0.64, P< 0.001). For CS weights, when the males and females were combined, the correlation coefficients were the highest between the CS weight-shell length (R= 0.73, P< 0.001), followed by CS weight-shell width (R= 0.65, P< 0.001) and CS length- shell height (R= 0.60, P< 0.001).

The expression of many morphological characters of bivalves is dependent upon environmental conditions, such as food concentration and water temperature (Boidron-Metairon, 1988; Strathamann *et al.*, 1992). The significant correlations between the CS length and shell sizes obtained from this study could result from the environmental conditions in which they could be influenced by the availabilities/amount of food during digestion (Rodrick, 1979). Besides, it was mentioned that the CS dissolved at its anterior end, releasing enzymes into the stomach (Wojtowicz, 1972; Rodrick, 1979) when food was available. Therefore, it was assumed that fluctuation of enzyme content in the CS could result in the variation of CS length and CS weight which was in accordance with food availability.

Various factors could also influence the relationships between the CS length and the shell parameters. According to Hummel *et al.* (1988), the CS length of *Macoma balthica* showed a linear relationship to the shell length during both the growing season and the rest of the year. Besides, Hummel *et al.* (1988) also reported that the CS length might be influenced by seasonal variations and tidal and daily variations. In bivalves, food is typically subjected to an intracellular digestive cycle in the digestive diverticula. Linear relationships between CS length and shell length had also been observed in the related species *Abra nitida* and *A. alba* (Kristensen, 1972) with a CS length about one third the shell lengths (about 33%). Moreover, a linear relationship was found between the CS dry weight and the total wet weight of *Crassostrea gigas* (Bernard, 1973).

One interesting question on the potential use of such relationships is 'Can the identification of the species be made based on the relationships between the CS length and allometric parameters?' It is argued that the CS length to the shell parameters could be potentially used for the identification of different mussel species. Based on the previous comparison, the percentage of the CS length to the shell length could be used to identify the different mussel species since different mussel species had a specific CS length to the shell length. Of course, the possibilities of subspecies occurrence could be investigated by allozyme or DNA microsatellite markers (Tan and Yap, 2006). Perhaps, the only observed limitation in the use of the length of the CS was that it would get dissolved easily after a period of improper storage.

Other potential uses of the ratios include as an eco-physiological indicator of food availability in the coastal waters. Ibarrola *et al.* (1998) reported that enzyme activities in the digestive gland of *Cerastoderma edule* increased rapidly (3 days) in the presence of food at any time of the year, suggesting that seasonal variation of enzyme activities would reflect a process of continuous adjustment of digestive enzyme levels to environmental food availability. Therefore, the CS weight could be influenced by the food availability in the coastal waters. However, it is believed that the ratios of the CS length to the shell parameters could be potentially used in the identification of *P. viridis* in the future, although further validation is necessary.

Conclusion

The present findings had provided an important reference for comparative purpose with other bivalve species. Possibly, the relationships between the length of the CS and the shell parameters could be used for the identification of *P. viridis* in comparison to other species besides as an indicator of food availability although further validation is required. The percentages of the length of CS and the shell parameters for *P. viridis* are important baselines for future reference.

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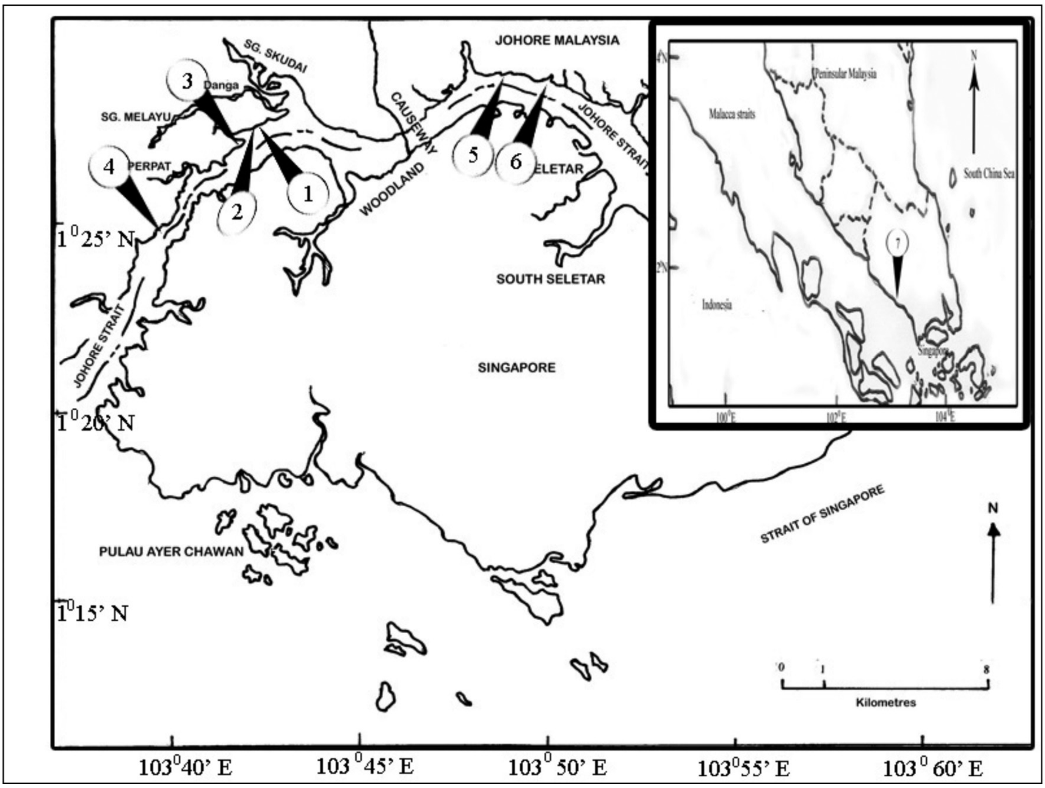

Figure 1: The sampling locations of *Perna viridis* along the Johore Straits and Senggarang.

Note: 1. Kg. Sg. Melayu-1; 2. Kg. Sg. Melayu-2; 3. Kg. Sg. Melayu-3; 4. Gelang Patah; 5. Senibong; 6. Kg. Masai; 7. Senggarang (Inset).

Table: 1. Shell lengths, shell widths, shell heights, crystalline style (CS) lengths and CS weights of *Perna viridis* analysed in the present study.

St	Sites	Gender	No.	Shell length (mm)	Shell width (mm)	Shell height (mm)	CS length (mm)	CS weight (mg)
1	Kg. Sg. Melayu-1	Male	5	59.32 ± 3.08	20.93 ± 1.44	28.3 ±0.47	40.40 ± 6.56	33.42 ± 6.36
		Female	5	59.02 ± 2.57	19.10 ± 0.89	28.39 ± 0.83	38.40 ± 3.61	20.2 ± 1.76
2	Kg. Sg. Melayu-2	Male	5	56.06 ± 4.77	18.82 ± 1.54	26.78 ± 1.47	37.00 ± 2.55	25.58 ± 7.03
		Female	4	64.43 ± 3.83	21.78 ± 1.01	30.33 ± 1.51	46.25 ± 3.94	39.25 ± 10.4
3	Kg. Sg. Melayu-3	Male	5	79.93 ± 1.58	30.04 ± 0.90	33.65 ± 1.62	65.60 ± 2.48	55.34 ± 4.51
		Female	5	88.26 ± 4.12	28.99 ± 1.54	35.39 ± 2.07	64.60 ± 3.39	61.14 ± 5.71
4	Gelang Patah	Male	4	49.48 ± 2.90	16.56 ± 0.34	24.73 ± 1.17	37.25 ± 2.32	31.85 ± 2.97
		Female	3	58.13 ± 4.94	20.53 ± 1.42	28.15 ± 2.61	41.67 ± 4.41	48.13 ± 13.59
5	Senibong	Male	5	59.07 ± 1.27	21.38 ± 0.71	28.31 ± 0.85	43.80 ± 1.98	33.0 ± 4.16
		Female	5	64.30 ± 1.12	21.12 ± 1.02	30.57 ± 0.90	46.40 ± 1.21	43.82 ± 4.09
6	Kg. Masai	Male	5	47.37 ± 1.06	16.04 ± 0.67	25.86 ± 1.34	36.00 ± 0.89	24.42 ± 0.09
		Female	5	57.78 ± 0.94	20.06 ± 1.09	28.70 ± 0.83	48.00 ± 3.39	35.44 ± 3.15
7	Senggarang	Male	6	66.10 ± 2.03	21.55 ± 0.93	29.57 ± 1.28	46.50 ± 2.95	46.30 ± 6.51
		Female	5	69.58 ± 3.15	21.62 ± 1.31	30.56 ± 1.03	47.80 ± 2.24	55.40 ± 5.27

Note: No= Number of mussels analysed.

Table 2: The overall shell parameters and percentages of crystalline style (CS) length to the shell parameters in female individuals of *Perna viridis*. Based on all seven sampling sites, N= 32.

Variables	Mean	SE	Minimum	Maximum
Shell length (mm)	66.46	2.11	48.25	97.40
Shell width (mm)	21.97	0.70	17.30	33.00
Shell height (mm)	30.43	0.63	23.00	40.50
CS length (mm)	48.00	1.78	30.00	75.00
CS weight (g)	0.04	0.00	0.01	0.07
CS length in relation to shell length (%)	72.30	1.62	55.10	98.12
CS length in relation to shell width (%)	218.56	4.58	172.73	270.88
CS length in relation to shell height (%)	157.39	4.35	103.81	209.79

Note: SE= standard error.

Table 3: The overall shell parameters and percentages of crystalline style (CS) length to the shell parameters in male individuals of *Perna viridis* in male individuals of *Perna viridis*. Based on all seven sampling sites, N= 35.

Variables	Mean	SE	Minimum	Maximum
Shell length (mm)	60.09	1.95	44.35	82.95
Shell width (mm)	20.90	0.81	14.35	32.10
Shell height (mm)	28.31	0.62	22.45	38.30
CS length (mm)	44.06	1.99	30.00	75.00
CS weight (g)	0.04	0.00	0.01	0.07
CS length in relation to shell length (%)	73.04	1.77	47.72	94.14
CS length in relation to shell width (%)	211.44	5.31	143.54	287.06
CS length in relation to shell height (%)	154.52	4.88	102.39	217.71

Note: SE= standard error.

Table 4: The Spearman's correlation coefficients based on log10 transformed data between the allometric parameters and crystalline style (CS) lengths and CS weight of *Perna viridis*. Based on all seven sampling sites.

	Female, N= 32		Male, N= 35		Male plus female, N= 67	
	CS length	CS weight	CS length	CS weight	CS length	CS weight
Shell length	0.76***	0.58***	0.78***	0.84***	0.81***	0.73***
Shell width	0.83***	0.51**	0.78***	0.77***	0.82***	0.65***
Shell height	0.52**	0.50**	0.63***	0.66**	0.64***	0.60***

Note: All parameters were log10 transformed prior to correlation analysis. Values given are the correlation coefficients (r) and their levels of significance (**=*P* <0.01, ***=*P* <0.001).