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Incidence of Hermaphrodite in Green Mussel *Perna viridis* along the West Coast of Peninsular Malaysia

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ABSTRACT

Incidences of hermaphroditism in green mussels (*Perna viridis*) were determined in four different areas (Sebatu, Port Dickson, Muar and Johor Bahru) along the west coast of Peninsular Malaysia. In Sebatu, 1000 specimens of green mussels were used for the visual determination of sex ratios. The male:female visual ratio was 1:0.79. Three hundred and seventeen individuals were also sexually identified using histology technique. Male to female ratio was 1:1.01. Two hermaphrodite specimens were recorded during the months of June and September, 2004. In Port Dickson, 319 specimens were visually sex-determined, the male: female ratio was 1.00:0.86. One hundred forty one were also histologically identified. Male: female ratio was 1.00:0.96. There was no hermaphrodite specimen encountered in this population. For the population of Muar and Johor Bahru, the entire specimen's numbers were visually sex-determined first and later confirmed by histological procedure. The male:female ratio was 0.72:1.00 and 1.00:0.95. No hermaphrodite specimen was found at either site. The pooled samples from different months showed no significant seasonal deviation in the sex ratio from a 1:1 ratio (Chi-square test, $p>0.05$).

Key words: Hermaphroditism, *Perna viridis,* sex ratio, histology, Peninsular Malaysia

INTRODUCTION

Mussels are extensively cultured along the west coast of Peninsular Malaysia (Ismail *et al*., 2000; Yap *et al*., 2002; Al-Barwani *et al*., 2007; Amin *et al*., 2005). The culture of green mussels (*Perna viridis*) in Malaysia was first introduced in the mid-seventies by the Fisheries Research Institute in Penang. Since then mussel culture has shown a significant development in different coastal waters of Peninsular Malaysia. The culture of green mussels in Malaysia is dependent exclusively on the natural spat-fall; Sebatu, Malacca is known for its rich natural spat-fall which has supported the industry at sustainable levels (Zaman, 2000). Green mussels are dioecious, with some occasional hermaphrodite specimens reported from Hong Kong, China and India (Lee, 1988; Anonymous, 1960; Narasimham, 1980; Nagabhushanam and Mane, 1991). Sexes cannot be identified by external morphology (Siddall, 1980; Vakily, 1989; Rajagopal *et al*., 2006). However, the internal morphology of the mantel can reveal the gender base on the different coloration of the male and female gonad which occupies a large section of it. The color of reproductive tissue of green mussels varies considerably, male (Fig. 1) gonadal tissue are usually milky to creamy white while

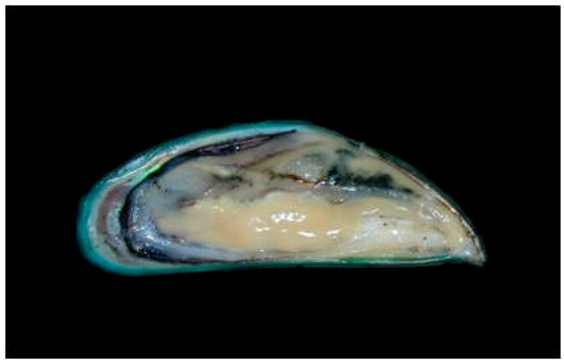

Fig. 1: Gonad of male *Perna viridis*

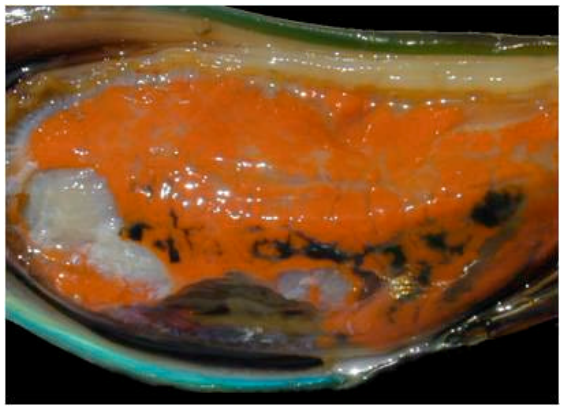

Fig. 2: Gonad of female *Perna viridis*

in females (Fig. 2) it is orange to red orange (Vakily, 1989; Rosell, 1991; Yap, 1999). An intermediate coloration or a color very similar to the mature male gonad could be found when the animals are immature or the females are at developing or spent stage (Vakily, 1989; Lee, 1988). At these stages, it is very difficult to correctly identify the sexes visually.

Hermaphroditism is common among individuals of other bivalves such as in the Anomalodesmata, Pectinidae, Pisidiidae and Tridacnidae (Morton, 1981; Lee, 1988), while it is considered stable gonochorism with occasional hermaphroditism in Mytilidae (Seed, 1969; Sunila, 1981; Lee, 1988). In case of green mussels various percentages of hermaphroditism were reported by different authors. Nagabhushanam and Mane (1991) have reported some minor incidence of hermaphroditism (0.8%) in India while Lee (1988) has reported a very low percentage (<0.1%) in Hong Kong. On the other hand, Anonymous (1960) and Narasimham (1980) have reported 14.3 and 8% cases of hermaphroditism in China and India, respectively. However, some authors in the region have classified green mussels as a dioecious species and no cases of hermaphroditism were reported by them (Huang *et al*., 1985). Since there is no information on hermaphroditism of green mussels from Malaysia, the objective of this study was to look for incidences of hermaphroditism in Sebatu, Malacca in the west coast of Peninsular Malaysia.

MATERIALS AND METHODS

Study area: Four study sites were examined for cases of hermaphroditism in green mussels. A total of 1400 specimens were collected from the four study sites in Sebatu, Port Dickson, Muar and Johor Bahru along the western coast of Peninsular Malaysia.

Sample process and analysis: Mussels were initially cleaned of all the encrusting organisms and their byssuses were removed. Biometric measurements such as length (maximum length along the anterior-posterior axis), height (maximum length along the dorsal-ventral axis) and width (maximum length through both valves) of each individual were measured by using vernier caliper to the nearest 0.1 mm. The total weight of each individual was then recorded. A small part of the male (Fig. 1) and female (Fig. 2) gonad from the mantel and the mesosoma were incised and fixed in 10% buffered formalin for 48 h. The gonadal tissues were then processed, embedded in paraffin wax, sectioned at 5 μm and stained in haematoxylin and eosin using standard methods (Bancroft *et al*., 1996). Histologically 316, 121, 60, 40 specimen were analyzed from Sebatu, Port Dickson, Muar and Johor Bahru, respectively.

Data analysis: Chi-square test was used to see if there was any seasonal deviation in the sex ratio from a 1:1 ratio. All analysis was done using SPSS software version 15.0.

RESULTS

In Sebatu, 1000 specimen of green mussels were examined visually for sex ratio. The numbers of male, female and undetermined were 524, 412 and 64, respectively. The male: female ratio was 1.00:0.79. Histologically (Fig. 3, 4), 317 individuals were identified for sex ratio. The numbers of identified male, female and immature were 156, 157 and 4, respectively. The total male to female

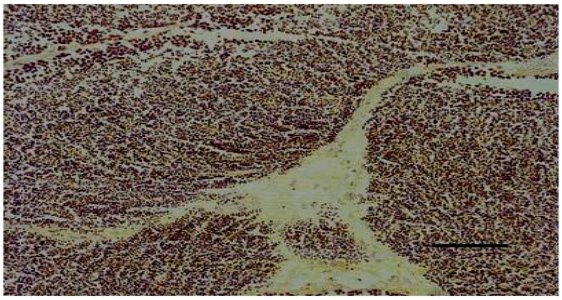

Fig. 3: Micrographs of male *Perna viridis* gonad tissue, Magnification x10, Scale bar: 100 μm

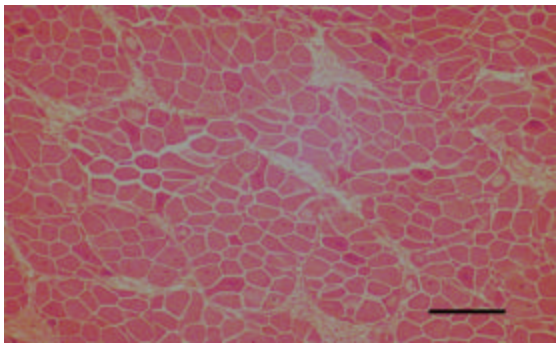

Fig. 4: Micrographs of female *Perna viridis* gonad tissue, Magnification x10, Scale bar: 100 μm

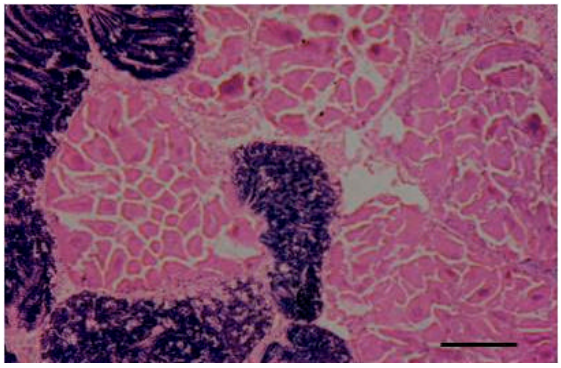

Fig. 5: Micrographs of hermaphrodite *Perna viridis* gonad tissue, Magnification x10, Scale bar: 100 μm

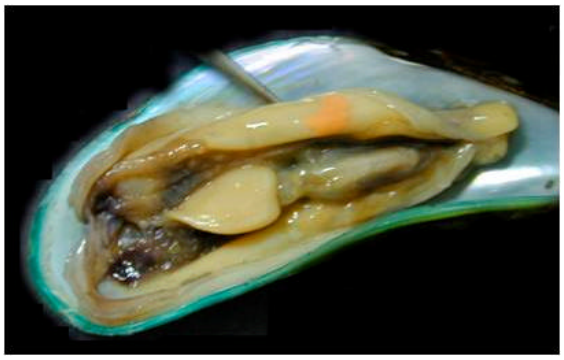

Fig. 6: Photo of hermaphroditic *Perna viridis*

Table 1: The monthly histological sex ratio in Sebatu, Malacca

Month	Male	Female	Immature	Total	Ratio (M:F)
October, 2003	7	14	0	21	1:20
November, 2003	8	12	0	20	1:1.5
December, 2003	11	10	0	21	1:0.91
January, 2004	16	5	0	21	1:0.31
February, 2004	8	11	0	19	1:1.38
March, 2004	8	12	0	20	1:1.50
April, 2004	7	12	1	20	1:1.71
May, 2004	13	11	1	25	1:0.85
June, 2004	12	8	0	20	1:0.67
July, 2004	12	9	0	21	1:0.75
August, 2004	6	12	2	20	1:20
September, 2004	15	9	0	24	1:0.60
October, 2004	14	8	0	22	1:0.57
November, 2004	10	13	0	23	1:1.3
December, 2004	9	11	0	20	1:1.22
Total	156	157	4	317	1:1.01

ratio determined histologically was 1.00:1.01 (Table 1). The occurrence of male and female gametes on the mantle was in different acinus (Fig. 5). Two hermaphrodite specimens were recorded during the months of June and September, 2004. Both specimens had both male and female gametes on one side of the mantle (Fig. 6). No trace of hermaphroditism was observed neither in the second side

of the mantle nor the mesosoma. The pooled samples for the different months showed no significant seasonal deviation in the sex ratio from a 1:1 ratio (Chi-square test, $p>0.05$).

In Port Dickson, 319 specimens that were visually sex-determined showed male, female and immature numbers to be 164, 142 and 13 individuals, respectively. The male:female ratio was 1.00:0.86. One hundred and forty one specimens examined histologically showed male, female and immature numbers to be 72, 69 and 0 individuals, respectively. The male:female ratio was 1.00:0.96. No hermaphroditic specimens were encountered in this population.

For the population of Muar and Johor Bahru, all specimens were visually sex-determined and observations confirmed histologically. Male, female and immature specimens were 18, 25 and 0 and 21, 20 and 0 for Muar and Johor Bahru, respectively. The male:female ratio was 0.72:1.00 and 1.00:0.95 for Muar and Johor Bahru, respectively. No hermaphroditic specimens were encountered at either site.

DISCUSSION

The male:female histological ratios were almost the same for Sebatu, Port Dickson and Johor Bahru. The overall sex ratios in the three sites were not significantly different from the 1:1 sex ratio. Regardless of this, there was some trend of a slight dominance of one gender over the other. In Sebatu, Port Dickson and Johor Bahru, the males showed a slight dominance over the females. While in Muar, the females showed a large dominance over the males. In Sebatu with histological gender identification, there was some slight overall dominance of females during the month of October and November in 2003 and February, March, April, August, November and December in 2004. The male dominated the ratio in the other seven months during the 15 month study periods (Table 1). Anonymous (1960) have reported a male dominance in sex ratio in the months of February to April which has coincided with the early phase of the gametogenetic cycle. However, Anonymous (1960) have based his identification on the coloration of the intermantle and mesosomal gonads which is believed to be unreliable for some period of time of the gametogenic cycle (Brousseau, 1982; Lee, 1988).

The histological ratio for Muar was similar to that obtained for Sebatu by visual identification with female dominating instead. The difference in male:female ratio of Muar could be due to the fact that the mussels there were from naturally bottom living individuals which may exhibit different ratios than those that are grown in suspended cultured (In all the other sites, the specimens were from suspended culture platforms). While in Sebatu, that ratio could because of immature or developing ovaries have coloration that is similar to that of mature male gonad, so visually it could be easily misidentified as a male. This is the reason behind the higher dominance of male sex ratio in the visual gender identification rather than in the more accurate histological gender identification.

Many researchers in the South East Asia, in the Caribbean and in the USA have failed to identify any hermaphrodite individuals of green mussels population in their localities (Huang *et al*., 1985; Agard *et al*., 1992; Rylander *et al*., 1996; Bigatti *et al*., 2005; Urbano *et al*., 2005). In this study, more than 1400 specimen were examined visually and histologically and only two cases of hermaphroditism were found. The high percentage reported by Anonymous (1960) could have resulted from the misidentification of the gender especially that he relayed on visual gender identification in his study. This study therefore indicates that hermaphroditism in green mussels is a rare phenomenon and could be classified as occasional hermaphroditism. This finding

is in line with the low percentage of hermaphrodite cases that Lee (1988) has encountered in his study. However, he has identified green mussels because of the low frequency of true hermaphrodite as a gonochoristic species.

Since green mussels were classified both as dioecious and hermaphroditic in different regions and different habitats, the actual identification is still debatable. Having some low reported cases of hermaphroditism make it difficult to draw conclusion as to what triggers this sexuality phenomenon in green mussels. Van der Schalie (1970) have hypothesized in fresh water mussels that the interaction between environmental influences and genetic sex-determining mechanisms might be responsible for occasional hermaphrodite occurrences. Kat (1983) posited that the occurrences are due to disruption of "single or complex of sex-determining hormones" owing to parasite infection. This hypothesis is not valid in this study since both hermaphroditic specimens of green mussels showed no evidence of parasite infestation.

CONCLUSION

The annual sex ratio of male to female was 1:1.01 at Sebatu, Malacca. Throughout the investigation, two hermaphroditic specimens were recorded in June and September at Sebatu. There was no hermaphrodite specimen encountered in the other three sides (Port Dickson, Muar and Johor Bahru).

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REFERENCES

Agard, J., R. Kishore and B. Bayne, 1992. *Perna viridis* (Linnaeus, 1758): First record of the Indo-Pacific green mussel (Mollusca: Bivalvia) in the Caribbean. Caribbean Mar. Stud., 3: 59-60.

Al-Barwani, S.M., A. Arshad, S.M.N. Amin, S.B. Japar, S.S. Siraj and C.K. Yap, 2007. Population dynamics of the green mussel *Perna viridis* from the high spat-fall coastal water of Malacca, Peninsular Malaysia. Fish. Res., 84: 147-152.

Amin, S.M.N., M.A. Halim, M. Barua, M. Zafar and A. Arshad, 2005. Population dynamics and exploitation level of green-lipped mussel (*Perna viridis*) using FiSAT from the offshore island of the Cox`s Bazar coast of Bangladesh. Pertanika J. Trop. Agric. Sci., 28: 103-110.

Anonymous, 1960. Perhminary observation on the seasonal changes in the gonad and sex reversal phenomenon in *Mytilus smaragdinus* Chemnits. Acta Universitita Amoy, 16: 57-63.

Bancroft, J.D., A. Stevens and D.R. Turner, 1996. Theory and Practice of Histological Techniques. 4th Edn., Churchill, Livingston, New York, London.

Bigatti, G., P. Miloslavich and P.E. Penchaszadeh, 2005. Sexual differentiation and size at first maturity of the invasive mussel Perna viridis (Linnaeus, 1758) (Mollusca: Mytilidae) at La Restinga Lagoon (Margarita Island, Venezuela). Am. Malacological Bull., 20: 65-70.

Brousseau, D.J., 1982. Gametogenesis and spawning in a population of *Geukensia demissa* (Pelecypoda: Mytilidae) from Westport, Connecticut. Veliger, 24: 247-251.

Huang, Z.G., S.Y. Lee and P.M.S. Mak, 1985. The distribution and population structure of green-lipped mussels (Bivalvia: Mytilacea) in Hong Kong waters. Proceedings of the 2nd International Workshop on the Malacofauna of Hong Kong and Southern China, April 6-24, 1983, Hong Kong University Press, Hong Kong, pp: 465-472.

Ismail, A., C.K. Yap, M.P. Zakaria, S. Tanabe, H. Takada and A.R. Ismail, 2000. Green-Lipped Mussel *Perna viridis* (L.) as a Biomonitoring Agent for Heavy Metals in the West Coast of Peninsular Malaysia. In: Towards Sustainable Management of the Straits of Malacca, Technical and Financial Options, Shariff, M., F.M. Yusoff, N. Gopinath, H.M. Ibrahim and A.N. Mustapha (Eds.). Malacca Straits Research and Development Centre (MASDEC), University Putra, Malaysia, pp: 553-559.

Kat, P.W., 1983. Sexual selection and simultaneous hermaphroditism among the Unionidae (Bivalvia: Unionidae). J. Zool., 201: 395-416.

Lee, S.Y., 1988. The reproductive cycle and sexuality of the green mussel *Perna viridis* (L.) (Bivalvia; Mytilacea) in Victoria Harbour, Hong Kong. J. Molluscan Stud., 54: 317-325.

Morton, B., 1981. The anomalodesmata. Malacologia, 21: 35-60.

Nagabhushanam, R. and U.H. Mane, 1991. Mussels in India. In: Estuarine and Marine Bivalve Mollusk Culture, Menzel, W. (Ed.). CRC Press, Inc., Boca Raton, Florida, pp: 191-200.

Narasimham, K.A., 1980. Dimensional relationships and growth of green mussel *Perna viridis* in Kakinada Bay. Indian J. Fish., 28: 240-248.

Rajagopal, S., V.P. Venugopalan, G. van der Velde and H.A. Jenner, 2006. Greening the coasts: A review of the *Perna viridis* success story. Aquat. Ecol., 40: 273-297.

Rosell, N.C., 1991. The Green Mussel (*Perna viridis*) in the Philippines. In: Estuarine and Marine Bivalve Mollusk Culture, Menzel, W. (Ed.). CRC Press, Inc., Florida, pp: 297-305.

Rylander, K., J. Perez and A. Gomez, 1996. Status of the green mussel, *Perna viridis* (Linnaeus, 1758) (Mollusca: Mytilidae), in North Eastern Venezuela. Caribbean Mar. Stud., 5: 86-87.

Seed, R., 1969. The Ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. II. growth and mortality. Breeding Settlement, Oecologia, 3: 277-316.

Siddall, S.E., 1980. A clarification of the genus *Perna* (Mytilidae). Bull. Mar. Sci., 30: 858-870.

Sunila, I., 1981. Reproduction of *Mytilus edulis* L. (Bivalvia) in a brackish water area, the Gulf of Finland. Annal. Zool. Fenn., 18: 121-128.

Urbano, T., C. Loderios, M. de Donato, V. Acosta, D. Arrieche, M. Nunez and J. Himmelman, 2005. Growth and survival of the mussels *Perna perna, Perna viridis* and an undefined morphotype in suspended culture. Cienc. Marinas, 31: 517-528.

Vakily, J.M., 1989. The Biology and Culture of Mussels of the Genus *Perna*. The World Fish Center, Manila, Philippines, ISBN: 9789711022709 Pages: 63.

Van der Schalie, H., 1970. Hermaphroditism among North American freshwater mussels. Malacologia, 10: 93-112.

Yap, C.K., 1999. Accumulation and distribution of heavy metals in green-lipped mussel *Perna viridis* (Linnaeus) from the West coast of Peninsular Malaysia. M.Sc. Thesis, University Putra Malaysia.

Yap, C.K., A. Ismail, S.G. Tan and H. Omar, 2002. Correlations between speciation of Cd Cu Pb and Zn in sediment and their concentrations in total soft tissue of green lipped mussel *Perna viridis* from the west coast of peninsular Malaysia. Environ. Int., 28: 117-126.

Zaman, M.K., 2000. Development in mollusk seed production in Malaysia. Proceedings of Seminar on Advance in Seed Production Technologies, April 12-13, 1995, Malaysian Fisheries Society, Department of Fisheries, Malaysia and University Pertanian Malaysia, pp: 68.