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Research Article

Study on Carapace Width Growth Band Counts Relationship of Orange Mud Crab, *Scylla olivacea* (Herbst, 1796) from Terengganu Coastal Waters, Malaysia

¹N. Hasyima Ismail, ¹A. Amin Safwan, ²N. Fairuz Fozi, ¹F.H. Megat, ^{1,3}H. Muhd Farouk, ⁴S.A. Kamaruddin, ¹M. Ikhwanuddin and ¹M.A. Ambak

¹Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

²Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

³Faculty of Applied Science and Computing, Tunku Abdul Rahman University College, Johor Branch, 85000 Segamat, Johor, Malaysia

⁴School of Fisheries and Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

Abstract

Background: Orange mud crab *Scylla olivacea* is one of the most important fisheries resources. A new development in ageing technique of crustaceans has been introduced. The detection of growth band deposited in hard structure of gastric mill in the cardiac stomach are found retained after moulting process can be used as age indicator and growth estimation. **Objective:** This study was carried out to determine the comparison between carapace width and growth band count of *S. olivacea* in Malaysia. **Materials and Methods:** Samples were collected from Setiu Wetlands, Terengganu, Malaysia from February until August, 2016. Samples were categorized based on their morphological measurements. The mesocardiac and zygo-cardiac ossicles in the gastric mill of *S. olivacea* was dissected out and preserved in solutions and underwent a cross sectioning process. A total of 76 of wild *S. olivacea* ranging from 6.56 to 12.84 cm in carapace width were analysed. The growth band counts were examined for each individual and ranging from 1 to 3 band counts. **Results:** A positive linear relation was observed between CW and GBC with $r^2 = 0.5178$, $p < 0.01$. Overall, there was a strong, positive correlation between CW and GBC. Increase in CW were correlated with increases in GBC respectively for this species. **Conclusion:** Therefore, the carapace width, growth band counts and body weight can be used to improve data on growth, recruitment, maturation and mortality. Thus, this study would able to improve new ageing technique and contribute greatly to improve the conservation and management of *S. olivacea* in Setiu Wetlands, Terengganu, Malaysia.

Key words: Mud crab, *Scylla olivacea*, carapace width, growth band counts, age, growth, crustaceans, gastric mill, conservation

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Corresponding Author: M.A. Ambak, Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The information on length or width relationships are essential in fishery stock assessment and management such as to estimate the weight at a given length or width of a species¹. The mathematical information derived from length or width and weight relationship could enhanced the general knowledge on well-being of individuals, biological, ecological factors, variation in growth according to sex, maturity, gonad development and breeding seasons of a particular species²⁻⁷. Such knowledge can be useful for studies on the life history and growth development of a species⁸. Extensive research has shown that the length-weight relationship on evaluating fish^{5,9-11} and crustaceans^{2,6,7,12-16}, mollusc⁴, including the horseshoe crab^{3,17-22}.

The growth band are deposited in a hard structure such as scales, bones, vertebrae and otolith in fishes and shells in bivalves²³ and this can be used to determine the age of an animal²⁴. Previously, the development of ageing technique in crustaceans were limited due to the presuming the loss of all integumental structure during moulting process^{14,15,25}. However, recently researchers have shown an increases interest in ageing estimation and growth development on crustaceans using growth bands deposited on hard structures in gastric mill and eyestalk of the animals^{14,15,25,26}. According to Campana²⁴, growth band deposition in otolith of fishes seems to be the most reliable method as age indicator in animals.

There is little published data on the growth and age estimation of *Scylla olivacea* in Malaysia^{13,27-29}. Thus, this study aims to determine the Carapace Width (CW), Growth Band Counts (GBC) relationship of *S. olivacea* collected from Setiu Wetlands, Terengganu, Malaysia and to understand the ageing and growth development of this mud crab species.

MATERIALS AND METHODS

Mud crab samples and sampling: A total of 76 samples of *S. olivacea* were collected from Setiu Wetlands Mangrove forest, Terengganu Coastal Waters, Malaysia (5°40'47.93" N 102°42'45.04" E) from February until August 2016. The samples of *S. olivacea* were categorized based on their morphological description provided by Keenan *et al.*³⁰. The crab size was measured by its Carapace Width (CW) using vernier callipers and each crab individuals were labelled with cable tie tag (Nylon cable ties, dimension 3 × 150 mm). The CW is the distance between the tips of 9th anterolateral spines of the carapace. Identification of *S. olivacea* are based on study by Keenan *et al.*³⁰. All samples were brought back to the

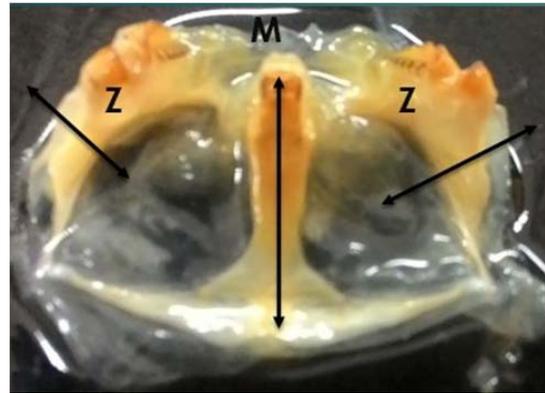


Fig. 1: Gastric mill ossicles of *S. olivacea* collected from Setiu Wetland. The black arrow indicates the cutting axis of zygocardiac (Z) and mesocardiac ossicles (M)

Science Fisheries Laboratory, School of Science Fisheries and Aquaculture, Universiti Malaysia Terengganu for further study.

Thin cross sectioning process: The samples were dissected out and exposed cardiac stomach extracted intact. Gastric mills were dissected out, cleaned and immersed in a preserved solution of 4% glycerol, 26% water and 70% for four days until all soft tissues could be removed^{14,15,25,26}. All structures of gastric mills (zygocardiac ossicles and mesocardiac ossicles) in Fig. 1 were separated and cleaned from excess tissues. The ossicles were then embedded in Buehler epoxy resin before preparing transverse sections for zygocardiac and mesocardiac ossicles with 150-300 µm thickness using Buehler Isomet Precision Saw^{14,15,25,26}.

The zygocardiac and mesocardiac ossicles of *S. olivacea* were mounted on a standard microscope slide and viewed under Olympus compound microscope (CX41) at 100-400X magnification. Digital images were taken using DinoCapture 2.0 software version 1.4.3 attached to the microscope. Images were digitally enhanced using Adobe Photoshop 12.0.4 × 32 to increase the contrast between adjacent bands. Growth bands were identified as paired (bipartite) light and dark zones in endocuticle. They were counted from basal (membranous layer and hypodermis) to the distal region of the endocuticle of the structure. This method were based on the studies by Kilada and Acuna¹⁴, Kilada and Ibrahim¹⁵, Leland *et al.*²⁵ and Kilada *et al.*²⁶.

Experimental design: All samples were identified based on their morphological identifications according to Keenan *et al.*³⁰ and were categorized based on their CW and sexes. The

samples were ranging from 6.00-6.99, 7.00-7.99, 8.00-8.99, 9.00-9.99, 10.00-10.99, 11.00-11.99 and 12.00-12.99 cm for CW. Each crabs were labelled using cable tie tag. Then, all samples were underwent dissecting process where the cardiac stomach were exposed. The gastric mill were extracted out from cardiac stomach and preserved in a solution of 4% glycerol, 26% water and 70% for four days. The gastric mill were then separated into zygo-cardiac ossicles and mesocardiac ossicles sections and prepared for the thin cross sectioning process.

Data analysis: Data obtained for all measurements were categorized based on their CW. The CW Growth Band Count (GBC) relationship were determined for both sexes and separately for females and males. The student t-test was carried out using SPSS Statistic Software version 22.0 to determine the differences in the regression CW and GBC between males and females of the *S. olivacea*. Coefficient of Variation (CV) is used to count the band independently by two readers. The two readings were used to increase the accuracy of the band counts and reproducibility of repeated measurements. The equation of CV as follows:

$$CV_j = 100\% \left(\frac{\sqrt{\sum_{i=1}^R (X_{ij} - \bar{X}_j)^2}}{\bar{X}_j} \right)$$

where, CV_j is the age precision estimate for the j th individual. The CV values of all individuals are averaged across the whole sample to produce a mean CV, X_{ij} , represents the i th age of the j th individual and represents the average age of the j th individuals^{15,24}.

RESULTS

A total of 76 individuals of *S. olivacea* were collected comprising of males (n = 45) and females (n = 31) from Setiu Wetlands, Terengganu. From the total of individuals collected, males show the highest occurrence (59%) followed by females (41%). The minimum, maximum and mean carapace width, CW (cm) and the analysis of length or CW-GBC relationship are given in Table 1. The CW of the total crabs were ranged from 6.6 to 12.8 cm. The Mean \pm SD (standard deviation) of CW was 9.1 ± 1.5 cm for both sexes.

The carapace width (CW) of *S. olivacea* varies with sexes with females being larger than males. The size of CW ranged between 9.0-10.0, 8.0-9.0 and 9.0-10.0 cm for both, male and female respectively. The length or CW-frequency distribution of the *S. olivacea* for both sexes are shown in Fig. 2.

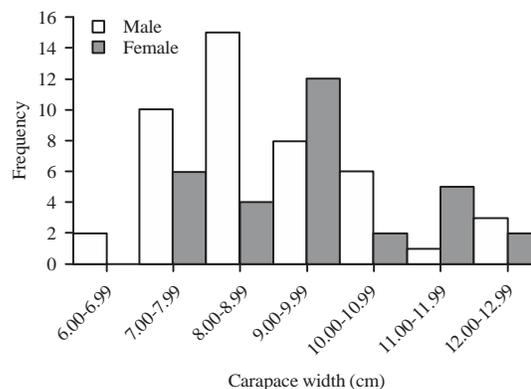


Fig. 2: Length or CW frequency distribution of male and female *S. olivacea* collected from Setiu Wetland (n = 76)

Table 1: The CW measurements with Mean \pm SD (standard deviation), minimum and maximum values of *S. olivacea* from Setiu Wetlands, Terengganu (n = 76)

Sex	n	Carapace Width, CW (cm)		
		Mean \pm SD	Min	Max
Male	45	8.9 \pm 1.5	6.6	12.8
Female	31	9.4 \pm 1.5	7.3	12.6
Total	76	9.1 \pm 1.5	6.6	12.8

The Carapace Width (CW) and Growth Band Counts (GBC) relationship for overall samples and for females and males collected were shown in Fig. 3. There was a significant linear relationship between the CW and GBC for overall samples ($p < 0.01$) and in both sexes ($p < 0.01$). Growth band were observed in the endocuticle layer of the cuticle. Growth band were recognized as narrower dark band in a broad translucent zone of gastric mill (Fig. 4).

All growth band were counts consistently between independent readers to avoid bias in counting. In order to minimize the bias in counting, the Coefficient in Variations (CV) equations were applied between the reading of independent readers using zygo-cardiac and mesocardiac ossicles. The CV values were 9.4% in the *S. olivacea* indicates that the less values of CV have no clear bias in counting. The growth band counts range between 1 and 3 in zygo-cardiac and mesocardiac *S. olivacea* and indicates that either structure could be used in counting the bands¹⁵.

The CV and GBC relationship of *S. olivacea* was estimated as $CW = 1.4991 \text{ GBC} + 5.7046$ and the coefficient of correlation of $r^2 = 0.5178$ shows the positive strong correlation between CV and GBC relationship for overall samples (n = 76). There were no significant different between females and males samples and the relationship of CV and GBC shows a positive correlations in both sexes. This suggest that in increasing the size, the growth band also increase.

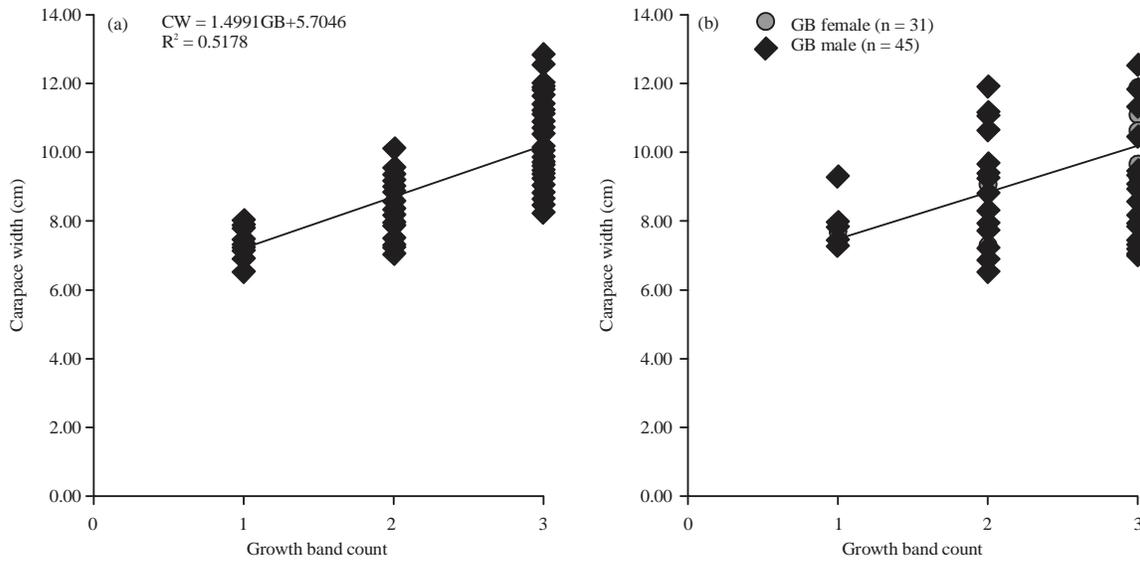


Fig.3(a-b): Carapace width and growth band relationship of *S. olivacea*. The curve in (a) CW and GBC relationship for overall samples (n = 76) and the curve in (b) Shows the CW and GBC relationship in females and males

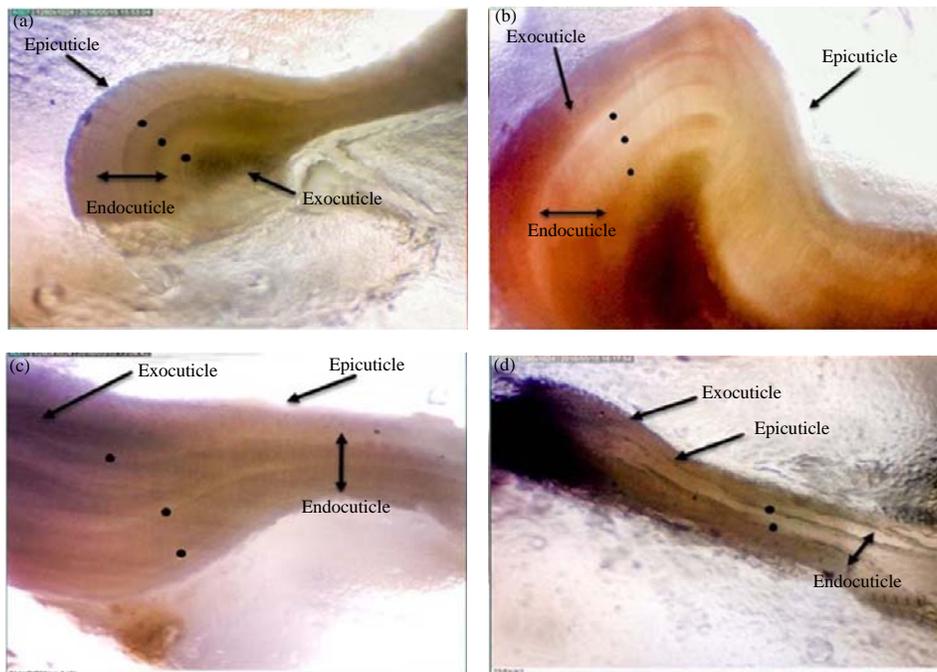


Fig.4(a-d): Growth band counts (black dots) shown in longitudinal thin sectioned of the gastric mill where consists of (a, b, d) Zycocardiac ossicles and (c) Mesocardiac ossicles of different individuals of the *S. olivacea*

DISCUSSION

In this study, the relationship between CW and GBC were revealed for the first time. The information on the relationship

of CW and GBC is insufficient due to the new development in method using the growth band count as age indicators in crustaceans²⁶. Therefore, the results from this study on the CW and GBC relationship of *S. olivacea* will facilitate the

knowledge of growth information and may improve the ageing technique of crustaceans.

The samples of *S. olivacea* were collected from Setiu Wetlands and the CW-GBC relationship were measured to determine the correlation between CW and GBC. The CW and GBC relationship were revealed for the first time using the growth band count in the mesocardiac and zyocardiac ossicles of the gastric mill in the *S. olivacea* stomach. The information from these relationships are very important in the future to determine the growth and age of *S. olivacea*.

The results showed that male crabs are found to be more abundant with 59% than female crabs with 41% from all samples collected from Setiu Wetlands. Similar results were documented where male crabs were found are more abundant with 33% compared to female crabs with 32% of *S. olivacea* in Setiu Wetlands¹³. Study from Khan and Mustaqeem³¹, shows that males of *S. serrata* are more abundant compared to the females of *S. serrata* in Karachi coast area (n = 488; male and n = 450; female). According to Hill³² and Ikhwanuddin²⁹, the less occurrence of female crabs in open water of mangrove areas is due to the offshore spawning migration during migration.

The growth band are widely used in fisheries to estimate growth and age of several of species and in crustaceans²⁶. The present study is the first report of the relationship between carapace width and growth band count of *S. olivacea* in wild population in Malaysia. The exponent 'b' values of linear regression at given CW and growth band indicate that the curve are allometric growth and shows that the increases the size, the growth band relatively increase and suggesting that the animal get older. According to Kilada and Ibrahim¹⁵, the age of blue swimming crab, *Portunus pelagicus* were determined by growth band in gastric mill and based on size at age relationship data which is an increase the size as the animals gets older.

Replicate growth band counts by two independent reader using Coefficient of Variation (CV) were applied in this study to assessing the bias between counting. The CV value resulted 9.4% for the *S. olivacea* in the present study. Similar CV values were reported in ageing studies for other crustacean species such as 8-11% in shrimp species, 15% in snow crab²⁶, 5-12% in most fishes²⁴, in bivalves with 5-7% CV values³³ and in *P. pelagicus* with 6-8%¹⁵. The similarity between the growth curve that were obtained from the only available linear regression CW and GBC relationship and CV values indicates that the band count are age indicator although differences between two sources are noticeable.

The growth band counts of *S. olivacea* ranged between 1 to 3 bands, implying that the longevity of this species

is 3 years similar with the growth band counts in *P. pelagicus* with three bands and indicating a longevity of 3 years¹⁵. Svane and Hooper³⁴ were also reported the same result with longevity of *P. pelagicus* with 3 year in South Australia and indicate that a variations of the longevity due to the similar habitat and ecological conditions in different location. Validation of the periodicity of the growth band was not applied in current study and this should be addressed in the future.

CONCLUSION

The result of this study indicated that the CW-GBC relationship have strong positive correlation and positive values of CV. This data could provide an additional information on age determination and growth estimation using growth band in mesocardiac and zyocardiac ossicles in gastric mill of *S. olivacea* although it was applied in current study due to small sample size. In addition, the similarity between growth band counts and estimated age of each individual of the species proved that this method has the potential to be applied routinely. However, to improve the ageing data using growth band counts, validation method on periodicity of the band deposition need to be done first and this information will give an impact in various fields, especially in fisheries stock assessment and management.

SIGNIFICANT STATEMENT

Age determination using growth band deposited in the hard structure of animals such as in fish, mollusc and bivalve widely known. However, previous knowledge stated that the applicability of growth band in crustaceans were limited due to the presumed loss all the exoskeleton during moulting process. Nowadays, various studies have shown that the growth band deposited in gastric mill of crustaceans can be used as age indicator. The present study provides data on relationship between Carapace Width (CW) and Growth Band Counts (GBC) of orange mud crab, *S. olivacea* as age indicator. Therefore, the data will contribute to the knowledge of crustaceans ageing for the future biological studies on age-structure-based stock assessments and for fishery conservation and management.

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