Sexual Polymorphism in a Population of *Strombus canarium* Linnaeus, 1758 (Mollusca: Gastropoda) at Merambong Shoal, Malaysia

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Various morphometric parameters of *Strombus canarium* Linnaeus, 1758 from Merambong Shoal, Malaysia, were measured and analyzed. The parameters include shell length, body whorl length, shell width, shell depth, shell lip thickness, aperture length, animal weight and shell weight. The population showed sexual polymorphism, and in addition to normal males and females, a 3rd morph composed of abnormal females with imposex characters were present. The latter (imposex females) accounted for 35.71% of the total adult female sub-population. Comparisons between males and normal females showed that the former had a significantly larger, heavier, and more-elongate shell than the latter. The male shells also had a significantly thicker lip with a higher degree of posterior and lateral lip flaring. Conversely, females allocated more energy
into tissue production than shell deposition relative to males. The 3rd imposex morph had a significantly larger and heavier shell, and a higher degree of thickening and flaring of the lip compared with both male and normal female shells. Impossex females also allocated less energy to gonad production relative to tissue production compared to normal females. http://zoolstud.sinica.edu.tw/47.3/xxx.pdf

Key words: Dog conch, Allometric analysis, Polymorphism, Imposex.

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The conch *Strombus canarium* Linnaeus, 1758 is a highly prized seafood in Malaysia and other areas within the region (Chuang 1961, Amini and Pralampita 1987, Erlambang and Siregar 1995, Zaidi et al. 2005). The species is usually abundant wherever it occurs, and is normally associated with sandy mud bottoms and seagrass beds (Abbott 1960, Amini and Pralampita 1987, Erlambang and Siregar 1995, Zaidi et al. 2005). It was the most abundant herbivorous mollusc within the study site and possibly contributes to the maintenance and well being of the seagrass bed ecosystem (Zaidi et al. 2005).

Gastropods within the genus *Strombus* are dioecious, exhibiting some degree of within-species sexual dimorphism (Abbott 1949 1960). Preliminary observations of *S. canarium* populations found wide variations in shell size and shape. Among the highly variable characters were shell length (variations in anterior siphonal canal development), shell depth, shell width (variations in lateral flaring of the shell-lip), aperture length (variations in posterior flaring of the shell-lip), and lip thickness, forming important morphometric characteristics of this species. There might be some variation between
sexes, but this has never been studied before. Therefore the main objectives of this study were to determine the morphometric variability within a population of *S. canarium*, and to evaluate whether certain characters are more sex-specific than others.

**MATERIALS AND METHODS**

**Study Site and sample collection**

The study was conducted at Merambong Shoal (01°19.979’N, 103°35.965’E) in the Johor Straits, Malaysia (Fig. 1). Sample collections were conducted using a transect line approach where all individuals within a 2-m width of a 50-m transect line were collected. Samples were first preserved in a container filled with ice and after 24 h in 10% formalin in seawater, and kept frozen prior to analysis. This preservation method proved practical as less mucus was secreted, and the animals stayed fresh even after a few months compared to formalin-preservation without freezing. Using this technique, the animal could be separated by slowly and carefully pulling the soft body until the columella muscle snapped, without the need to break the shell. The shell, animal, and gonadal tissues were then blotted damp-dry and weighed (wet-weight) to the nearest 0.001 g on an analytical balance. Samples were oven-dried at 60°C to a constant weight, then re-weighed (to obtain the dry-weight), further burned in a muffle furnace at 450°C for 8 h, and re-weighed to obtain the ash-free dry-weights (AFDW).
Fig. 1. Map showing the study site (arrows), the Merambong Shoal, west Johor Straits, Malaysia.

Morphometric variability

Samples of *S. canarium* were divided into different sexes and growth stages, i.e., juvenile, sub-adult, and adult, respectively. Growth stages were recognized according to the shell thickness, shell-lip flaring, coloration and degree of erosion (CFMP 1999), and by observation of the gonads, seminal vesicles, and capsule gland. The incidence of imposex was recognized by the presence of a pseudopenis on the female’s egg groove, based on descriptions by Kuwamura et al. (1983). Various linear morphological characteristics of the shell were measured to 0.01 mm using a digital vernier caliper (Fig.
The measurements included shell length (SL), body whorl length (BW), shell width (SW), shell depth (SD), shell outer lip thickness (OL) and aperture length (AL).

Fig. 2. Linear measurements used for comparing shell morphology. Flaring of the shell-lip shown by the arrows: (A) posterior flaring and, (B) lateral flaring of the shell lip.

The linear measurements and a combination of various ratio values were then compared among juvenile (male and female), sub-adult (male and female), and adult (male, female, and imposex female) sub-populations. The generated ratios included: relative shell width (SW/SL), relative shell depth (SD/SL), and relative body whorl length (BW/SL). The ratio of animal weight to shell weight (A/S) and the ratio of gonadal weight to animal weight (G/A) were also analyzed to respectively determine differences in somatic tissue production versus shell deposition, and differences in the allocation of tissue resources (Reed 1993b 1994). As the degree of flaring to the shell-lip (lateral- and posterior- flaring, see Fig. 2) is an important species-specific character (Appeldoorn...
1988), it was also analyzed by measuring the aperture length and width relative to total shell length (AL/SL), body whorl length (AL/BW), shell width (AL/SW), and shell depth (SW/SD).

**Statistical analyses**

Prior to any statistical analyses, data distributions were tested for normality and homogeneity of variances. Similarities in variances (and covariances) were tested using Bartlett’s test for univariate and Box’s M test for multivariate analyses. Variations between groups were first analyzed using multivariate approaches, i.e., the parametric canonical variate analysis (CVA) or nonparametric multidimensional scaling (NMDS), using PAST (PAleontological STatistic) software (Hammer et al. 2005). Differences in specific morphometric parameters between groups were then further analyzed via the univariate method, i.e., one-way ANOVA or nonparametric Kruskal-Wallis tests, followed by the appropriate post-hoc analyses, at $p < 0.05$ probability levels.

**RESULTS**

**Allometric analysis**

Shell length and shell width were both good predictors of AFDWs for male and female sub-populations of *S. canarium*. Regression analysis of log-transformed data showed a strong relationship of AFDW with shell length and AFDW with shell width (Table 1). However, the slopes of the linear regressions (log-transformed data) markedly differed when different predictors (shell length or shell width) were used
(paired *t*-test, male: \( t = 11.40, \text{d.f.} = 197, p < 0.001 \); female: \( t = 15.28, \text{d.f.} = 239, p < 0.001 \)). Male and female *S. canarium* showed positive allometric patterns (\( b > 3 \)) when shell length was used as a predictor, and negative allometric patterns (\( b < 3 \)) when shell width was used as a predictor. The slope (‘\( b \’) was higher in males when shell length was used as a predictor, but higher in females when shell width was used as a predictor.

Table 1. Correlations between log-transformed values of ash-free dry-weight (AFDW) against shell-length (SL) and shell-width (SW) for *Strombus canarium*. Retransformed values of AFDW, SL, and SW are provided in the form of a conventional allometric equation: \( W = aL^b \)

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>( R^2 )</th>
<th>( p )</th>
<th>Allometric equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male ((N = 197))</td>
<td>logAFDW = 3.48logSL (- 6.03)</td>
<td>0.98</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Female ((N = 239))</td>
<td>logAFDW = 3.38logSL (- 5.9)</td>
<td>0.98</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Morphometric characters

There was no significant difference in any of the morphological characters compared between male and female conch at the juvenile and sub-adult stages (\( p > 0.05 \)). The incidence of imposex was not observed during these early stages of development.

Within the adult population, the incidence of imposex was observed to be 35.71% of females exhibiting a male character (a penis). Thus morphological comparisons within the adult group were made between males, normal females, and imposex females.
Multivariate ANOVA (MANOVA) of linear morphological characters showed a highly significant difference between the 3 morphs (Wilks $\Lambda = 0.4153$, $F_{[12, 256]} = 11.70$, $p < 0.001$). A CVA was then conducted (Table 2). A scatter plot of the CVA coefficients is presented in figure 3, where each specimen (morphs) was plotted as a point in a space of reduced dimensionality. The 95% confidence ellipses show large overlap between morphs, with imposex females towards the upper right and males and normal females towards the lower left of the plotted area. The 1st canonical discriminant function coefficient accounted for 75.3% of the variance in morphology among the groups. Its eigenvector elements showed both positive and negative values, indicating that the CVA described differences in ‘shape’ rather than ‘size’, where SD was the most important discriminating parameters. The 2nd function accounted for 24.7% of the total variations, with OL forming the most important discriminating character. The CVA did not interpret axes subsequent to the 2nd one, as they described only small amounts (i.e., < 0.02%) of variation. In summation, the CVA suggested that SD and OL were the most important characters (linear measurements) separating the 3 different types of S. canarium morphs (see Table 2).
Fig. 3. Scatter plot of the 1st 2 axes of the canonical variate analysis and its 95% confidence ellipses. Data points represent individual discriminant function scores of the linear measurement data of each group (males, females, and imposex females). AL, aperture length; BW, body whorl; OL, shell-lip thickness; SD, shell depth; SL, shell length; SW, shell width.

Table 2. Canonical variate analysis (CVA) loadings based on a correlation matrix of 6 shell linear characters of males, females, and imposex *Strombus canarium*. Columns represent the 1st 2 axes of the canonical discriminant function coefficients.

<table>
<thead>
<tr>
<th></th>
<th>Axis 1</th>
<th>Axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>0.8726</td>
<td>0.2857</td>
</tr>
<tr>
<td>Percent (%) of variation</td>
<td>75.32</td>
<td>24.66</td>
</tr>
<tr>
<td>Cumulative Percent (%) of variance</td>
<td>75.32</td>
<td>99.98</td>
</tr>
<tr>
<td>Eigenvectors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL (Shell length)</td>
<td>-0.0033</td>
<td>0.0511</td>
</tr>
<tr>
<td>BW (Body whorl length)</td>
<td>0.2337</td>
<td>-0.2632</td>
</tr>
<tr>
<td>SW (Shell width)</td>
<td>0.0616</td>
<td>0.3828</td>
</tr>
<tr>
<td>SD (Shell depth)</td>
<td>0.9446</td>
<td>0.1091</td>
</tr>
<tr>
<td>OL (Shell lip thickness)</td>
<td>0.0258</td>
<td>0.7877</td>
</tr>
<tr>
<td>AL (Aperture length)</td>
<td>-0.2206</td>
<td>0.3864</td>
</tr>
</tbody>
</table>
For the ratio values, a nonparametric multivariate (NMDS, Kruskal, 1964) analysis was applied, as the data were not normally distributed, even after being transformed. The NMDS analysis starts by ranking (ordering) distance values between all pairs of data points using any distance measure. These ranks are then used in an iterative procedure in order to place the points in a low-dimensional space in which ranked distances are preserved (Hammer et al. 2005). A scatter-plot of this analysis is presented in figure 4, based on the Bray-Curtis similarity coefficient. The 95% ellipses showed that males and imposex females were more closely distributed, while normal females were located towards the lower left area of the plot. The nonparametric MANOVA (NPMANOVA) showed a highly significant difference between $S.\ canarium$ morphs (NPMANOVA, $F = 5.218, p < 0.001$), indicating that at least 1 type of morph significantly differed from at least another one.
Morphological comparisons among the 3 morphs were then further analyzed using a univariate approach (Table 3). All characters significantly differed except the ratio of body whorl length to shell length (BW/SL; Kruskal-Wallis, $H = 3.0, p > 0.05$). Imposix females showed significantly greater shell dimensions compared with males and normal females ($p < 0.05$) except for shell depth, which did not significantly differ from normal females but was significantly greater than males (one-way ANOVA, $F = 18.14, p < 0.01$). On the other hand, makes and normal females did not significantly differ from one another in most of the linear characters compared ($p > 0.05$), again with the exception of shell depth.

**Fig. 4.** Scatter plot of the 2 axes of the non-parametric multidimensional scaling analysis (NMDS) and 95% confidence ellipses of each group (males, females, and imposix females).
### Table 3. Comparisons of various morphometric characters among morphs of mature *Strombus canarium*. Data were analyzed using one-way ANOVA except (*) where a Kruskal-Wallis non-parametric test was used. Values are the mean ± SD and median ± Z for the respective method of analysis. Different superscripts indicate a significant different at the $p < 0.05$ probability level. AL/SL, relative aperture length-shell length; AL/BW, relative aperture length-body whorl; AL/SW, relative aperture length-shell width; SD/SL, relative shell depth; BW/SL, relative body whorl length; SW/SL, relative shell width; A/S, animal weight/shell weight; G/A, gonad weight/animal weight.

<table>
<thead>
<tr>
<th>Character</th>
<th>Male</th>
<th>Female</th>
<th>Imposex</th>
<th>F / H</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell length</td>
<td>54.67 ± 3.76a</td>
<td>55.56 ± 3.72a</td>
<td>58.16 ± 3.1b</td>
<td>3.23</td>
<td>0.043</td>
</tr>
<tr>
<td>Body whorl length</td>
<td>43.21 ± 2.9a</td>
<td>44.29 ± 2.58a</td>
<td>46.0 ± 3.03b</td>
<td>4.59</td>
<td>0.012</td>
</tr>
<tr>
<td>Shell width</td>
<td>34.61 ± 2.27a</td>
<td>35.38 ± 1.99a</td>
<td>37.65 ± 1.49b</td>
<td>7.39</td>
<td>0.001</td>
</tr>
<tr>
<td>Shell depth</td>
<td>24.71 ± 1.63a</td>
<td>26.17 ± 1.57b</td>
<td>27.31 ± 1.34b</td>
<td>18.14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lip thickness</td>
<td>3.32 ± 1.39a</td>
<td>2.83 ± 0.87a</td>
<td>5.06 ± 0.98b</td>
<td>11.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Aperture length</td>
<td>45.52 ± 3.21a</td>
<td>45.82 ± 2.88a</td>
<td>48.84 ± 2.8b</td>
<td>3.75</td>
<td>0.026</td>
</tr>
<tr>
<td>Shell weight</td>
<td>11.50 ± 2.98a</td>
<td>11.86 ± 2.56a</td>
<td>16.03 ± 2.48b</td>
<td>9.28</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Animal weight</td>
<td>5.54 ± 1.59a</td>
<td>6.60 ± 1.52b</td>
<td>8.12 ± 1.73c</td>
<td>12.56</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total weight</td>
<td>18.11 ± 4.47a</td>
<td>19.67 ± 4.23a</td>
<td>25.79 ± 3.62b</td>
<td>11.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AL/SL</td>
<td>0.83 (2.04)b</td>
<td>0.82 (-3.05)a</td>
<td>0.84 (1.79)b</td>
<td>10.61</td>
<td>0.005</td>
</tr>
<tr>
<td>AL/BW</td>
<td>1.05 ± 0.02b</td>
<td>1.03 ± 0.02a</td>
<td>1.06 ± 0.02b</td>
<td>19.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AL/SW</td>
<td>1.32 ± 0.04b</td>
<td>1.30 ± 0.04a</td>
<td>1.29 ± 0.05ab</td>
<td>5.81</td>
<td>0.004</td>
</tr>
<tr>
<td>SW/SL</td>
<td>0.63 (-1.35)a</td>
<td>0.64 (0.09)a</td>
<td>0.65 (2.41)b</td>
<td>6.21</td>
<td>0.045</td>
</tr>
<tr>
<td>SD/SL</td>
<td>0.45 (-6.55)a</td>
<td>0.47 (5.16)b</td>
<td>0.48 (2.88)c</td>
<td>43.68</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BW/SL</td>
<td>0.79 (-1.73)a</td>
<td>0.79 (1.54)a</td>
<td>0.80 (0.43)a</td>
<td>3.00</td>
<td>0.223 NS</td>
</tr>
<tr>
<td>A/S</td>
<td>0.49 ± 0.12a</td>
<td>0.57 ± 0.13b</td>
<td>0.51 ± 0.12ab</td>
<td>4.91</td>
<td>0.009</td>
</tr>
<tr>
<td>G/A</td>
<td>0.23 (-1.86)a</td>
<td>0.26 (2.63)b</td>
<td>0.19 (-1.32)a</td>
<td>7.57</td>
<td>0.023</td>
</tr>
</tbody>
</table>

NS, not significant
Normal females had a significantly greater shell depth than males (one-way ANOVA, $F = 18.14$, $p < 0.01$). Impossex females and males both showed a significantly greater degree of posterior flaring of the shell-lip compared with normal females, as shown by the AL/SL, AL/BW, and AL/SW values ($p < 0.05$). There was also a significantly higher degree of lateral flaring of the shell-lip in impossex females (one-way ANOVA, $F = 6.21$, $p < 0.05$). No significant difference was detected in lateral lip flaring between males and normal females (one-way ANOVA, $F = 6.21$, $p > 0.05$). For the general shell shape (SD/SL), all morphs significantly differed from one another, with the extremity of males possessing more-elongate shell and impossex females a more-globular shell (one-way ANOVA, $F = 43.68$, $p < 0.05$).

Impossex females were also significantly heavier (whole animal weight) compared with males and normal females ($p < 0.001$), most likely due to a relatively heavier shell and body mass. However, the animal/shell (A/S) ratio did not significantly differ between males and normal females, and in terms of gonad production (G/A), it was significantly inferior compared to normal females (one-way ANOVA, $F = 7.57$, $p < 0.05$). Although males and normal females did not significantly differ in terms of total weight and shell weight, the body mass of normal females was significantly heavier than males (one-way ANOVA, $F = 12.56$, $p < 0.001$). Normal *S. canarium* females also showed greater animal/shell (A/S) and gonad/animal (G/A) ratios, indicating greater gonadal and somatic tissue reserves compared with males ($p < 0.05$).

**DISCUSSION**
Shell length, wet weight, and dry weight are measurements commonly used to assess growth or increases in biomass of shelled molluscs (Smith et al. 1992, Cardoso et al. 2002, Dolbeth et al. 2005; Malaquias and Sprung 2005). These measures estimate the organic content or the biomass of an individual. A more-accurate method to determine the organic content is to measure the AFDW (Paine 1964), which is, however, not always feasible. Measuring the AFDW requires the destruction of the animal, which is not suitable for long-term monitoring of the growth of groups of animals or of endangered species. The present study therefore provides useful information in estimating biomass (AFDW) from length and width data. Both shell length and shell width were shown to be good predictors of AFDW for the species studied. The use of shell width as a reference for growth assessment is therefore recommended for fishery data collection of this species, along with the conventional assessment of shell length growth.

Sexual dimorphism was clearly evident within the studied S. canarium population, which however was only apparent in the adult stage. Within the Strombidae, the shell stops growing in length upon reaching maturity (Abbott 1960, Appeldoorn 1988). At this juncture, growth in length is minimal, and subsequent shell growth is in the form of shell thickening and outer lip flaring. Nevertheless, morphometric comparisons between male and female characters of adult S. canarium detected significant differences in only a few of the features studied, including shell depth, animal weight, and the degree of shell lip flaring.

Our results indicated that males are only slightly smaller than normal females, but with no significant difference between them. However, most of the larger individuals sampled were normal females and almost all small adults (> 40 mm) were males. This
agrees with previous studies of other *Strombus* species: e.g., Abbott (1949) of *S. gibberulus gibbosus* Linnaeus, 1758; Randall (1964) of *S. gigas* Linnaeus, 1758; Kuwamura et al. (1983) and Reed (1995) of *S. luhuanus* Linnaeus, 1758; and Reed (1993a) of *S. pugilis* Linnaeus, 1758. This condition appears to be a general characteristic of *Strombus*. However, previous observations of *S. canarium* populations in Indonesia by Erlambang (1996) reported that males were slightly larger than females, in contrast with our results.

The present study showed that *S. canarium* males were more dorso-ventrally flattened than normal females, which is inconsistent with other studies. Reed (1993a) found no significant difference in shape between males and females of *S. pugilis*. Her study on *S. luhuanus* however, found that females were significantly more slender than males (Reed 1995). This study also suggests that *S. canarium* females allocate more energy to tissue and gonad production compared to males, as shown by the significantly higher ratio of animal weight to shell weight, and gonad weight to animal weight. A previous study of *S. pugilis* found no significant differences in allocation of gonad tissue between males and females (Reed 1995). The male, on the other hand, allocated more energy to shell deposition, as shown by the significantly thicker shell and higher degree of shell flaring.

The population studied showed sexual polymorphism in morphological characters when the 3rd morph (imposex female) was included. Imposex is the superimposition of male sex characters onto a female, a phenomenon brought about by chronic exposure to tributyltin (Gibbs and Bryan 1986 1996, Fioroni et al. 1991). Imposex females significantly differed in terms of both shell size and shape, compared to males and normal
females. Polymorphism has been reported in only a few *Strombus* species, where the imposex group was in many instances referred to as a masculinized female (Kuwamura et al. 1983; Reed 1992 1993b 1994 1995). Kuwamura et al. (1983) first described this condition in *S. luhuanus*, but considered it to be a normal condition as > 70% of adult females were affected. The condition has also been reported in *S. pugilis* (Reed 1993a) and a few other species (Reed 1993b 1994). Due to their markedly larger size, the importance of imposex individuals for mariculture has even been suggested (Reed 1994 1995).

Previous studies reported high levels of organotin in the study area (Tong et al. 1996, Wood et al. 1997), which is located between the Port of Tuas, Singapore, and the Port of Tanjung Pelepas, Malaysia. Many imposex cases in other gastropods have been reported (Tan 1997 1999), reaching 100% incidence in some species. Our results showed the percent of imposex individuals in *S. canarium* is still very low compared to those reported by Tan (1997 1999). This might indicate different susceptibilities to organotin in different species, as was reported by Liu and Suen (1996) in their surveys of Prosobranch gastropod imposex in Taiwanese waters.

Imposex in its severest form can, in many instances, be detrimental to mollusc populations, by affecting reproduction (Bryan et al. 1986, Gibbs and Bryan 1986). Our results showed that the allocation of energy to gonad production was significantly reduced in imposex females compared to normal females. In addition, the presence of a pseudopenis along the path of the egg groove might affect the female’s ability to lay egg masses, which however, was not analyzed in this study. Further study is therefore urgently required to address the impacts of imposex on the population.
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