Population Structure of *Canarium labiatum* (Röding, 1798) (Mollusca: Neostromboidae: Strombidae) on Green Island, Great Barrier Reef, Queensland

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Abstract

Canarium labiatum is a small gastropod of the Strombidae family that is commonly encountered in the inter-tidal zones of tropical Queensland, Australia, yet little is known of its population structure. A targeted survey of the *Canarium labiatum* population on Green Island, located near Cairns, Queensland, was conducted on 12 August 2015. Ninety adult specimens were collected, of which 49 were female and 41 were male. The sample demonstrated significant sexual axial-length size dimorphism, with a bias towards larger females. While we collected more females than males, this did not represent a statistically significant bias, and rather may reflect the clustering nature of the sample. In addition, there was no evidence of pseudohermaphroditism in females within the population under consideration. Interestingly, 11.1% of the sample did not show banding and brown/grey-blue maculations on a light grey shell, the typical colour pattern associated with *Canarium labiatum*. This paper fills a knowledge gap in Queensland's *Canarium labiatum* population structure and provides a basis for a wider study into the size dynamics of Strombidae in general, but *Canarium* in particular.

Keywords: *Canarium labiatum*, clustering, colour, imposex, pseudohermaphroditism, sex bias, size dimorphism

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Introduction

Strombidae Rafinesque, 1815 is a frequently encountered marine gastropod family common to most tropical oceans. Members of the Strombidae express sexually dimorphic characteristics in both physiology and shell morphometrics, particularly in relation to the mean body size and length of the adult shell (Abbott, 1949, 1960, 1961; Reed, 1993a; Mutlu, 2004). The shells of individuals within a population may vary greatly, with occasional examples having rare colour phenotypes (Abbott, 1960). Some species, such as *Strombus pugilis* Linné, 1758 (Reed, 1993b) have demonstrated that there are more than two sexes within a population, with the reporting of masculinised females, a condition referred to as pseudohermaphroditism, or imposex, and defined

as the imposition of male sexual characteristics on females in gastropods (Jenner, 1979; Nicolaus & Barry, 2015). There is little or no knowledge regarding any of these population characteristics in members of the stromboid genus *Canarium* Schumacher, 1817, worldwide, with most work having been conducted during the mid to late twentieth century on taxonomy and basic physiology (Abbott, 1949, 1960, 1961; Geary & Allmon, 1990; Reed, 1993a).

Evidence from fossil beds and large collections of extant taxa indicate that sexual axial-length size dimorphism in Strombidae is a pleisiomorphic state, with a strong bias towards larger females (Geary & Allmon, 1990). Strombidae vary greatly in the mean size of adults within a population, and in the expression of sexual dimorphic characteristics in both physiology and shell morphometrics (Abbott, 1949, 1960, 1961; Reed, 1993a; Mutlu, 2004; Arularasan et al., 2011). However, it is necessary to explore the phenomenon of sexual dimorphism in novel taxa within the wider Neostromboidae (Maxwell et al., 2019) to establish the extent of this phylogenetic constant within the wider clade. Greater understanding of the phenomena of sexual size dimorphism will inform on the evolutionary limitations of size divergence.

The extent of pseudohermaphroditism expression in Queensland's Strombidae is another area that has not been explored. In Strombidae, pseudohermaphroditism is often associated with the superimposition of a penis onto females (Reed, 1993b; Cardenas et al., 2005). The presence of pseudohermaphroditism has varying effects on taxa, ranging from sexual impotence to no physiological effect on reproductive biology (Vlasconcelos et al., 2006). In masculinised female *Strombus pugilis*, the size of the dysfunctional external male sexual organ ranges from 2–12 mm, with no difference in ovarian structure between masculinised and normal females (Reed, 1993a). The presence of this penile gland in *Strombus pugilis* has no effect on the sexual function of the masculinised female as it is located on the edge of the egg groove (Reed, 1993b). Males and normal females are smaller than masculinised females when considering both size and shell to body mass ratios (Reed, 1993b; Cardenas et al., 2005). For example, in *Conomurex luhuanus* (Linné, 1758) populations, shell size of males was larger than normal females, but lighter in mass compared to masculinised females, which were larger in all size traits (Reed, 1995). However, Reed (1993b) found no evidence for this state in *Lobatus costatus* (Gmelin, 1791) after the examination of 500 specimens, suggesting an absence of masculinised females. Interestingly, there is no information on the presence of pseudohermaphroditism in *Canarium*.

Another population dynamic that has remain unexplored in many Strombidae is the occurrence of colour morphs. Rare colour phenotypic expression is well documented in the Strombidae (Kreipl et al., 1999). However, the relative abundance of these colour forms within a population has not been fully explored in many species. While some species are famous for their variability, such as *Canarium urceus* (Linné, 1758) which can be found in all colours of the rainbow and blends of those primaries (Abbott, 1960), other species have limited phylotypic plasticity in colouration. For example, *Canarium klineorum* Abbott, 1960 changes in shade and depth of colour, but there is limited variability in the base colour of this taxon. The level of colour variation within many *Canarium* populations in Queensland is not yet known.

One such overlooked species, *Canarium labiatum* (Röding, 1798), is a gregarious and locally common species across its known range of the Central Indo- and Eastern Pacific with typical banding and brown/grey-blue maculations (Abbott, 1960). This species has a high tolerance for environmental variability, such as temperature, indicated by the extent of its range from the equatorial shallow waters past the 30° latitudes (Abbott, 1960). It is also found in a diverse range of habitats, from coral outer reefs (Sudbury Cay, Queensland) to near-coastal muddy rocky reefs (Yule Point, Queensland).

This paper has four aims that will elucidate the knowledge gap concerning the life history and population characteristics of a discrete population sample of *Canarium labiatum* on Green Island, Queensland, Australia. The first seeks to test whether there is sexual size dimorphism in shell length. The second examines sex ratios to determine whether there is a sex-ratio bias. The third examines the expression of phenotypic plasticity in shell colouration within the sample. The fourth seeks to establish whether there is preliminary evidence of pseudohermaphroditism within the *Canarium labiatum* population under consideration.

Abbreviations

AM – Australian Museum, Sydney, New South Wales
QM – Queensland Museum, Brisbane, Queensland
QV – Queen Victoria Museum & Art Gallery, Launceston, Tasmania
SM – Collection of Stephen J. Maxwell, Cairns, Queensland

Methods

A targeted survey of a *Canarium labiatum* population located near Cairns, Queensland, Australia (16°46′S, 145°58′E) was conducted by SM on 12 August 2015. The population was identified in previous years (2013–2014) and was found to be localised and present in the general area (Maxwell & Watts, unpublished data; Figure 1). This information provided the starting point on the island from which a search for an initial *Canarium labiatum* cluster commenced. Once the first cluster ($n > 5/m^2$) was identified, the immediate area was searched using an opportunistic method, a process of searching an area until specimens were located, to determine the limits of the identified localised population to which the cluster belonged. This resulted in a 60 m^2 search area. Once the population boundary was established, all members of the population were collected within the defined area using a secondary fingertip search for four hours.

The type of clustering may affect the distribution of sex ratios between males and females. Within congregations of the same taxon, four distinct categories for clustering can be discerned: mixed age; juvenile; mating; and non-mating clusters (Brownell, 1977; Catterall & Poiner, 1983). Therefore, it is important to note the clustering nature of the sample on collection, particularly the age demographic and presence of sexual activity, as this may affect whether a sex-ratio bias is observed or not.

After collection, the total axial length of each specimen was measured. The animal was then placed on its dorsum and sexed as it righted itself: the presence of a verge indicated a male, while the presence of an oviduct indicated a female. Masculinised females are readily distinguished from males and normal females by a deformed and vestigial verge (Reed, 1995). The masculinised female verge is muscular, may be multi-lobed, and may contain superficial channels with ciliated epithelia (Reed, 1993b). During this process, we also noted the colouration of the ventral side of the shell. Atypical colouration was considered if the shell was a consistent solid colour or lacked the banded, typically brown/grey-blue maculations on a light grey shell (Abbott, 1960).

Figure 1. Green Island, showing the region of the constant presence of a *Canarium labiatum* population (Image modified from Google Earth, accessed January 2020).

Summary statistics for shell length were generated for each sex, and the means and standard errors for each sex presented to statistically demonstrate the presence of sexual dimorphism using SPLUS v2007 (Insightment Corp.). A box plot of each sex was generated to illustrate the size–frequency distributions of each sex. A pooled-variance, two-sample t-test was conducted to determine whether there were significant differences in the size of each sex. The proportion of males was tested against a hypothesised proportion of 0.5 using an exact binominal test to determine sex bias. Colour ratios were calculated as a percentage of the total sample. Pseudohermaphroditism was scored as 'present' or 'absent'.

Target Taxon

Mollusca Linné, 1758
Neostromboidae Maxwell et al., 2019
Strombidae Rafinesque, 1815
Canarium Schumacher, 1817 – Type: *Strombus urceus* Linné, 1758, p. 745, No. 440 (Maxwell et al., 2020)

Canarium labiatum (Röding, 1798)

Type: *Lambis labiata* Röding, 1798 (Martini, 1777, 3 pl. 78, Figures 804–805)

Type location: Amboina, Indonesia (Abbott, 1960, p. 68)

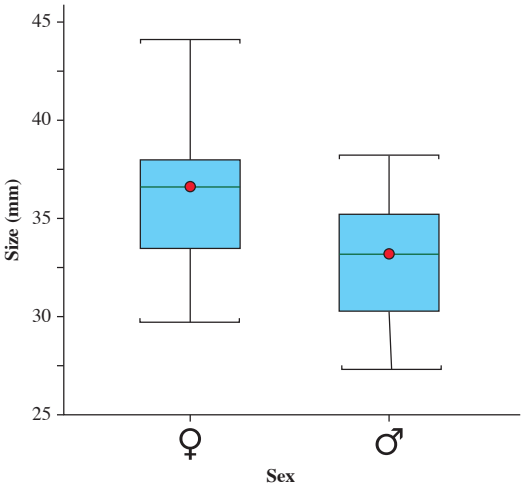
Description: The shell ranges in length from 15 to 55 mm. Solid, smooth to axially plicate, the shell has a distinctive orange columella. The columella has 25 to 40 weak, but distinct, raised lirae over its entire length, which are prominent at the ends of the columella. The outer aperture contains spiral lirae that are typically dark stained over an orange background, but in rare examples the aperture is entirely orange. The inner aperture lacks these lirations. The outer lip is well formed and thickened.

Remarks: There are seven institutional records contained within the *Atlas of Living Australia* (ALA, www.ala.org.au) for *Canarium labiatum* from Green Island, the first from 1901 to the most recent, 1978 (1901, AM – C.9665; 1929, AM – C.400361; 1961, AM – C.400152; 1969, QM – MO.83431; 1973, QM – DM.11576062; 1975, AM – C.400362; 1978, QV – QVM.9.9932). At present, no literary records for the occurrence of this species on Green Island are known to the authors.

Results

The sample of *Canarium labiatum* contained primarily adults (96.8 %; total $n = 90$; female $n = 49$; male $n = 41$), with no evidence of copulation, indicating a non-mating cluster. The sample suggested an average population density of 1.5/m^2 within the study perimeter. Three sub-adults and three dead specimens were observed within the search area but were excluded from further analysis as their sex could not be determined. The mean size of females was 35.91 mm (± 0.45 SE), in contrast to males, which had a mean size of 32.81 mm (± 0.46 SE). This difference in size, biased towards larger females, was statistically significant ($t_{2,88} = 4.79$, $p < 0.001$). The size distribution of each sex indicates that females have a wider size distribution range than males (Figure 2).

Figure 2. Boxplots of the axial lengths of each sex of *Canarium labiatum* within the study population on Green Island.



The number of males collected was smaller than the number of females (1:1.19), although this difference was not statistically significant ($p = 0.46$; $\alpha = 0.95$). While most (88.9%) of the sample exhibited typical colouration of maculation in greys and greens, 11.1% showed atypical colouration, with five yellow-shelled specimens (5.6%), four chocolate-coloured specimens (4.4%), and one coffee-coloured

specimen (1.1%; Figure 3), recognised by the colour of the ventral side *in situ* (Figure 4). Of the 90 specimens collected, there was no evidence of pseudohermaphroditism.

Figure 3. Colour forms of *Canarium labiatum* observed within the study population on Green Island: (A) typical maculated (37.5 mm, female, SM 23.043ag); (B) typical banded (37.0 mm, female, SM 23.043h); and the more uncommon (C) coffee (31.5 mm, male, SMC 25.070q); (D) yellow (34.5 mm, female, SM 23.043m); and (E) chocolate (44.5 mm, female, SM 23.043i) forms.

Discussion

This study confirms the presence of sexual axial-length size dimorphism in a non-mating, primarily adult aggregation of *Canarium labiatum* on Green Island, Queensland. The distribution of female sizes was broader than males, with the distributions of both sexes overlapping. This makes the assessment of the sex of a shell based on size problematic, a pattern that is not uncommon in this group of molluscs, given several examples of stromboid size-dimorphic distributions in both extant and extinct taxa (Geary and Allmon, 1990; Reed, 1993a). However, while female size may overlap male size significantly, males tend to occupy only the lower half of the size distribution in the sample studied. Therefore, selecting the largest members of a sample adult population, irrespective of clustering type, will result in a biased selection towards females. The study population showed a non-significant bias towards females over males. The different clustering possibilities have implications for the determination of sex bias towards more females and, thus, can be problematic to statistically establish from a single sample (Abbott, 1949; Wiedemeyer, 1998; Mutlu, 2004; Maxwell et al., 2017).

This study found that 11.1% of all specimens showed an atypical colour pattern to the typical bands and maculations. This is the first time that the colour proportions within a population of *Canarium labiatum* have been reported. This historical lack of knowledge on colour ratios is problematic, given the propensity of recreational collectors and

Figure 4. *Canarium labiatum* observed within the study population on Green Island: (A) Ventral views of living yellow and coffee colour forms; (B) Dorsal view of a yellow sub-adult *in situ* (Image: U. Weinreich, 12 August 2015).

commercial fishermen to frequently target rarer and more aesthetically pleasing specimens because of the higher price they command, with typical phenotypes not being collected. As such, knowing the colour proportions within a population has implications for understanding the potential impact of selective harvesting on the entire population.

That no pseudohermaphroditism was found indicates one of three possibilities:

1. The presence of TBT (tributyltin) and its derivative compounds are not present at concentrations high enough to affect the population. TBT is a marine pollutant commonly known to cause pseudohermaphroditism, and may reflect the distance to sources of TBT (Vlasconcelos et al., 2006; Amiard & Amiard-Triquet, 2015).
2. The sample size was too small to observe this rare characteristic (Reed, 1993b).
3. This phenotype is simply not naturally present in this population.

We suggest that further studies are required, such as testing the water quality of this region and surveying other *Canarium labiatum* populations to establish the reason for the lack of pseudohermaphroditism.

Conclusion

In the current study, female *Canarium labiatum* were larger than males and more abundant. The colour and pattern variability of most of the population was typical, with greenish maculations, with only a limited number (11.1%) of atypically coloured phenotypes observed. We found no evidence for pseudohermaphroditism in females. *Canarium labiatum* offers researchers an opportunity to explore with ease many of the life history traits of Strombidae due to its accessible intertidal habitat and abundance. This study fills a knowledge gap in overlooked members of the Strombidae and an opportunity to establish the extent of sexual axial-length size dimorphism and sex bias within the wider clade, as well as the potential for these population traits to have an impact on size evolution within the Strombidae.

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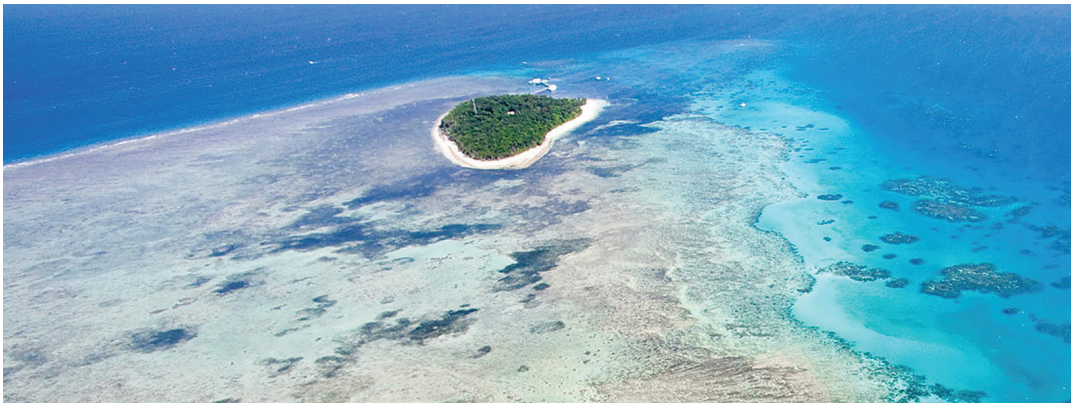
Green Island is a small (12 ha) coral cay formed on the sheltered side of a platform reef. It became part of the Great Barrier Reef World Heritage Area in 1981 (Source: https://greatbarrierreef.org/islands/green-island/).

The island and reef are part of the traditional sea country of the Guru-Gulu Gungandji Aboriginal people, who know the island as *Wunyami*. Today the Guru-Gulu Gungandji people retain a strong spiritual connection with their country and are active in managing the island.



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