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Sex-ratio bias in *Laevistrombus canarium* Linné, 1758 (Gastropoda: Strombidae) from Far North Queensland, Australia

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ABSTRACT

In general, sex-ratios in herbivorous gastropods show a strong female bias, particularly in the family Strombidae of the Indo-Pacific. Previous studies of Indo-Pacific strombs point to an underlying female bias in most species. However, the full spatial extent and potential mechanisms driving this phenomenon are currently unknown. Therefore, we studied two populations of *Laevistrombus canarium* Linné, 1758 over a four year period to explore sex-ratio bias and to investigate the underlying mechanisms. Furthermore, the current study investigated methods to test for sex-ratio bias in strombs where samples are limited and sampling is problematic We sampled individuals from two reefs in Far North Queensland, Australia, with different benthic compositions, despite their close proximity (approximately 4 km apart). In agreement with previous studies, we also found a strong bias towards females. This bias remained consistent across both locations and changes in benthic substrate. Our findings are consistent with models suggesting inherent genetic factors regulate natural patterns of sex imbalance in these strombs. These findings also suggest that there are as yet unidentified stromb life-history characteristics associated with the origin and maintenance of this phenomenon. ❒ *Life history, Phylogenetic constraints, Sampling effort, Sex imbalance*

A significant sex imbalance favouring females occurs in many gastropod populations (Abbott 1949; Griffith & Castagna 1962; Wiedemeyer 1998; Mutlu 2004). This is particularly common in the Strombidae, where a consistent ratio of at least three males to four females has been observed in many taxa (Abbott 1949; Wiedemeyer 1998; Mutlu 2004; Cob *et al*. 2009). Despite this phenomenon being regularly documented, the mechanisms underlying it are currently unknown.

Sex imbalance in some populations of gastropods is highly spatially or temporally variable. For example, in populations of *Strombus pugilis* Linné, 1758, although monthly counts have shown a significant bias towards females throughout most of the year, anomalous counts have also occurred where males were more numerous than females (Cardenas *et al*. 2005). Historically, this variability has been attributed to low population densities combined with the natural clustering behaviour of these taxa. This is because sparsely distributed individuals that randomly encounter each other preferentially form small clusters in which sex-ratios can be artificially skewed as a sampling artefact (Catterall and Poiner 1983). Previous studies in which sex-ratio biases have been observed may also be subject to this sampling phenomenon, as these studies have often only sampled small numbers of individuals from a single cluster because of either sampling constraints, or because sex-ratios have not been the focus of the original data collection.

Sex-ratios in some stromb taxa may also be impacted by anthropogenic processes (Randall 1964). For example, populations of the large Caribbean *Lobatus* Swainson, 1837 tend to have a more balanced sex-ratio than those observed in Indo-Pacific strombs (Abbott 1949; Griffith & Castagna 1962; Wiedemeyer 1998; Stoner & Ray-Culp 2000; Mutlu 2004). It is suggested that fishing could be affecting the natural sex balance within these populations, because commercial fisherman in the Caribbean are known to select for large female *Lobatus gigas* Linné, 1758, leading to a male biased sex-ratio through overharvesting of females (Randall 1964). However, the full impact of fishing is questionable, as unfished populations of *L. gigas* are also known to show no sex-ratio bias (Randall 1964; Stoner & Ray-Culp 2000).

Given these disparate findings it is important that appropriately robust sampling be conducted across both space and time to fully understand sex-ratio variability within Strombidae. There has been one study specifically into stromb sex-ratio on *Laevistrombus turturella* Röding, 1798 (incorrectly identified as *L. carnarium* Linné, 1758) where a long-term sex bias towards females was found with an average of 1/1.88 ± 0.16 males to females (Cob *et al.* 2009). However, it is unclear from the literature whether other previous surveys publishing sex-ratio data have been undertaken using multiple samples or robust sampling procedures, making the drawing of conclusions on the generality of this phenomenon problematic.

Stromb mating aggregations occur seasonally and are consistently larger than clusters observed at other times of year (pers. obs.). Therefore, they are one type of aggregation that may be less susceptible to the problem of sampling bias, assuming that there is no inherent sex-ratio bias in these aggregations due to mating-system characteristics. Thus, sampling mating clusters offers a potential cost and time effective method to establish the actual sex-ratio for a given limited sized populations at a specific location, or when only limited sampling is possible.

The current pilot study investigated methods to test the sex-ratio bias in strombs where samples are limited and sampling is problematic. In particular populations of the Indo-Pacific stromb *Laevistrombus canarium* were studied over three years at two different sampling locations with different benthic substrates. Both populations were not subject to fishing or commercial collecting. We aimed to establish the level and pattern of sampling required to accurately determine sex-ratios in these two *L. canarium* populations and to determine whether a consistent sexual imbalance of approximately three males to four females occurred. We also aimed to determine whether sampling large mating aggregations at a single point in time would accurately reflect population sex-ratios obtained from longer-term sampling of smaller non-breeding clusters over multiple years, thus providing a cost- and time-effective method of studying this phenomenon in the future.

MATERIALS AND METHODS

Field sampling involved nine collecting trips at low tide to two coastal reefs adjacent to Port Douglas, Queensland, Australia between June 2013 and May 2016. Collecting in coastal northern Queensland is problematic for three reasons: 1) the prevalence of estuarine crocodiles, 2) the inability to dredge for samples due to substrate, and 3) the limited number of tides that expose and enable access to the colonies.

We conducted six collecting trips to Alexandra Reef (16.53 S, 145.49 E) and three to Four Mile Beach (16.51 S, 145.47 E). Two non-mating cluster samples were obtained from Four Mile Beach and three from Alexandra Reef over the full study period (total n = 5). One mating cluster sample was obtained from each location in June/July 2013 (total n = 2) and an additional two mating cluster samples were obtained from Alexandra Reef over the full study period. These two localities have different benthic compositions despite their close proximity (approximately 4 km apart). It is unlikely that adults regularly move between the two sampling locations due to unfavourable intervening habitat and living specimens are rarely observed in the interspace between the two populations (pers. obs.). However, due to a lack of data on patterns of larval dispersal, it is possible that juveniles could recruit to both locations from a single larval source population (Delgado *et al.* 2006; Paris *et al*. 2006; Aldana-Aranda & Perez-Perez 2007).

On all sampling occasions, once an individual *L. canarium* was located, the nature of the benthic substrate (e.g. sandy vs. silty) was noted and one metre wide transects were walked from the high-tide mark to the waters-edge covering the extent of the area exposed by the low tide. Adult strombs were sexed on site by the absence, or presence, of a verge seen as the animal righted post-release (Abbott 1960; Cob *et al.* 2008). Mating and non-mating clusters were identified by the presence or absence of copulation activity.

The first analysis used each non-mating cluster sample at each time/site as a replicate and combined the data from both sites. This pooling was necessary to obtain a sufficiently robust sample of non-breeding clusters over the three year period to accurately establish a measure of the background sex-ratio in the Port Douglas region. The median sex-ratio obtained from this pooled data set (n = 5) was then compared to that expected in a sex balanced population (1 male : 1 female) using a Wilcoxon One-sample Signed rank test.

Next, an independent samples Kolmogorov-Smirnov test was used to test for differences between the median sex-ratios obtained using the larger mating cluster samples as replicates, as compared to the smaller non-mating cluster samples.

Data from the Alexandra Reef site, which had the largest number of non-mating cluster samples, was then used to test if the numbers of males and females observed in individual mating clusters differed significantly from the ratio obtained by combining all non-mating cluster samples at this same location using a chi-squared analysis.

RESULTS

The sex-ratio in these two populations of strombs was variable in both space and time (Table 1). However, analysis of the non-mating clusters pooled across sites showed a consistent imbalance in sex-ratio in favour of females (1 : 1.8), which differed significantly from an expected balanced (1:1) sex-ratio ($W_{5,1} = -2.060$, $p < 0.039$,

TABLE 1. Sex-ratios of two populations of *Laevistrombus canarium* (n = 9) from North Queensland, Australia *Designates a mating cluster

Locality	Date	Sex		Sex Ratio (M:F)	Substrate
		Male (n)	Female (n)		
Four Mile Beach*	07/2013	19	22	0.86	Rubble sandy mud
Four Mile Beach	09/2013	3	5	0.6	Rubble sandy mud
Four Mile Beach	07/2014	3	6	0.5	Sandy mud
Alexandra Reef*	06/2013	8	17	0.47	Rubble and sand
Alexandra Reef	09/2013	6	12	0.5	Rubble and sand
Alexandra Reef*	07/2014	15	31	0.48	Weed and sand
Alexandra Reef	07/2014	8	11	0.73	Weed and sand
Alexandra Reef*	08/2015	15	38	0.39	Sand
Alexandra Reef	05/2016	14	28	0.5	Rubble, weed & sand

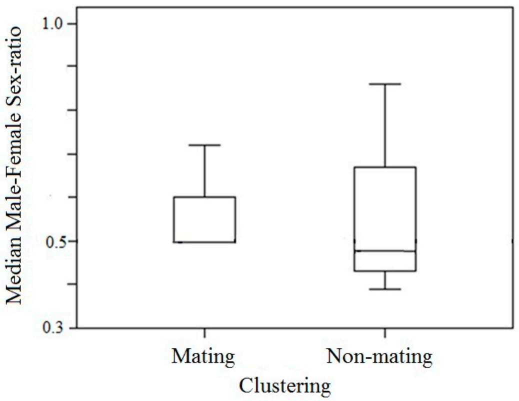

FIG. 1. Box plot of the median ratio of males to females for the populations of *Laevistrombus canarium* showing the mating and non-mating cluster samples from Alexandra Reef, North Queensland, Australia.

figure 1). The median sex-ratio derived from the mating cluster samples also showed an imbalance favouring females (1 : 1.9) that did not differ significantly from the ratio obtained using non-mating clusters ($Ks_{4,5}$= 0.225, p = 0.164).

At Alexandra Reef no statistical difference was observed in the proportions of males and females observed in each individual mating cluster sample and the overall proportions of each sex observed when all of the non-mating cluster samples at this site are combined ($\chi2 = 8$, df = 6, p = 0.2381). This suggests that each of the larger individual mating cluster samples provides a relatively accurate estimate of the overall female sex bias within the population, and that this sex bias has remained relatively consistent at ~ 0.5 at this site over the three-year sampling period.

DISCUSSION

This study confirmed the presence of a significant sex imbalance favouring females in *Laevistrombus canarium* populations from the Far North Queensland coast, and the existence of a significant sex-bias towards females across the *Laevistrombus* complex (Cob *et al*. 2009). Importantly, the results demonstrate that the limited number of individuals that can be obtained during any single non-mating cluster sample are able to test hypotheses concerning the existence of a sex-ratio bias but are insufficient to quantify that bias. This has implications for planning further studies given the logistical difficulties and time constraints involved in obtaining sufficient non-mating cluster samples. Alternatively, our results demonstrate that a single sample of a large mating cluster provides data representative of the sex-ratios observed from non-mating samples pooled over longer time periods and across different sampling locations. Consequently, mating cluster samples can be used as a time- and cost-effective method of determining actual population sex ratios over much shorter time periods than would otherwise be required.

Based on mating cluster samples, the sex-ratio bias in *Laevistrombus canarium* populations was consistent across both space and time. This suggests that the general phenomenon of sex-ratio bias we observed is not simply a sampling artefact associated with sampling small clusters of individuals at single points in time. In addition, the sexual imbalance in *L. canarium* populations remained similar at each location despite different benthic substrates, suggesting that substrate conditions are unlikely to be significantly affecting sex-ratios.

One explanation for the observed sex biases found in this study could be that there is differential survival of each sex during the larval and/or immediate post-settlement period. Stromb mortality is highest during the larval dispersal stage and the period soon after settlement, with predation at this time being capable of eliminating entire breeding cohorts (Stoner *et al*. 1998). However, despite documented high mortality during these early developmental periods, evidence for differential sex-specific survival that could account for a consistent sex imbalance is lacking (Cob *et al.* 2009, 2009a). Further, because this phenomenon has been little studied, there is no evidence to support sex-ratio imbalance as a consequence of non-random recruitment of either sex to specific locations.

The consistent female sex-bias found in *L. canarium* populations from Far Northern Queensland is most likely a consequence of genetic regulation of this phenomenon both in *L. canarium* and throughout the Strombidae, as proposed by Yusa (2007). There is at least two alternatives to the XY sex-determination mechanisms found in the Caeneogastropoda, ZW-ZZ and XX-XY. Given that matings between WX females and XY males produces only one quarter male offspring, this may account for the sex-imbalance (Thiriot-Quievreux 2003, Bull in Yusa 2007). However, the sex-chromosomal arrangement found in strombids is unknown, and the presence of such constraints in this species is still conjecture, based primarily on a lack of support for alternative hypotheses.

Despite the preliminary nature of our study and limited samples sizes, our results confirm that the sexual imbalance observed in different locations reflects real sex-ratio biases found in larger more accessible populations (Cob *et al*. 2009). Therefore, investigating the evolutionary processes responsible for the origin and maintenance of such a trait, provide an exciting avenue for future research.

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