Growth and reproductive of *Babylonia areolata* Link 1807 in Songkhla and Pattani, the lower part of Gulf of Thailand

Rujinard Sriwoon¹, ²* and Naruporn Poonsud¹

¹ Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

² Plankton Research Unit, The Centre for Biodiversity of Peninsular Thailand, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

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Abstract

The ivory shell, *Babylonia areolata*, was sampled from commercially harvested resource areas in the lower part of the Gulf of Thailand: Rusamilae (RSM) and Songkhla (SKL). This study aimed to survey the presence of imposex and compare the size, frequency distribution, sex ratio, and gonadal stages of ivory shell. Shell length frequency distributions of *B. areolata* caught in RSM from October to December 2016 (n=135) ranged from 32.04 to 58.32 mm, and in SKL from October 2016 to January 2017 (n=149) ranged from 37.8 to 61.95 mm. The largest ivory shell that measured 61.95 mm in shell length (SL) was recorded at SKL. The mean SL was larger at the RSM site (50.31 mm) than at the SKL site (44.67 mm). Both of the SKL and RSM populations showed a bimodal length-frequency distribution with the smaller peak at a shell length less than the minimal landing size (MLS) at the RSM site and a larger peak at a shell length greater than the MLS. The males and females from RSM reached maturity at smaller sizes (44 mm and 36.49 mm, respectively), whereas the females at SKL reached maturity at 51.00 mm. In order to preserve the local fishery and to characterize the Babylon stock in the area, the reproductive biology of the species was studied in relation to sex and commercial size (45 to 61 mm). Five reproductive stages were defined for females on the basis of histological analysis. The shell lengths with mature gonads were 44 mm and 36.49 mm for males and females at RSM and 51 mm for females at SKL which exceeded the minimal landing size (45 mm). The sex ratio was significantly in favor of females; therefore, female mortality may not be directly attributable to imposex. Imposex accounted for 2.78% at RSM and 5.94% at SKL. This is the first report of imposex in *B. areolata* at SKL. Females from SKL showed mature gonads at a size of about 1.5 times larger (51.00 mm) than females from RSM (36.49 mm). This finding is especially important for a sustainable management of this fishery, taking into account that there are differences in size at maturity between populations.

Keywords: *Babylonia areolata*, imposex female, reproductive, Gulf of Thailand

1. Introduction

The ivory shell *Babylonia areolata* (Neogastropod: Buccinicace) inhabits the sandy or muddy bottom of shallow waters from the upper to the lower part of the Gulf of Thailand. *B. areolata* is one of the most extensively cultured marine mollusks in the Southeast Asian countries, and it is the second most economically important marine gastropod for human consumption in Thailand (Chaitanawisuti, Kritsanapuntu, & Natsukari, 2002a; Kritsanapuntu, Chaitanawisuti, & Natsukari, 2009). The commercial aquaculture of the spotted Babylon in Thailand has been developed due to the growing demand and expanding domestic and export markets. A large variability in spawning event, hatchability, and larval and juvenile survival rates of the spotted Babylon has been observed. A low survival rate of 0.34–9.82% was reported in the commercial culture of this species during the crawling stage (Chaitanawisuti *et al.*, 2002a; Chaitanawisuti & Krisa-
napuntu, 1998, Chaitanawisuti & Krisanapuntu, 1999; Chaitanawisuti, Krisanapuntu, Kathinmai, & Natsukari, 2001; Chaitanawisuti, Krisanapuntu, & Natsukari, 2002b; Poontong & Ntongmeesub, 1996). The juvenile behavior at a shell length less than 5 mm will crawl up the wall of a tank and cannot move back to the water. Therefore, a large number of juvenile snails die along the edge of a nursing tank (Chaitanawisuti, Krisanapuntu, & Natsukari, 2004). A higher survival rate was reported of *B. areolata* from the veliger larvae stage to 60-day-old early juveniles in tanks with a fine sand substrate and nylon net attached to the edge of the tanks to prevent them from leaving the tanks than tanks without a sand substrate (Sutthinon, Taparudee, & Yashiro, 2007). Because of the increase in commercial requirements and the decline in the natural stock, much attention has been paid to developing an appropriate aquaculture system for this species (Chaitanawisuti et al., 2004). So far, the primary studies have focused on breeding technology (Krisanapuntu, Chaitanawisuti & Natsukari, 2007), nutrition (Sangsawangchote, Chaitanawisuti, & Piyarattivitvorakul, 2010; Xue, Ke, Wang, Wei, & Xu, 2010; Zhang, Zhou, & Cheng, 2009), taxonomy (Gittenberger & Goud, 2003), toxicology (Tanhan, Sretarugs, Pokethiti-yook, Krutarchue, & Upathan, 2005; Supanopas, Sretarugs, Sa, Krutarchue, Pokethitiyook, & Upathan, 2005), ecology (Xue et al., 2010), phylogenetics (Huualkasin, Tongchuaia, Chotigeat, & Phongdara, 2008), and genetic diversity and structure (Chen, Luo, Wang, & Ke, 2010; Chiu, Kuo, Lin, Huang, & Wu, 2015).

The spotted Babylon *B. areolata* is a gonochoric with sexual dimorphism based on the presence of a penis in males and distinctive sexual glands in both sexes. The courses of the reproductive cycle and the duration of mating are highly variable according to location. The reproductive cycle of *B. areolata* in the upper and lower parts of the Gulf of Thailand is somewhat similar with a spawning peak in April and August at the upper side of the Gulf and April and June at the lower side (Suwanjarat, Muengo, & Thonsrboon, 2007). In males, the percentages of maturation and spawning decrease from July to December. The gonad index (GI) of the females is greater than the males in both populations. The breeding season of *B. areolata* occurs from January to May (in the upper part) and to June (in the lower part) which is related to the higher water temperature (Wu, Chen, Chen, Zhang, & Guo, 2005; Xu et al., 2010). The sex ratio in the upper part was 0.62:1 (male:female) with significantly more females, whereas the lower area had a sex ratio close to 1:1. Reproductive failure may occur in females with severe imposex which results in a decline of the population or even mass extinction (Gibbs & Bryan, 1996). This species is considered to be a bioindicator of trIBUTyltin (TBT) contamination in Thai waters because the females may be imposex-affected, i.e. characteristics of males are superimposed onto the females (Swennen, Sampantarak, & Ruttanadakul, 2009). The frequency of imposex occurrence in the Gulf of Thailand has increased significantly from 1996 to 2006 with the highest occurrence in the eastern part of Bay of Bangkok followed by Pattani Province. ImposEx in neogastropods is used to determine the relative TBT distribution and has been reported in the families of Muricidae, Nassariidae, Melongenidae, Turridae, and Buccinidae in the Gulf of Thailand. In the family of Buccinidae, TBT contamination has affected *B. areolata* (Link, 1807), *Nassaria pusilla* (Roding, 1798), and *Phos senticosus* (Linnaeus, 1758; Swennen et al., 2009). ImposEx females demonstrated oocyte degeneration indicated by the presence of numerous lipid droplets in their ovarian tissues. The appearance of cortical granules in the ooplasm and microvilli on the vitelline envelope seems to be the unique feature of *B. areolata* that differs from other gastropods in the Buccinidae (Muengo, Suwanjarat, & Klepal, 2011).

The present study investigated the gonadal stages of *B. areolata* in the Rusamilae and Songkla populations to evaluate them in relation to the population characteristics (presence/absence of imposex) and environmental conditions. We also compared these reproductive traits (size frequency distributions, sex ratio, gonadal stages) between the two populations. The results provide more information about the life history of this species.

### 2. Materials and Methods

#### 2.1 Sample collection

Babylon samples were collected from local fishermen from October to December 2016 in Songkla Province and from October to November 2016 and again in January 2017 in Rusamilae in Pattani Province (Figure 1). Every month, about 50 specimens were randomly collected with the exception of December 2016 which was affected by the monsoon season on the Gulf of Thailand. Therefore, in December 2016, 34 samples were collected at Songkla while the collection of samples at Rusamilae was postponed to January 2017.

#### 2.2 Laboratory techniques

Based on the first examination, the individuals were measured for the total shell length (SL) and shell width (SW) with Vernier calipers (±0.01 mm). The shell was mechanically broken and the whole body of each individual was removed to confirm its gender. Due to the occurrence of imposex in one of the samples from Pattani (Swennen et al., 2009), the sex was determined based on the presence of the albumen gland in females as well as by the absence and presence of the penis in males. The percentages of imposex-affected females were calculated as the number of females with a penis or vas deferens or both with respect to all female samples of each population. The sex of all individuals was later confirmed by histological examination of their gonads. The individual total weight was also recorded. The flesh of each specimen was carefully separated from the shell and weighed immediately. The entire gonad was fixed in Bouin’s fluid for 24 h and subsequently stored in 70% ethanol for histological analysis. The tissues were dehydrated using an ascending series of ethanol concentrations and then embedded in paraffin. Sections were cut at 6 μm with a microtome, stained with hematoxylin-eosin (Bancroft & Gamble, 2002), and observed under a light microscope. Sections were classified according to gonadal development stage. Gonadal development was classified into five stages: Undifferentiated, Proliferation and Growth I, Growth II,Evacuation, and Post-evacuation. Data obtained from the histological analysis are expressed as percentage of individuals in each stage of gonadal development per collection and are presented as frequency distribution histograms for each month.
3. Results and Discussion

3.1 Body size and sex determination

A total of 284 individuals from the two populations were histologically analyzed. The mean±SD shell lengths were 50.31±5.63 mm (range 32.04–58.32 mm) for the Rusamilae samples (n=135) and 44.67±2.74 mm (range 37.8–61.95 mm) for the Songkhla samples (n=149). The mean±SD shell widths were 29.70±3.4 (range 20.88–35.06 mm) for the Rusamilae samples (n=135) and 28.02±2.74 mm (range 20.69–36.66 mm) for the Songkhla samples (n=149).

Length frequency distribution was displayed for three months: October 2016 (n=51), November 2016 (n=50), and December 2016 (n=34) at Songkhla (Figure 2 a-c), and October 2016 (n=50), November 2016 (n=50), and January 2017 (n=49) at Rusamilae (Figure 2 d-f). During the study period, the areas showed a length-frequency distribution from 33 to 63 mm.

The 135 samples collected from October to December 2016 in Songkhla showed a length-weight relationship from 39 to 63 mm in length (Figure 3a) and a weight range from 2.95 to 16.59 g (total weight) (Figure 3b). On the other hand, the 149 samples collected at Rusamilae from October to November 2016 and in January 2017 showed a length-weight relationship from 33 to 59 mm length (Figure 3c) that ranged from 2.02 to 16.75 g (total weight) (Figure 3d).

The sex of each individual was confirmed by histological observation of the gonads. In Rusamilae, 72.30% (n=108) were females and 27.7% (n=41) were males. In Songkhla, 75.37% (n=101) were females and 24.63% (n=33) were males. The incidence of imposex was calculated as the proportion of imposex females among the total number of females in a sample. Out of the females analyzed, 2.78 and 5.94% were imposex at Rusamilae and Songkhla, respectively (Figure 4).

The mean shell lengths of the male and female B. areolata were 47.32±4.46 mm and 43.66±5.90 mm, respectively, for Rusamilae and 48.11±5.50 mm and 51.18±5.47 mm, respectively, for Songkhla. The proportions of males were 0.44 for Rusamilae and 0.36 for Songkhla, and the sex ratio was significantly in favor of females (X²=29.43; X²=34.51, P<0.05). Therefore, imposex may not be directly attributed to female mortality. The Rusamilae sample collected in January 2017 (n=49) showed that the sex ratio was close to unity (X²=0.51, P>0.05) and no imposex individuals were found. Similarly, the Songkhla samples collected in November 2016 (n=50) showed a sex ratio of 1:1 and no incidence of imposex.

3.2 Gonadal stages

Based on histological observations, gonad sections were classified into five stages: Undifferentiated, Proliferation and Growth I, Growth II, Evacuation, and Post-evacuation.
Figure 2. Shell length frequency distribution of *Babylonia areolata* in Songkhla: a: October 2016 (n=51); b: November 2016 (n=50); c: December 2016 (n=34) and in Rusamilae; d: October 2016 (n=50); e: November (n=50); January 2017 (n=49). Size classes of 2 mm from 33 to 63 mm were considered.

Figure 3. Shell length frequency distribution of male and female ivory shell: a: Songkhla sampled from October to December 2016 (Male = 38, Female = 97); c: Rusamilae sampled in October and November of 2016, and January 2017 (Male = 41, Female = 108). Relationship between shell length and growth for *Babylonia areolata* at Songkhla and at Rusamilae; b: length-weight at Songkhla; d: length-weight at Rusamilae. Length of sampled animals ranged from 33 to 63 mm and body weight ranged from 2.02 to 16.75 g.
Undifferentiated individuals were characterized by the lack of differentiated gonadal tissue. During Proliferation and Growth I (Figure 4a and b), a few separate follicles embedded in connective tissue were observed. The gonadal tissue was composed of oogonia and primary oocyte, but no vitellogenic oocytes were present. At this stage, the follicular lumen was small and oogonia or oocytes showed a well-developed nucleus (Figure 4d). At the Evacuation stage (Figure 4e), free oocytes in the lumen and large oocytes were found still attached to the wall. The lumen of some follicles became emptier and some pre-vitellogenic oocytes were observed near the follicle wall. In males with gonads in the Evacuation stage, spermatooza were in the lumen and a few spermatids were still on the wall (Figure 4f). At the Post-evacuation stage (Figure 4g), the follicles lost their typical shape. Varied between large empty follicles to empty small follicles, they dispersed throughout the network of connective tissue. The gonadal tissue was composed of a thin layer of spermatogonia and spermatooza (Figure 4h).

The five gonadal stages were recognized in the female individuals from the Songkhla and Rusamilae populations. However, the size at which each gonadal stage occurred was different between populations (F=3.03, P<0.05) (Figure 5). The shell lengths of the female individuals with undifferentiated gonads ranged from 44 to 54.50 mm (mean=48.51±4.09) and 32.24 to 44.37 mm (mean=37.72±4.12) for the Songkhla and Rusamilae populations, respectively. The minimum sizes of mature females (at Growth II, Evacuation, and Post-evacuation) were 51.00 and 36.49 mm for the Songkhla and Rusamilae populations, respectively. Once the females reached maturity, their gonads presented with different stages of development independent of their body size (F=0.74, P>0.05). Imposex-affected females from the Songkhla and Rusamilae populations had only Proliferation and Growth I stage found in the non-affected females. Also, imposex-affected females did not show any difference in their development in comparison to the non-affected females.

Four gonadal stages (Proliferation and Growth I, Growth II, Evacuation, and Post-evacuation) were recognized in the male individuals from Songkhla and Rusamilae populations. However, the size at which each gonadal stage oc-
curred was different between populations (Figure 6). The mean±SD shell lengths of the male individuals with undifferentiated gonads were 51.62±1.31 mm (range 52.55–56.47 mm) at Songkla and 47.02±4.31 mm (range 37.30–57 mm) at Rusamilae. The minimum size of mature males (at Growth II, Evacuation, and Post-evacuation) was 44 mm in the Rusamilae population.

Both mature and immature gonads (in Proliferation and Growth I) were present in all of the three months in the Rusamilae population but only in October in the Songkla population. Evacuation of oocytes occurred mainly in October in the Rusamilae and Songkla populations. In females, Post-evacuation was found during October and November (except December 2016 in the Songkla population and January 2017 in the Rusamilae population), with the presence of reabsorbed oocytes and other gametogenic remains.

The determination of sexual stages is currently based on histological observation for an accurate qualitative analysis of gametogenesis. A comparison between the historical and phenotypic sexes (presence or absence of a penis) can identify the cases of imposex which frequently describes this species. During our three-month study in Songkla (n=135) and Rusamilae (n=149), cases of imposex were observed. We investigated the case of imposex in the lower Gulf of Thailand and morphological alterations such as pseudopenis which are evident in females affected by imposex. However, oviduct blockage or occlusion of vulva by vas deferens formation was not observed. Similar conditions were described in *B. japonica* from Japan (Horiguchi et al., 2006) in which no blockage of the oviduct was observed in imposex-affected females. However, this state differed from *B. areolata* in Thailand (Muenpo et al., 2011) and *B. spirata* in Pakistan (Afzar, Siddiqui, & Ayub, 2012) in which oviduct blockage and occlusion of the vulva were found in specimens showing advanced stages of imposex. Muenpo et al. (2011) reported vas deferens sequence stages 1s, 1c, 2a, 2b, 2c, 3a, 3b, 3c, 4b, and 5b were found in *B. areolata*. No male tissue had developed in any imposex-affected females at stage 5 and the ovarian spermatogenesis in *B. areolata* might occur at higher environmental TBT concentrations and duration of exposure. In this study, oogenesis in the imposex female *B. areolata* was similar to that of the normal female.

TBT is an anti-fouling agent in paints applied to boats, lumber preservatives, and slimes in cooling systems. The deleterious effects, especially imposex, of TBT released by anti-fouling paints, were reported on marine animals. Imposex is a phenomenon whereby male sex characteristics are induced which leads to sterility in the female. Several studies reported that contamination from TBT in the seawater at a very low concentration of 1–2 ng/L can pose serious effects on a wide number of non-targeted marine animals, such as imposex in gastropods, shell-thickening, and spot failure in oysters, and other known toxic effects induced by this toxic pollutant at trace concentrations. Even a low concentration of TBT induces the occurrence of pseudopenis (imposex) in gastropods. At a high degree of imposex, TBT commonly causes the imposex females to have reproductive failure (Barroso, Moreira, & Bebianno, 2002; Axial, Micallef, Muscat, Vella, & Mintoff, 2003) that can finally lead to a population decline in some gastropod species (Barroso & Moreira, 2002; Horiguchi et al., 2006). Local extinction caused by TBT that induced female sterilization was also previously reported in several regions (Barroso & Moreira, 2002). The intensity of imposex or the degree of imposex correlates to the proximity of harbors and marinas where high TBT concentrations have been detected (Blackmore, 2000; Leung et al., 2006; Pellizzato, Centanni, Marin, Moschino, & Pavoni, 2004; Ramon & Amor, 2001; Swennen et al., 1997). The degree of imposex also increases consistently with the tissue concentrations of TBT (Leung et al., 2006; Pellizzato et al., 2004). The concentration of TBT in the sediment of the estuary of the Pattani river mouth was found to be 1.630 ng/g dry weight in 1996 (Kan-Atireklap, Tanabe, Sanguansin, Tabacanon, & Hunsprugs, 1997b), 593 ng/g dry weight in 2017 (Hajisamoh, Siddiique, & Shakya, 2018). TBT contaminated mud from the shipping lane increased the imposex incidence in Rusamilae from no imposex in 1996 (Swennen et al., 1997) to 1.3% in 2006 (Swennen et al., 2009) to 2.78% in 2016 (this study). The concentration of TBT in the sediment of the estuary of Songkla Lake was found to be 36 ng/g dry weight in 1995 (Kan-Atireklap et al., 1997b), 90 ng/g dry weight in 2012 (Hajisamoh, 2013), and 70–139 ng/g dry weight in 2016 (Yaeda, Suksaraj, Vitayavirasuk, & Ophitakhorn, 2017). TBT contaminated mud from the shipping lane increased the imposex incidence in Songkla from no imposex in 2006 (Swennen et al., 2009) to 5.94% in 2016 (this study). None of TBT concentrations exceeded the critical level of 2.5 μg/g dry weight which is the level that can pose a threat to the growing rate of living organisms (Hajisamoh et al., 2018). It can be concluded from the present study that a low concentration of TBT induces pseudopenis (imposex) in *B. areolata*. In addition, regulation for the safe disposal of contaminated sediments and of anti-fouling paints after cleaning ships is urgently needed in Thailand.

The population of *B. areolata* studied showed no differences in their gonadal stages despite the differences in their demographic and life-history traits. The results of this
work confirmed that the gonadal development is directly related to the body size of each population, i.e. immature stages are among the larger sizes in the Songkhla population than in the Rusamiable population. Despite our sampling being restricted to market-sized animals (over 45 mm), the percentage of mature animals clearly depends on animal size. Males appeared smaller than females (48.1±±5.50 mm vs. 51.18±±4.77 mm) in the Songkhla samples, but males were larger than females (47.32±±4.66 mm vs. 43.66±±0.90 mm) in the Rusamiable samples. The females from Songkhla showed mature gonads that were about 1.5 times larger (51.00 mm) than the females from Rusamiable (36.49 mm). Due to increased exploitation of B. areolata in the Gulf of Thailand, fishing yields have decreased severely from the year 2004 which led to the relevance of fishing restrictions to be reconsidered. Subsequently, even though the minimal landing size was set at 45 mm, several more restrictive measures were established. However, it has become obvious that the management of ivory shell fishing needs to consider not only the size distribution data but also gonadal maturation. This fishing activity and size-specific gonadal maturation in females is in accordance with Buccinum undatum caught in west Cotentin in 2005. The shell length common whelk that corresponded to 50% of animals having mature gonads (49 mm and 52 mm for male and female, respectively) exceeded the minimal landing size (45 mm). Females of B. undatum under 49 mm in shell length were sexually mature and produced only a few eggs (Heude-berthelin, Hegron-Mace, & Legrand, 2011). Considering that recruitment largely depended on maturity and fecundity of the females, this difference between the males and females should be taken into account in the improvement of protective measures in fishing activity. This finding is especially important for sustainable management of this fishery, taking into account that there are differences in size at maturity between populations.

Volutidae are a good example of the biological characteristics of ivory shell, such as growth and reproduction, are highly relevant and useful for management of fisheries. As a result of the increased harvesting of B. areolata in the lower Gulf of Thailand, fishing yields have decreased severely. Fishing restrictions need to be considered. Subsequently, more restrictive measures were established, for example, the minimal landing sizes of 33 mm, and limiting the number of fishing days and fishing licenses. For the same reason, the size distribution parameters should be considered with caution because the mode of fishing is based on environmental factors, animal motility, and food availability. These data confirm the necessity of strict management of the Babylon stock in highly active fishing areas such as the lower Gulf of Thailand. Finally, water temperature is known to impact the physiology of this species. Therefore, investigations may also be of great importance in the context of climatic change.

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January to May (in upper part) or June (in lower part). In this study, the females from the Songkhla population started their reproductive activity earlier than the Rusamiable population. Regarding differences in seasonality, temperature may trigger reproduction.


