Imposex study on *Thais aculeata* (Gastropoda: Muricidae) in Ambon Bay, Eastern Indonesia

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Abstract. Study on imposex of *Thais aculeata* was conducted in September to October 2016 in Talake Shipyard, Ambon Bay. The aim of this study was to investigate and describe the imposex insidence in muricids *T. aculeata*. Level of imposex was calculated using some indices i.e. RPLI (Relative Penis Length Index), RPSI (Relative Penis Size Index) and PI (Percentage of Imposex). A total of 110 individuals *T. aculeata* consisted of eight size classes were found, which 39 females individuals were identified to be exposed of imposex. Females with imposex were characterized by the presence of pseudopenis in the form of a small bulge on the top right of the tentacles. Pseudopenis relatively smaller than that of male penis size i.e. 2.13 mm while male penis size was 4.95 mm. Percentage of imposex of *T. aculeata* was 41.05%, RPSI was 7.97; RPSI was 49.58, and sex ratio of male and female individuals was 1:6.3 The correlation between class size shell with imposex was moderate relationship (R = 0.44). Ratio between number of male individuals and female with imposex was significantly different, but ratio between normal females and females with imposex; and shell length average between normal females and females with imposex was not significantly different, respectively.

Key words: muricid, *Thais aculeata*, imposex, Talake Shipyard, Ambon Bay

1. Introduction

The term "imposex" was first proposed by Smith (1971) [1] to explain an imposition or existence of male sexual characteristics in the development of female gastropods e.g. penis and vas deferens which are functionally infertile and morphologically change. The first observations were made on gastropods *Nassarius obsoletus*. Occurrence of imposex mainly related to TBT (tributyltin) pollution in the waters where the snails existed there.

TBT was commonly used in shipping activities at ports or docks as antifouling agents on ship body's paints. TBT can prevent or retard the damagae of the vessel, but the effects of TBT use are very dangerous to the water conditions, especially to the biota including the non-targeted biota in the waters, for example is molluscs [2]. Besides imposex, TBT also caused an abnormality of molluscs shell thickening process [3]. Several other studies also reinforced the argument that TBT was one of the causes of the occurrence of imposex on molluscs [4, 5, 6].

Studies on imposex due to TBT contamination have been conducted in several regions of the world [7, 8, 9, 10, 11, 12, 13]. In Southeast Asian, the effects of TBT have been documented in many countries including Indonesia, Malaysia, Singapore, Philippines and Thailand, in many species of muricid gastropods, *Thais luteostoma, T. bitubercularis, T. clavigera, T. rufotincta, T. distinguenda, T. gradate and T. jubilaea, Morula musiva, Cronia margariticola, Drupella rugosa, Morula musiva, M. granulata, M. margariticola, Chicoreus capucinus* and *Naquetia capucina* [14, 15, 16, 17, 18, 19, 20, 21]. Most observations were performed on gastropods than bivalves because gastropods especially Neogastropods were more sensitive to TBT contamination than bivalves and other types of molluscs [22]. Neogastropod species used in this study was *Thais aculeata.*

Snail *T. aculeata* Deshayes, 1844 is a member of Family Muricidae. The recent scientific name that accepted according to WoRMS (http://www.marinespecies.org/) is *Thalessa aculeata* (Deshayes & Milne-Edward, 1844). Even so, the name that is commonly used is *Thais aculeata*. This snail has an ovate to biconical shape, the tip is medium tapered, its circumference is surrounded by spiral spikes, thick faskiola, outer lip has five inner denticles that extend to the aperture or openings as lirae. Dark brown or black or purple with patches or lines between nodules with yellowish white color in the inner body part and maximum length reaches 6 cm [23, 24, 25].

The investigation of imposex in muricid *T. aculeata* species was the first study in Ambon Island. This species was commonly found the costal water which has heavy shipping traffic and maritime activities. The aim of this study was to identify the imposex phenomenon on *T. aculeata* regarding limited information in this area. The imposex standard indices will be used to evaluate the incidence of imposex in coastal shipping-related area considering that it was an inexpensive method to establish occurrence of TBT pollution in marine environment.

2. Materials and Method

2.1. Study sites

Fieldwork was conducted in Talake Shipyard (S: 03°41′59″, E: 128°10′17″) in Ambon Bay, Maluku in September to October 2016, considered to the samples availability, population size and predominant shipping-related area (Figure 1).

Figure 1. Study sites in Talake Shipyard, Ambon Bay.

2.2. Sampling and handling procedure.

Individuals of *T. aculeata* were randomly collected from the intertidal region at low tide. All visible muricids were collected reaching the open areas of the shore and more cryptic areas such as under over-hangs and behind pilings. To have a reliable data, at least 30 specimens was collected. It has been shown an optimum number of sampel for imposex determination [26, 27] and to avoid oversampling of potentially stressed populations. Then, samples were transported to the laboratory in plastic bags and kept frozen prior to analysis. The samples obtained were then preserved, taken to the laboratory for shell dimensions measurement including shell length (SL), shell width (SW) and shell height (SH) (Figure 2). The samples were counted and identified according to Abbot and Dance (1990) [24], Dance (1976) [23], Dharma (1988; 1992; 2005) [28, 29, 30], Roberts et al. (1982) [31], Wilson and Gillet (1971) [32] and Wye (2000) [33]. The process of identification and sample analyzes were carried out in the Reference Collection, Center for Deep-Sea Research, LIPI, Ambon. In addition, physical-chemical profile and bottom substrate around the study sites were also measured to determine hydrographic conditions and habitat characteristics in the study site.

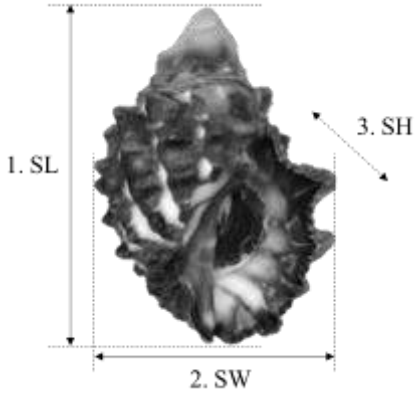

Figure 2. Measurement of shell dimensions of *T. aculeata.* 1. Shell length (SL); 2. Shell width (SW); 3. Shell height (SH).

2.3. Data analysis

2.3.1. Size class frequency and sex ratio. Each shell dimension and total weight of *T. aculeata* was measured and weighed using digital caliper and digital scale, respectively. Size class was determined based on the Sturge rules [34] including; (1) determine the range (R) = maximum length - minimum length; (2) determine the number of classes (C) = 1 + 3.32 logN, N = number of samples; (3) class interval (CI) = range (R) / number of classes (C). Next, the end of the first class and frequency of each class interval were determined, and the individual number of each station was calculated according to the number of class formed. Sex ratio was calculated based on the proportion of males and females individual number. Then, the relationship between shell size, sex ration, and imposex variation were tested using Chi-square analysis [35].

2.3.2. Imposex determination. In the laboratory, shell length and width were measured using vernier calipers to the nearest 0.1 mm, and cracked using a small bench vice. Soft parts of animals were removed from their shells and the salient features of the reproductive system analysed. Sex was determined based: maless by the presence of penis, seminal vesicle and yellowish colour of the testis; females by the presence of an egg capsule gland, ovaries and albumin gland of whithish colour. Imposex females were established by the presence of a pseudopenis located dorsal to the right tentacle [36, 37, 16, 38].

Penis length was measured in both males and females exhibiting imposex. Subsequently, the average length of the penis in males and in females was estimated [39]. Imposex was quantified using

three index initially developed for the neogastropod *Nucella lapillus* included: RPLI (Relative Penis Length Index), RPSI (Relative Penis Size Index) and PI (Percentage of Imposex). These indices later adapted for the use in other neogastropods [40, 41].

3. Results

3.1. Description of study site

Ecologicaly, coastal area near Talake shipyard had a fairly wide tidal zone, with more than 300 meters coastline length horizontally and about 80 meters perpendicular to the coastline. Based on visual investigation, it was the biggest and the busiest shipyard in Ambon. Several ships were docked and repaired in this place, which directly connected to the Ambon Bay. As a result, its waters characteristics were influenced by water mass and dynamics of Ambon Bay. The result of measurement of chemical physical parameters was presented in Table 1.

Table 1. Chemical and physical waters parameters of study site.

Parameters	**Value**
1. Temperature (°C)	28.5
2. Salinity (‰)	32.0
3. pH	7.23
4. Dissolved Oxygen/ DO ($mg.l^{-1}$)	6.17
5. Phosphate/ PO_4 ($mg.l^{-1}$)	0.02
6. Nitrate/ NO_3 ($mg.l^{-1}$)	0.01
7. Turbidity (FTU)	0.43
8. Current ($cm.s^{-1}$)	8.31
9. Persentage of substrate (%): Muddy sand	
▪ Gravel	28.2
▪ Sand	55.6
▪ Clay	16.2

3.2. Size class frequency

A total of 110 individuals of *T. aculeata* were found in this study and divided to eight size class based on Sturge rules. Individual frequency of each size class was showed in Figure 3. The highest number of individuals (31 individuals) was in the sixth class (28.32-31.66 mm), while the lowest (6 individuals) was found in the first class (11.58-14.92 mm).

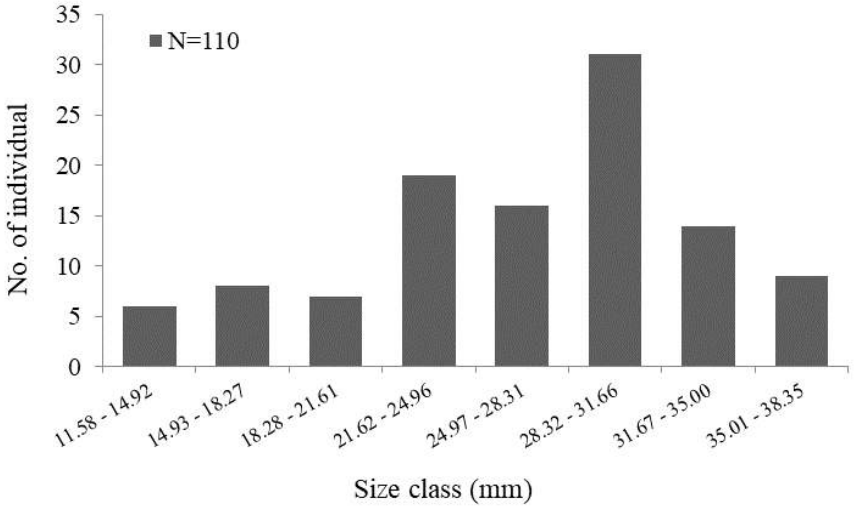

Figure 3. Frequency of individual *T. aculeata* on each size class.

3.3. Sex ratio

The number of females compared to males *T. aculeata* was 95 and 15 individuals. The sex ratio analysis showed that *T. aculeata* females outnumbered males in the overall sample, with an overall sex ratio of 6:1 (Females: Males) with X^2 counted > X^2 table (58.18>3.84) (Table 2).

Table 2. Sex ratio and Chi-square analysis with probability of *T. aculeata* in Talake Shipyard

Number of individuals		M:F	e_i	X^2 value	Probability
Males	15	1:6.33	55.0	29.09	<0.05
Females	95			29.09	
Total	110			**58.18**	

*M: Males; F: Females; e_i: expected frequency, X^2: Chi-square value.

3.4. Imposex of T. aculeata

Imposex was observed in *T. aculeata* collected from all area of Talake shipyard in Ambon Bay. A total of 39 of 95 females individual were induced by imposex with percentage of 41.05, which most have smaller pseudopenis than penis of males individual (Figure 4).

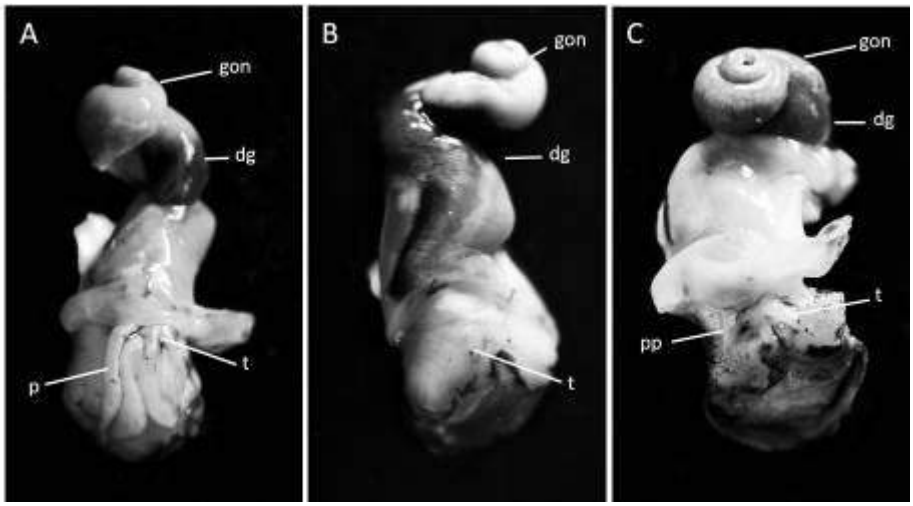

Figure 4. Morphology of soft tissue *T. aculeata.* A) males; B) normal females; and C) females with imposex; gon-gonad; dg-digestive gland; p-penis; pp-pseudopenis; t-tentacle.

A comparison between sex ratio, average of penis length, PI, RPSI and RPLI of *T. aculeata* was showed in Table 3.

Table 3. Sex ratio, average of penis length, PI, RPSI and RPLI of *T. aculeata*

Individual	Average of penis length (mm ± SD)	PI (%)	M:F	RPSI	RPLI
Males penis	4.95 ± 2.02	41.05	1.09:1	7.97	49.58
Females pseudopenis	2.13 ± 0.93				

The results of correlation analysis between size class and imposex variation showed moderate relationship (R=0.44), which mean that size class not influenced enough for imposex incidence in *T. aculeata* from Talake shipyard. The result of Chi-square analysis showed that the ratio between normal females and females with imposex was not significantly different (X^2count < X^2table=3.04<3.84) (Table 4).

Table 4. Ratio of normal females and females with imposex, and Chi-square analysis with probability of *T. aculeata* in Talake Shipyard

Number of females individual		Nf:Fi	e_i	X^2 value	Probability
Normal females	56	1.43:1	47.5	1.52	>0.05
Females with imposex	39			1.52	
Total	95			3.04	

*Nf: Normal females; Fi: Females imposex; e_i: expected frequency; X^2: Chi-square value.

Then, the results of Chi-square analysis showed that ratio between number of males individual and female with imposex was significantly different (X^2count > X^2table=10.67<3.84) (Table 5); and ratio of shell length average between normal females and females with imposex was not significantly different (X^2count < X^2table=0.028<3.84) (Table 6).

Table 5. Ratio of males individual and females with imposex, and Chi-square analysis with probability of *T. aculeata* in Talake Shipyard

Number of individual		M:Fi	e_i	X^2 value	Probability
Males	15	1:2.60	27.0	5.33	<0.05
Females with imposex	39			5.33	
Total	54			10.67	

*M: Males; Fi: Females imposex; e_i: expected frequency: X^2: Chi-square value.

Table 6. Shell length average of normal females and females with imposex, and Chi-square analysis with probability of *T. aculeata* in Talake Shipyard

Females individual	SL average (mm ± SD)	Nf:Fi	e_i	X_2 value	Probability
Nf	25.86 ± 7.58	1:1.04	26.46	0.014	>0.05
Fi	27.07 ± 3.66			0.014	
Total	52.93			0.028	

*SL: Shell Length; Nf: normal females; Fi: Females imposex; e_i: expected frequency: X^2: Chi-square value.

4. Discussion

The rocky intertidal region is the main habitat for *Thais* [42, 43, 44]. These snails are also easily found in habitats filled with barnacles and oysters which are the main food. In addition, *Thais* sp. can also be found in mangrove forests [3, 45]. In this study, muricids *T. aculeata* were found widespread especially in tidal areas of Talake Shipyard, most of them attached on hard substrates including rock, or dock structure. The highest number was found in size class 28.32-31.66 mm, which mean that samples were dominated by adults snail. Liu and Chen (1992) [46] stated in their study that *Thais* was categorized as adults if its body size reached at least 23 mm. Growth of snails occured much faster at young stage compared to adult, so most of adult snails were found in the study area.

In addition, female individuals were more dominant than male snails with ratio 6:1. It was accordance with Gibbs et al. (1987) [41], that generally female individuals were more dominant in one population of gonocrostic gastropods (each individual has a different genitals). Besides, the difference in sex ratio could also be caused by the growth different between male and female snails, which Kusumastuti et al. (2013) [47] stated that the average growth of female was faster than males snails especially in individual more than 25 mm in size.

The results showed that imposex phenomenon occured in *T. aculeata* population in Talake Shipyard, with a percentage of imposex 41.05%. Females with imposex had a pseudopenis in the form of a small bulge above the right side of the tentacles; and a vas deferens. Pseudopenis was relatively smaller size compared to the penis size of male individuals. Similar result was also found by Biggati and Penchaszadeh (2005) [5] in their study that penis size and vas deferens of imposex female individuals was relative to the male individuals, but generally smaller. For example, the development of vas deferens and penis on *Odontocymbiola magellanica*. Histological observations of imposex female individuals indicated the presence of a small vas deferens and penis, less than 9 mm. Wagiman (2004) found that penis length of male individual was 2.0 - 7.2 mm and pseudopenis of female was 0.0 - 3.3 mm. Also, the average length of female pseudopenis was relatively smaller than the average length of male penis, i.e. female pseudopenis of 2.13 mm and male penis of 4.95 mm.

RPSI of *T. aculeata* in Talake Shipyard was 7.97 that smaller than RPSI obtained by Kusumastuti et.al. (2013) [47] in Tanjung Mas Semarang (93,17). On the other hand, RPSI in Talake Shipyard was higher than RPSI obtained by Widianwari (2010) [48] in Jakarta Bay. Results of both studies stated that high RPSI was correlated with shipping activities in those areas, particularly in term of TBT compound pollution.

Moreover, all size class tend to have equal opportunity exposed to imposex in female individuals. It was seen by a moderate correlation between shell size and imposex occurrences; and the correlation between average shell length of normal female and imposex females which not significantly different. Imposex was found mostly on female individuals with shell length 28.16 - 31.30 mm and ticker shell shape. Wagiman (2004) [3] found that TBT can cause thickening and abnormality of Neogastropods shell included *Thais* species. In dogwhelks *Nucella lapillus* population, the percentage of female individuals decreased when occurrence of imposex increased [49]. Shim et al. (2000) [50] found that sex ratio of muricids *T. clavigera* snail increased significantly when TBT pollution was recorded in high concentrations in Korea.

Percentage of imposex of *T. aculeata* in Ambon Bay based on this study was relatively lower than other locations in Indonesia, for example in Tanjung Mas Semarang of 50.84% [47] and Teluk Jakarta in Muara Baru 92.3% [48]. Wagiman (2013) revealed that areas with high shipping activities showed a percentage of imposex up to 100 percent in all muricids *Thais* sp. Whereas, frequency of imposex was lower or less than 50 percent in the areas with low shipping activities. It indicated that *Thais* species was more sensitive to TBT pollution and can be used as bio-indicators of TBT pollution.

5. Conclusion

The highest number of individuals was in size class 28.32-31.66 mm, while the lowest was found in size class 11.58-14.92 mm. Sex ratio between females and males was 6:1 or significantly different. A total of 39 of 95 females individual was induced by imposex with percentage of 41.05%, RPSI was 7.97; and RPSI was 49.58. Imposex of *T. aculeata* in Ambon Bay was relatively lower than other locations in Indonesia, but all size class of female individuals tend to have equal opportunity exposed by imposex. Further study was needed to investigate the incidence of imposex in other shipping-related areas and in different Neogastropods species, especially in Maluku waters.

Acknowledgement

This study was a part of research project entitled "The study of imposex on molluscs species in Ambon Island waters, Maluku". It was funded by the DIPA of Center for Deep-Sea Research, Indonesian Institute of Sciences 2016. We would also like to thank you to Daniel J. Tala and Francy Nendissa for field work and laboratory assistance.

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