IMPOSEX INCIDENCE IN *Thais tuberose* (Roding, 1798) *Cronia margariticola* (Broderip, 1832) IN THE COASTAL BARANGAYS OF NASIPIT, AGUSAN DEL NORTE, PHILIPPINES

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ABSTRACT

The imposex incidence in Thais tuberose (Roding, 1798) and Cronia margariticola (Broderip, 1832) were studied to assess the incidence of imposex in the coastal Barangays of Nasipit, Agusan del Norte. Almost all sites were showed incidence of imposex development. The most affected and highest incidence of imposex using T. tuberose and C. margariticola is Brgy. Camagong with the value of 73.33% and 66.66% in Incidence of Imposex (IOI); 32.35% and 34.21% in Conversion of Potential Female of Imposex Index (CPFII); and in Relative Penis Length Index (RPLI) of 72.10 and 61.71% respectively. Followed by Sta. Ana, Apagan, Punta, Amontay have the most cases of imposex. The study identified higher occurrences of imposex near and adjacent the shipyard. Results showed that T. tuberose is more sensitive to imposex compared to C. margariticola, thus, suggesting a better bioindicator.

Keywords: *cronia margariticola, thais tuberose, imposex, biondicator*

1.0 Introduction

Imposex or pseudohermaphrodism is a widespread phenomenon among prosobranch molluscs characterized by the development and superimposition of non-functional male accessory sex organs (i.e vas differences and/or a penis) on female or juvenile gastropods (Astilla et al., 2005). The incidence of imposex in gastropods is a worldwide concern and has been studied in many gastropods (e.g. Nias et al., 1993; Minsink et al.,1996; Bryan et al.,1993; Schulte-Oehlmann et al., 1997; Short et al., 1989; Matthiessen et al., 1998; Stroben et al.,1992). This phenomenon is caused by exposure to Tributyltin compound and results in accumulation of testosterone in the gastropods (Gibbs & Bryan, 1996; Nias et al., 1993; Horiguchi et al., 1994). Tributyltin is the most commonly used antifouling agent used in paints for the underwater hull of large boats (Berge and Walday, 1999). It was used to kill the algae, mussels and barnacles found in the hulls of the vessels which is a historic problem that hinders the operation of marine vessels.

Several studies have been conducted, Gibbs and Bryan (1987) confirmed a long term field and laboratory experiments with *Nucilla lapillus*, exposed to Tributyltin which showed the bioaccumulation within female correlated with an increase in the development of imposex. Also, Matthiessen et al., (1998) explained that gastropods bioaccumulate TBT and its endocrine

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disruptive effects result in elevated testosterone levels giving rise to imposex. Nias et al., (1993) reported that TBT has been the major cause of imposex in *N. lapillus*. Such occurrence would therefore affect the reproductive capacity of the affected population, especially when imposex is known to be irreversible (Blackmore, 2000). Also, Smith (1996) added that severe imposex may result to population decline or even mass extinction. In addition, Mensink et al., (1996) concluded that severe stages of imposex at higher TBT concentrations can lead to female sterilization and death. During the exposure in early life stages of high concentration of TBT, a complete sex change might occur (Stroben et al., 1992).

Despite the pollution of TBT in the marine environment, it is still considered a problem worldwide, particularly in seaports although, some countries have led to regulatory measures in using TBT. France and United Kingdom prohibit the use of TBT paints on vessels under 25 meters in length. In the Philippines, there is no monitoring of TBT pollution. The use of TBT is very rampant. In the study of Astilla et al., (2005) in Mactan Cebu, the first study conducted in the Philippines, showed 100% development of female penis developed in the population in the Caltex depot.

The use of *Cronia margariticola* by Pandey and Evans (1996) in Indonesia and Astilla et al., (2005) in Mactan Island, Philippines in monitoring TBT in marine waters by documenting imposex exhibited sensitive to imposex. The use of *Thais tuberose* by Liu et al., (1997) found that imposex in this species is more severe than other species despite similar TBT burdens. The *T. tuberose* species is the first study to be conducted as biomonitoring of TBT pollution in the Philippines. These two species of gastropods are present and common in the costal water in Nasipit, Agusan del Norte which has the heavy shipping traffic of marine vessels. Also, it was presumed as the source of TBT pollution. The outcome of this study is to compare the sensitivity of these two species of gastropods in response to TBT pollution. The use of imposex standard indices will be established to evaluate the incidence of imposex in different coastal Barangays of Nasipit, Agusan del Norte considering that it is an inexpensive method to establish occurrence, patterns and changes in TBT pollution in marine environment.

2.0 Research Design and Methods

2.1 Study Site

The nine (9) coastal Barangays of Nasipit, Agusan del Norte were selected as sampling sites considering the presumed source of organotin pollution (the seaport). The nine sampling stations are the Barangay Talisay, Camagong, Sta. Ana, Apagan, Punta, Amontay, Aclan, Ata-tahon and Cubi-cubi. The Geographical Positioning System (GPS) was used to record the distance and the location of each barangays in the study area.

2.2 Collection of Samples and Processing

Collection of *T. tuberose* and *C. margariticola* were done using a random sampling method in each station. Sampling was carried out while the tide is at low shore due to the fact that the specimen is binding on rocks and in sea grasses further down the shoreline. These

gastropods species are found only in the intertidal zone. All samples were placed in a container filled with seawater and sealed for transit. The collection of samples was done through handpicking from identified stations. To have a reliable data 30 specimens in every site was collected (Castro, 2002). The specimen were preliminarily narcotized with magnesium chloride solution 3.5% (Huet et al., 1995) and preserved in a refrigerator. After 24 hours, the samples were placed in 10% formalin solution of seawater and kept frozen prior to analysis.

2.3 Recognition of Sexes

In analysis, the length of each gastropod was measured from the apex to the distal end of the siphonal canal using a vernier caliper measured to the nearest 0.1 mm. The shell of the specimen was being crushed with a hammer. The soft part was placed in a petridish for recognition of sexes using the dissecting microscopes. Penis length were measured by the vernier caliper to the nearest 0.1 mm. Males were distinguished by the presence of seminal vesicles and red orange gonad and females were identified by the presence of a white egg capsule gland and albumen gland, and cream–colored gonad with yellow ovaries (Smith, 1980). Imposex is established by the presence of a penis on females located dorsal to the right tentacle.

2.4 Data Analysis

To determine the occurrence of imposex, the following indices for indirect TBT biomonitoring were calculated: The Relative Penis Length Index (RPLI) is an index that quantifies the degree of imposex in the population and is obtained from the equation: (Mean length of female penis)/(Mean length of male penis) x 100 (Minchin et al., 1996; Gooding et al., 1999).

The Conversion of Potential Female Imposex Index (CPFII) is a new index developed to calculate the potential females of a given area of population converted to imposex. Equation is obtained in (number of imposex animals)/(Number of Animals + Number of Imposex Animals) x 100 (Tewari et al., 2002).

The Incidence of Imposex (IOI) was calculated using the following formula by Shim, et al., (1999): (Number of Imposex Animals)/(Total Numbers of Females) x 100.

Linear Regression Analysis was used to correlate the RPLI vs shell length and the distance from the source and RPLI. The degree of imposex is analyzed through bivariate factors – across sampling locations in the *T. tuberose* and *C. margariticola*.

3.0 Results and Discussions

A total of three hundred seventy (369) of *Cronia margariticola* individuals and three hundred sixty nine (370) of *Thais tuberose* were collected from the nine (9) coastal Barangays of Nasipit Agusan del Norte. Imposex were detected in two gastropods. As shown in Figure 1, the IOI, CPFII and RPLI in *T. tuberose* of 35.5%, 16.5%, and 46.8% are relatively higher than *C. margariticola* of 23%, 12.3% and 34.5% respectively in all sampling sites. Moreover, a relatively high RPLI observed, shows a significant development of the penis among female gastropods used in the study; that

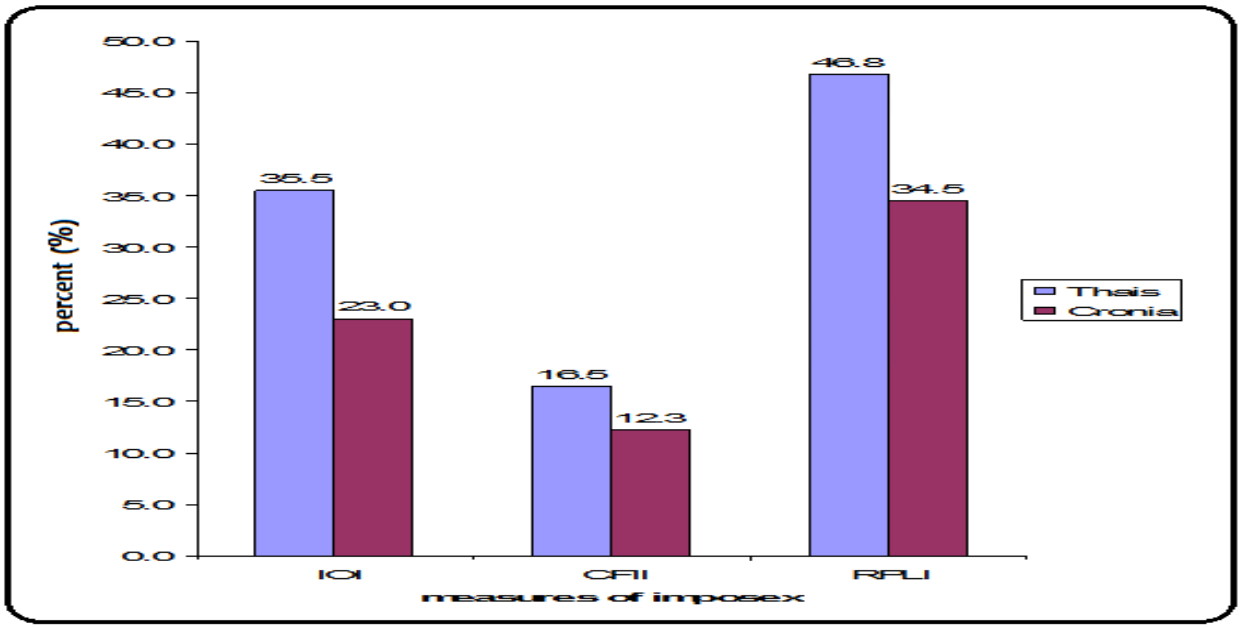


Figure 1. Comparison of the incidence of imposex indices of *T. tuberose* and *C. margariticola*

is, penis developed among female species is more than half of the actual male penis among the populations. The penis length of the females are smaller compared to male penis. The longest pseudopenis was 0.58mm in *C. margariticola* and 0.62mm in *T. tuberose*. This result was supported by Blackmore (2000) who stated that penis size in females is always smaller in males.

The *T. tuberose* species exhibited high value of imposex indices. This result was consistent in the previous studies conducted by Liu et al., (1997) in Taiwan, that imposex was much more severe in *Thais* species than the other neogastropoda, despite similar organotin burdens. The *C. margariticola* exhibited less imposex incidence due to its sensitivity in organotin pollution. According to Tan (1999) the differences in habitat, diet, and physiology have been suggested as causes of interspecific differences in imposex. In addition, Li and Collin (2009) stated that another factor that can influence the expression of imposex is age. The species that are longer lived or slower growing may be more likely to have high levels of TBT and thus exhibit imposex. The Incidence of Imposex (IOI); Conversions of Potential Female Index (CPFI) and Relative Penis Length Index (RPLI) of *T. tuberose* and *C. margariticola* are presented in Table 1. The highest Incidence of Imposex (IOI) of the *T. tuberose* and *C. margariticola* is in Brgy. Camagong with the value of 73.33% and 66.66%. This is followed by Brgy. Talisay of 66.66% and 41.66% respectively. Least incidence was obtained from barangay Cubi-cubi, Ata-atahon and Aklan. A marginal degree of imposex was obtained in Barangay Aklan, 0% in *C. margariticola* and 6.89% IOI in *T. tuberose.* Moreover, Barangays Cubi-cubi and Ata-atahon showed no incidence of imposex in both gastropods. It should be noted that barangay Sta. Ana showed a high degree of imposex in terms of IOI (64.00%) for *T.*

Table 1. Imposex indices of *Thais tuberose* and *Cronia margariticola* across the nine coastal barangays

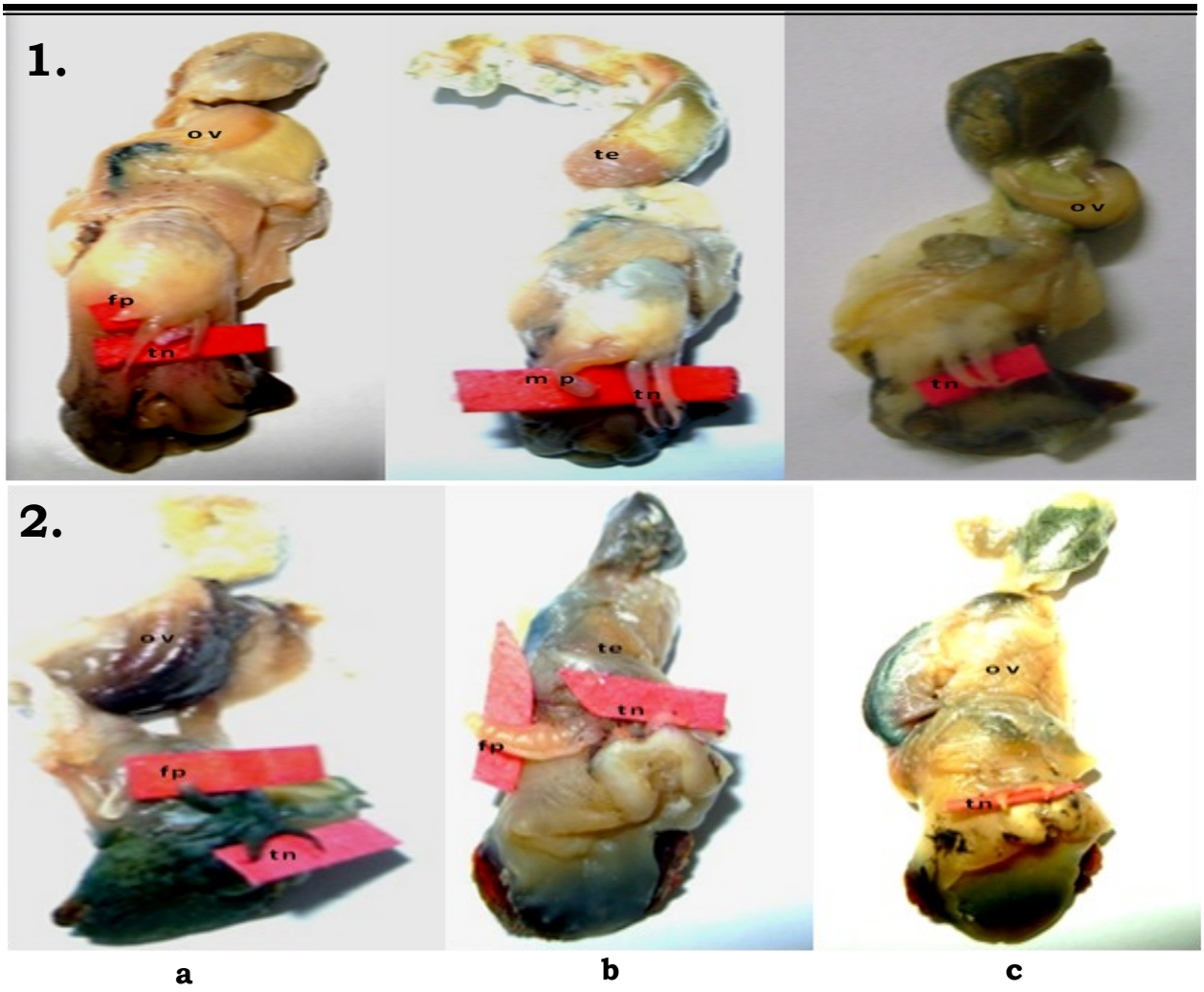
Sampling Locations	Distance (m) from the shipyard to the sampling locations	MEAN					
		Cronia margariticola			*Thais tuberose*		
		IOI	CPFII	RPLI	IOI	CPFII	RPLI
Brgy. Talisay (S1)	251	41.66	22.22	52.12	66.66	29.27	67.06
Brgy. Camagong (S2)	302	66.66	34.21	61.71	73.33	32.35	72.1
Brgy. Sta. Ana (S3)	350	38.46	19.6	51.4	64	27.58	60.63
Brgy. Apagan (S4)	1220	30.77	16	50.52	46.42	24.07	58.76
Brgy. Punta (S5)	1667	21.43	13.04	48.67	34.48	18.87	58.64
Brgy. Amontay (S6)	1715	8	5.4	45.85	27.77	12.19	57.99
Brgy. Aklan (S7)	1975	0	0	0	6.89	4.16	45.58
Brgy. Ata- Atahon (S8)	4550	0	0	0	0	0	0
Brgy. Cubi- Cubi (S9)	5063	0	0	0	0	0	0

tuberose but relatively lower in IOI (38.46%) for *C. margariticola*.

The extent of imposex is determined using the index of Conversion of Potential Female to Imposex Indices (CPFII). The value ranges in *Thais tuberose* and *Cronia margariticola* are 32.35% and 34.21% in Brgy. Camagong to 29.27% and 22.22% in Brgy. Talisay and Brgy. Sta. Ana has the value of 27.58% and 19.60% respectively. The Relative Penis Length Index (RPLI), is shown on Table 1. Brgy. Camagong had the highest percentage value with a total RPLI of 61.71% and 72.10% followed by Brgy. Talisay with a total value of 52.12% and 67.06% and Brgy. Sta. Ana with the total value of 52.12% and 60.63% respectively.

Barangay Camagong, being proximal to the shipyard, has a dense shipping traffic, thus the occurrence of biological effects (predominantly imposex) in offshore waters. The dense traffic of ships logically explains the high concentration of TBT as antifouling agent hydrolyzed in the water. It could be that majority of the collected samples from Brgy. Camagong are juveniles when the sexual differentiation is on the process, TBT has been known as endocrine disrupter (WHO, 1999). There was a possibility that the wolfian duct designed to become a vagina for females with the influence of estrogen was disrupted thus imposition of male organ occurred in both *C. margariticola* and *T. tuberose*. This is supported by Matthiessen and Gibbs (1998) who reported that bivalves and gastropods are especially sensitive to organotin or the TBT larval stage are more vulnerable than adults. The internal anatomy of *C. margariticola* and *T. tuberose* showing the male, female and imposex test animals are shown on Figure 2.

This study revealed that the highest incidence of imposex barangays are near and adjacent from the seaport. ANOVA on the linearity (table 2) in the relationship between the indices of imposex and distance from the seaport to sampling sites were established. The Incidence of Imposex (IOI) ($P < 0.001$; $r = -0.89$ & -0.78); Conversion of Potential Female Index ($P < 0.001$; $r = -0.90$ & -0.80); and Relative Penis Length Index ($P < 0.001$; $r = -0.97$ & -0.80) has significant results in *T. tuberose* and *C. margariticola* respectively.



Legend:
(a) *imposex;* (b) *male;* (c) *female;* (k) *kidney;* (o) *ovary;* (p) *penis;* (te) *testes;* (tn) *tentacles.*

Figure 2. Internal structure of (1) *C. margariticola and* (2) *T. tuberose*

Table 2. Regression analysis and ANOVA result of imposex indices between shell length and distances

Parameters	*Thais tuberose*			*Cronia margariticola*		
	R2	R	P value	R2	R	P value
Shell length vs. RPLI	52.12%	-0.622	0.0005	52.34%	-0.683	0.001
IOI vs. distance from seaport	80.64%	-0.898	0.003	62.25%	-0.789	0.002
CPFII vs. distance from seaport	82.29%	-0.907	0.008	64.30%	-0.802	0.009
RPLI vs. distance from seaport	94.96%	-0.974	0.003	64.30%	-0.802	0.009

The highest mean shell length is obtained in Brgy. Ata-atahon (mean 2.5 cm.) in *T. tuberose* and Brgy. Cubi–cubi (mean 6.3 cm) of *C. margariticola.* The lowest mean shell length is Brgy. Camagong with a value of 5.38 and 2.10 cm respectively. Using regression analysis between the Relative Penis Length Index (RPLI) and imposex shell length (r = -0.62 & - 0.63) are negatively correlated in *T. tuberose* and *C. margariticola* respectively. It implies that as the RPLI increases the shell length decreases or vice versa. The result of this study particularly on the mean shell length needs further study. This result is inconsistent to the result conducted by Li and Collin (2009) that the *Acanthais brevidentata*, showed that females with imposex are larger than normal females. However, several factors trigger the growth of gastropods such as exposure to wave action and or in predation pressure (Blackmore, 2000).

This result was supported by numerous studies in relation to the distance of the sampling locations in presumed source, the seaport. According to the study of Li and Collin (2009) in Taiwan, that frequency and severity of imposex declined with distance away from canal anchorage. In addition, Astilla et al., (2005) in Pandey and Evans (1996) stated that within the port area, they observed 75% imposex incidence, followed by the site adjacent to the port area which had 61% imposex incidence, then by fishing villages where 30% imposex incidence was detected, and lastly, in remote areas where there were some small boat activity which registered 15% imposex. In Mactan Island, Philippines, Astilla et al., (2005) reported that Caltex Depot has the most severe imposex and this can be attributed to its proximity to a shipyard where considerable painting and repair activities take place. In addition, De Castro et al., (2005) in Northeast Brazil that the increase in the incidence of imposex was probably caused by the establishment of Pecém Harbor and its increasing shipping activities. Also, Bryan et al., (1987) concluded that there is a strong relationship between the degree of imposex and proximity to harbors.

The use of TBT in marine vessels is very rampant worldwide. In the Philippines, there is no regulation in the use of Tributyltin in the shipping activities. The prohibition of TBT in large harbor areas is necessary. For example in the country Japan, the regulation prohibiting the use of TBT-based antifouling is implemented and significant decrease of RPSI was observed (Champ, 2000). The regulation of implementation of prohibition of TBT in the Philippines is necessary considering the effect of imposex in the marine environment and health hazard to human.

4.0 Conclusion

Imposex were detected in two species of gastropods, the *T. tuberose* and *C. margariticola*. Using the standard indices; the IOI, CPFII and RPLI in *T. tuberose* yielded a value of 35.5%, 16.5%, and 46.8% are relatively higher than *C. margariticola* of 23%, 12.3% and 34.5% respectively in all sampling sites. The *T. tuberose* species exhibited high value of imposex indices compared to *C. margariticola* suggesting good bioindicator in TBT pollution. Highest occurrence of imposex are near and adjacent from the seaport such as the Brgy Camagong, Talisay, Sta. Ana, Apagan, Punta and Amontay. ANOVA on the linearity revealed

significant difference (P < 0.001) in RPLI, CPFII and IOI and distance from the seaport to sampling sites.

5.0 Recommendations

In view of the result of this study, the following are recommended; conduct histological examinations to the affected female tissues; use linear measurements for comparing shell morphology; use Vas Deference Sequence; look into the lifecycle of the imposex gastropods and longer sampling period and all nearby Barangays where seaport are located should be studied not only in Agusan del Norte but in the entire Philippines.

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