Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Study on a new mechanism of sterilization in imposex affected females of tropical marine neogastropod, *Thais* sp.

Ferdaus Mohamat-Yusuff [1,2]*, Syaizwan Zahmir Zulkifli[1,3], Tsuuguo Otake[1], Hiroya Harino[4] and Ahmad Ismail[3]

[1]International Coastal Research Center, Atmosphere and Ocean Research Institute, The University of Tokyo, 2-106-1 Akahama, Otsuchi, Iwate 028-1102, Japan

[2]Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[4]Department of Biosphere Sciences, School of Human Sciences, Kobe College, 4-1 Okadayama, Nishinomiya, Hyogo 662-8505, Japan

*Corresponding Author E-mail: ferdius@upm.edu.my

Publication Info

Paper received:
29 September 2012

Revised received:
28 June 2013

Re-revised received:
05 August 2013

Accepted:
19 August 2013

Abstract

The morphological expressions and histopathological analysis of the gonads of a tropical marine neogastropod species (*Thais* sp.) from East Malaysia revealed new evidence of mechanical sterility in the imposex affected females. The gradual development of imposex was classified into five stages (Stage 0 to Stage 4) with three types of sterility conditions; Type A caused prohibition of copulation and capsule formation; Type B prohibits the releasing process of eggs; and gonads in Type C are infertile. Further analysis is needed to confirm, if the gonad malformation in imposex affected snails is generated specifically by tributyltin (TBT) or by other possible factors. The levels of imposex incidence (stages and percentages) were greater in a marina and decreased with increasing distance from the marina. Organotin tissue burden across the sexes showed that dibutyltin (DBT) as well as TBT might be the elements inducing imposex in *Thais* sp. from Miri in East Malaysia.

Key words

East Malaysia, Imposex, Organotin, Sterility, *Thais* sp.

Introduction

The use of biological indicators is one of the efforts to monitor pollutant levels in the environment. These organisms could be changed in terms of physical, biochemical, physiological, behavioral and other related processes due to exposure and bioavailability of pollutants that exist in the surrounding environment. In Malaysia, several organisms have been proposed as bioindicators, such as *Telescopium telescopium* (Ismail and Safahieh, 2005), *Nerita lineata* (Ismail and Ramli, 1997), *Uca annulipes* (Ismail *et al*., 2003), *Dotilla myctiroides* (Zulkifli *et al*., 2012a), *Thais* spp. (Mohamat-Yusuff *et al*., 2010, 2011), *Oryzias javanicus* (Khodadoust *et al*., 2012, 2013), *Anguilla bicolor bicolor* (Arai *et al*., 2012), *Monopterus albus* (Sow *et al*., 2012a; Sow *et al*., 2013a) and *Periophthalmodon schlosseri* (Zulkifli *et al*., 2012b).

The imposex phenomenon of females developing male genital organs (a penis and a vas deferens) has been documented to occur in gonochoric gastropods all around the world during last several decades and has been widely reported to be a contamination effect of organotin (OT), and specifically tributyltin (TBT) (Horiguchi *et al*., 2012; Ismail *et al*., 2004; Mohamat-Yusuff *et al*., 2010). General patterns of imposex expression are classified into seven stages (S_0-S_6) with three types (a, b, c) by referring to the sequence of the vas deferens development (VDS). Six stages of imposex have been defined, with three types in the early three stages and in Stage 5, and two types each in Stage 4 and 6 (Shi *et al*. 2005). Still, the classification system is continuously being modified due to diverse expression of imposex development, which strongly depends on the species and on geographical differences.

© Triveni Enterprises, Lucknow (India)

Thais sp. is one of the commonly found marine neogastropod in Malaysia (Mohamat-Yusuff *et al*. (2011, 2012). This genus has been used as bioindicator for organotin contamination for years. An example is the imposex study conducted by Horiguchi *et al*. (1994) using snails *T. clavigera* and *T. bronni* from the Japan coast, in which 5 developmental stages were defined by taking into account only the female's penis length and shell height because the vas deferens was fully developed in all affected snails. A study conducted by Tan (1997) using *T. gradata* from Singapore coast did not use VDS to define different stages because no vas deferens structures were found in the samples of that population. However, in recent years Mohamat-Yusuff *et al*. (2010) presented a new imposex scheme for *T. gradata* from the southern coast of Peninsular Malaysia. This study classified imposex expression into 7 stages by observing both the penis and vas deferens along with changes in the structure of the vulva.

Though, the end of imposex development has been less studied and literature has focused on the morphological expression of imposex affected snails. Very few articles have addressed the gonad status of females with imposex characteristics. Gibbs *et al*. (1987) reported that under laboratory conditions, females of *Nucella lapillus* experienced suppression of oogenesis and were supplanted by spermatogenesis leading to sperm production at TBT levels above 3-5 ng Sn/l. Their finding was supported by field observations. A study conducted by Horiguchi *et al*. (2006) proposed that ovarian spermatogenesis in females exposed to TBT or TPT may be one of the reasons why the spawning ability of females *Babylonia japonica* from Japan decreased (Horiguchi *et al*., 2006). Nevertheless, morphological and histological evidence of the gonad tissues leading to reproductive failure due to imposex in *Thais* sp. has never been documented.

Thus, the objectives of this study were to examine the morphological appearance of gradual imposex development in *Thais* sp. from East Malaysia and to determine the factors leading to sterilization of severe imposex affected females along with histological analysis of the gonad. This study also determined the concentration of organotin tissue burden in *Thais* sp.

Materials and Methods

Study area and sampling method : Miri city is a government administrative centre for Miri District in Miri Division of the State of Sarawak (Malaysia). This area is active in petroleum upstream- and downstream-related activities, processed timber, oil palm production, and tourism. The coastal area is busy with sea vessels used to transport goods. In the present study, 321 individuals of *Thais* sp. were collected by hand during low tide at three locations along the Miri coastal areas (Fig. 1). The time spent in collecting specimens, per worker, was recorded as described by McClanahan and Muthiga (1992) with minor modifications. The number of samples collected was not limited during the specific time spent. Three workers walked in same direction but at different intertidal depths along the sampling site. Population density was also measured.

The selection of three locations for sampling was done by taking into account their distance from boating activity including areas without any maritime activity. Marina Bay (4°23.409'N; 113°58.153'E) is a yachting area that provides 76 berths for small

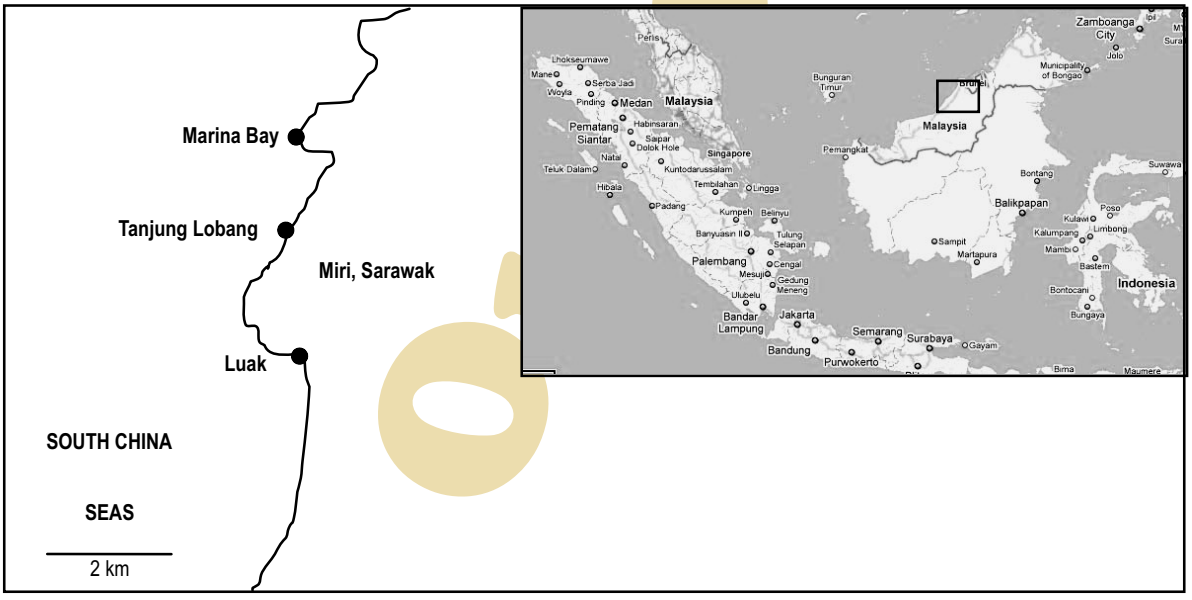


Fig. 1 : Sampling locations (black dots) for the specimens of *Thais* sp. along the Miri coastal area in Sarawak, East Malaysia

boats up to 18 m and is under construction to support larger boats up to 34 m. Tanjung Lobang (4°22.409'N; 113°58.074'E), which is located about 2 km from Marina Bay, is a recreational beach where no boating activities takes place. Luak (4°21.303'N; 113°58.102'E) is about 4 km from Marina Bay and is also a recreational beach without any boating activities.

Imposex measurement : The gradual imposex development was recognized by the formation of a penis and/or the sequence of the vas deferens development along with gonad status. In the advanced levels of imposex, females were identified by the presence of a sperm ingesting gland and vulva despite having a fully developed vas deferens and penis as earlier observed by Gibbs *et al*. (1990). All observation and imaging work was performed under a dissecting microscope connected to a digital camera (DXM1200F, Nikon, Tokyo, Japan), and the captured images were analyzed using the ACT-2U software (Version 1.70). For histopathological analysis of gonads, about 20 randomly selected specimens from each site were fixed with Bouin's solution, sectioned (7 µm thick) and stained with haematoxylin and eosin. The remaining unfixed samples were used for organotin (OT) analysis. OT analysis was conducted as described by Harino *et al*. (2005) with some modifications. The concentrations of OT in this study were expressed as Sn^{4+} in wet weight. Differences in imposex incidence and OT compounds among shell heights and between sexes were examined for statistical significance by calculating the mean ± S.E. and performing one-way analysis of variance (ANOVA). The correlation analysis using Pearson or Spearman Rho's product was not possible due to low sample numbers.

Results and Discussion

Percentage of imposex incidence are presented in Table 1. In general, no imposex specimens from Luak were found, but more than 80% of the females in Tanjung Lobang and 100% females from Marina Bay were diagnosed as imposex. The imposex expressions in *Thais* sp. with a combination of development of penis, VDS and gonad status revealed 5 stages of imposex including Stage 0 for normal females and Stage 4 as the highest imposex level. The gradual development belonged to three types: Type A, Type B and Type C as illustrated in Fig. 2. Each end of the respective type is the sterile condition. The examinations found that only 7.1% of imposex affected females from Marina Bay were diagnosed as Stage 1 due to development of a penis bulk behind the right tentacle. Stage 2 was characterized by development of a vas deferens on the basal areas of the penis and also on the outer area of the end oviduct. The gonad status of Stage 2 specimens showed that the ovaries functioned as normal ovaries. There was a specimen having an open structure of the bursa copulatrix and no vas deferens from the pallial oviduct instead of having a similarly appearing penis bulk and a vas deferens at Stage 2 of imposex (Fig. 3(I)). This expression was classified as the Stage 2-Type A and was found in 3.6% of the imposex specimens from Marina Bay. Imposex Stage 3 was characterized having bigger sizes of penis, complete with flagellum at the tip of penis and a fully developed vas deferens on the mantle floor and this was the dominant imposex stage found in this study. By referring to the end condition of the oviduct, specimens with a vas deferens grown into the pallial oviduct were classified as Stage 3-Type B, which were 16.6% in Tanjung Lobang and 21.4% in Marina Bay of the imposex specimens. Stage 3-Type C snails showed a vas deferens growing past the oviduct in the area between oviduct and rectum. This was found in 33.3% of the Tanjung Lobang imposex specimens and 21.4% of the Marina Bay imposex specimens. An advanced level of Type B (Stage 4-Type B) was seen in a specimen with unreleased egg's capsule as shown in Fig. 3iv and was also found in 33.3% of Tanjung Lobang imposex specimens and 21.4% of imposex specimens from Marina Bay. The histological analysis of the gonad tissues from the specimens at Stage 3-Type B and Stage 4-Type B demonstrated apoptosis cells in the ovaries (Fig. 3(x)). The specific location was in the area near germinative epithelium. The number of apoptosis cells were greater with increasing levels of imposex stage and also spread into wider areas in between the ovaries in correspondence with the imposex level.

In case of imposex Stage 3-Type C, the histological analysis of the gonad showed the presence of a vas deferens structure that was full of sperm. This was seen even though the ovaries were apparently functioning as normal ovaries based on the vitellogenic stage of the follicle cells. The highest level imposex of Type C (Stage 4-Type C) was found only in the samples from Marina Bay (21.42%), which were characterized by a fully grown penis despite having vulva and sperm ingesting gland as shown in Fig. 3(viii). Further investigation on the gonad tissues showed that the gonad was an ovo-testis and showed

Table 1 : Sample size (N), population density (D = N hr^{-1} $person^{-1}$), mean shell height (mm), percentages of females, percentages of imposex affected females, percentages of imposex stages of Thais sp. from three sampling locations at Miri, Sarawak

Location	N	D	Shell height (mm)	Female (%)	Female as imposex (%)	Stage 1 (%)	Stage 2 (%)	Stage 3 (%)	Stage 4 (%)
Luak	116	120	25.5±0.53	72.4	0	ND	ND	ND	ND
Tg. Lobang	104	248	26.9±0.15	36.9	86.8	ND	16.7	16.7 33.3[b]	33.3[a]
Marina Bay	101	568	28.0±0.46	59.4	100	7.0	3.6[*] 3.6[a]	21.5[a] 21.5[b]	21.4[a] 21.4[b]

Remarks: ND - not detected; [*] imposex - Type A; [a] imposex - Type B; [b] imposex - Type C

some alteration due to the presence of massive macrophage materials surrounding the ovo-testis, a lot of apoptosis cells in the ovarian structure (Fig. 3(x)), and testicular necrosis in the testicular structure (Fig. 3(xi)).

Data for the levels of OT in the specimens examined were pooled together and divided according to different sexual conditions. Butyltins tissue burdens are displayed in Fig. 4(i) and phenyltins tissue burdens are displayed in Fig. 4(ii). Among the six types of OT compounds, MBT and DBT were consistently present at high levels in all samples: 87.5-173 µg kg^{-1} wet wt. of MBT and 18-269 µg kg^{-1} wet wt. of DBT. High levels of TBT were only detected in imposex samples in the range of 21-33 µg kg^{-1} wet wt, and very low concentrations were found in non-imposex male and female samples, in the range of 1-3 µg kg^{-1} wet wt. TPT was not detected in all the specimen; MPT was detected in large concentration only in females of Stage 2 and Stage 3 imposex (25 µg kg^{-1} wet wt., 154 µg kg^{-1} wet wt. and 94 µg kg^{-1} wet wt.). Very low concentrations of MPT were detected in samples of non-imposex male specimens. DPT was the highest OT species extracted in *Thais* sp. especially in samples of Stage 2 and Stage 3 imposex (538 µg kg^{-1} wet wt. and 350 µg kg^{-1} wet wt.). There were significant differences of five organotin species between imposex stages, males and females (ANOVA, $p<0.05$).

This study identified three imposex conditions that showed a high possibility of leading to sterilization in infected individual. These three conditions were found in snails that were collected from Marina Bay that showed a high concentration of all three butyltins (mono-, di- and tri-) in their tissues. The first condition was deformities of the pallial oviduct, specifically the opening structure of the bursa copulatrix, which was classified as Stage 2-Type A. This malformation caused prohibition of copulation and capsule formation. A similar incomplete closure of oviduct was well documented in *O. erinacea* from southwest England in late 1980's. No further explanation was ever documented in other species, especially for genus *Thais*. A study using *T. clavigera* and *T. bronni* did not describe such an oviduct malformation, except for a split of the oviduct (Horiguchi *et al*., 1994).

The second condition was development of vas deferens tissue on the oviduct end and deep into the pallial oviduct wall, which tightens the extrusion channel. In this condition, copulation

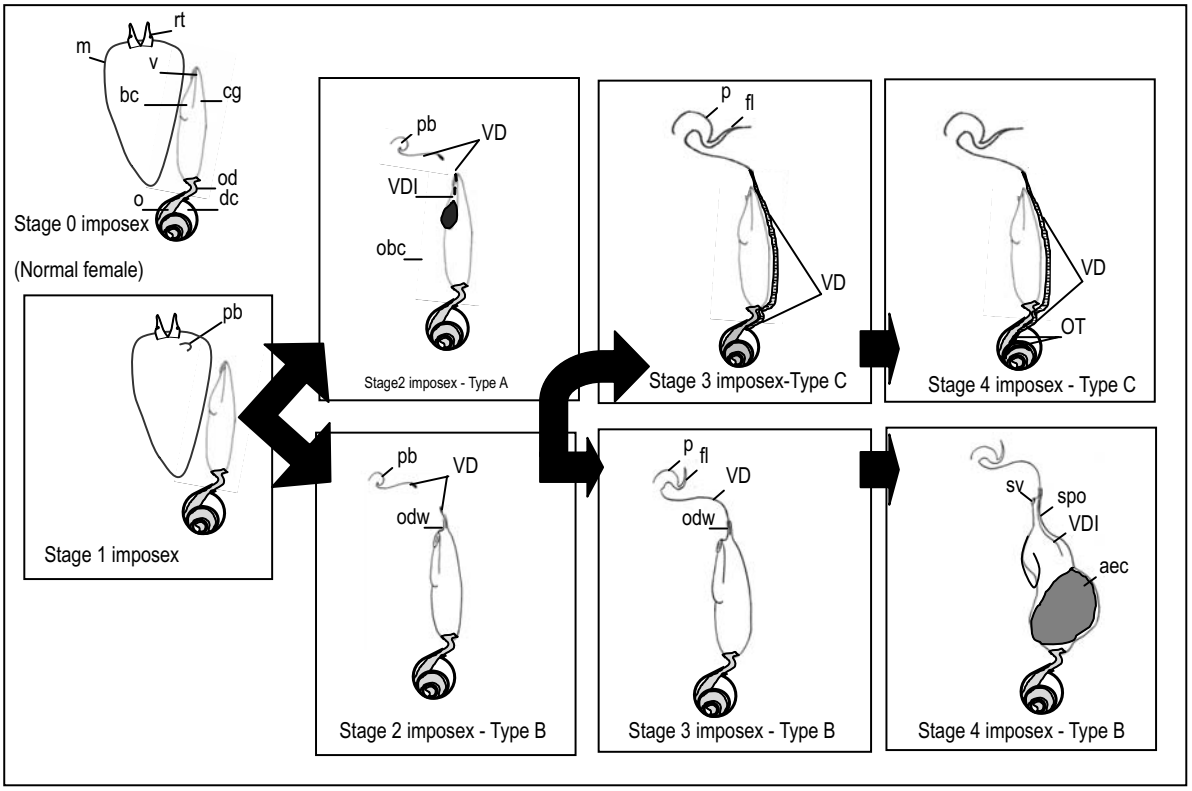
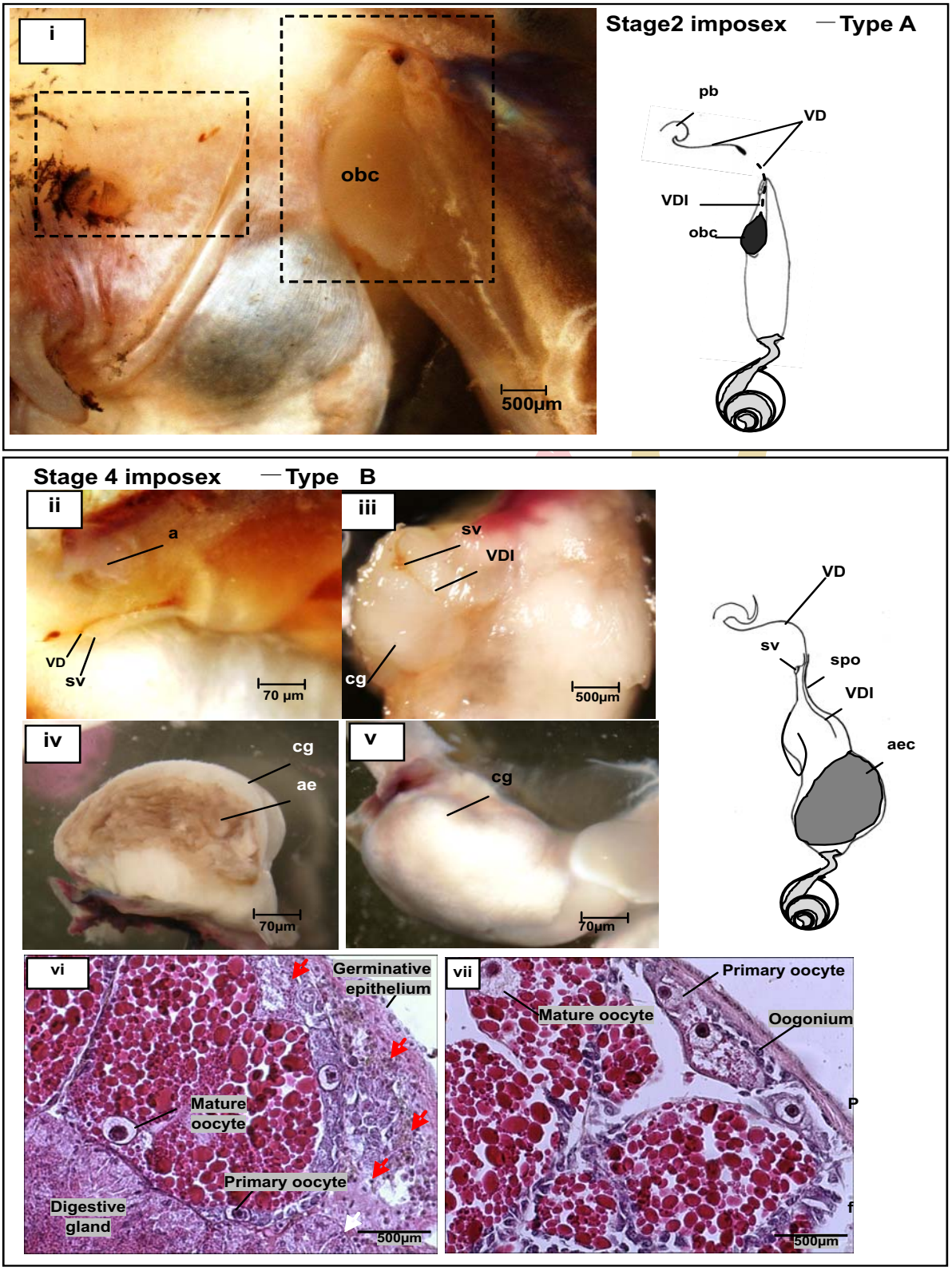


Fig. 2 : Gradual development of imposex stages in *Thais* sp. Stages leading to sterilization are at the ends of each type. a; anus, aec; aborted eggs capsule, bc; bursa copulatrix, cg; capsule gland, fl; flagellum, m; mantle, o; ovary, OT; ovo-testis, od; oviduct, odw; oviduct wall, p; penis, pb; penis bulk, pr; prostate gland, rt; right tentacle, sv; shrunken vulva, spo; shrunken pallial oviduct, VD; vas deferens, VDI; vas deferens in gonaduct wall


i
obc
500µm
Stage2 imposex — Type A
pb
VD
VDI
obc
Stage 4 imposex — Type B
ii
a
VD
sv
70 µm
iii
sv
VDI
cg
500µm
VD
sv
spo
VDI
aec
iv
cg
ae
70µm
v
cg
70µm
vi
Germinative epithelium
Mature oocyte
Primary oocyte
Digestive gland
500µm
vii
Primary oocyte
Mature oocyte
Oogonium
P
f
500µm

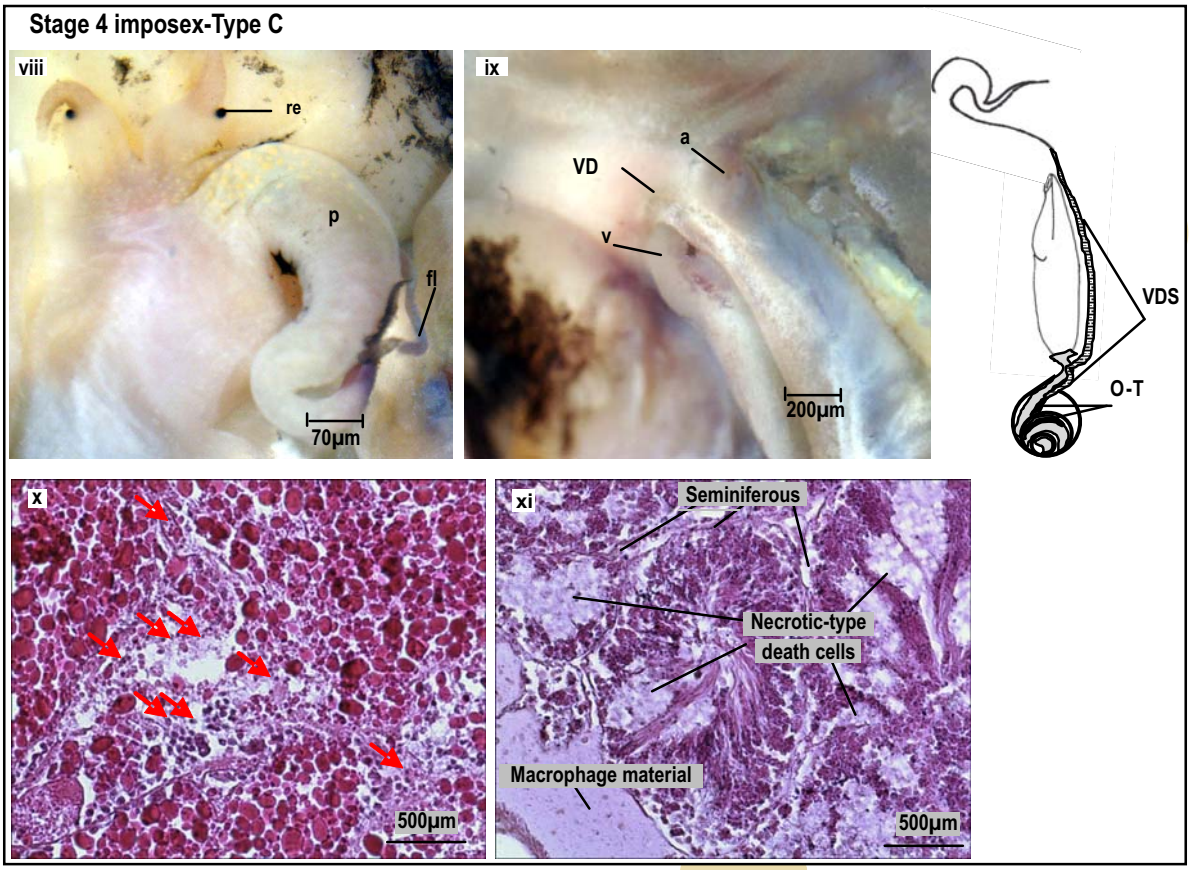


Fig. 3: *Thais* sp. Conditions leading to sterilization. (i) Opening structure of oviduct. (ii) Shrunk oviduct end. (iii)VD grew in the oviduct wall. (iv) Aborted eggs capsule in capsule gland. (v) External appearance of capsule glands with aborted eggs mass. (vi) Ovary of Stage 4-Type A imposex. (vii) Ovary of normal female. (viii) Morphology of penis of Stage 4-Type B imposex. (ix) VD position between oviduct and rectum. (x) Ovary with apoptotic-type death cells. (xi) Testicular necrosis in Stage 4-Type B imposex. ( ) apoptosis cells.

is totally prevented, leading to blockage of the fertilization process. In the most advanced stage, unreleased egg capsules were present in the oviduct. Imposex expression in genus *Thais* has been reported for the first time in this study. However, a similar condition was observed in other gastropods of different genera such as *Cantharus cecilie* (Shi *et al*., 2005) and *Nassarius reticulates* (Huet *et al*., 1995). The histological analysis of the gonad tissues from the specimens of Stage 3-Type B and Stage 4-Type B demonstrated apoptosis cells in the ovaries. The specific location was observed near germinative epithelium. The number of apoptosis cells were greater with increasing levels of imposex stage and also spread wider within the ovaries.

The third condition was vas deferens growing past the oviduct, but not disturbing the extrusion channel. The expression of vas deferens passing by the oviduct (VDSP) was also seen in other snail species such as *Lepsiella vinosa* (Nias *et al*., 1993), *Hexaplex trunculus* (Axiak *et al*., 1995), *Buccinum undatum* (Mensink *et al*., 1996), *Morula granulate* (Reitsema and Spickett, 1999), *Bolinus brandaris* (Ramo´ n and Amor, 2001), *Neptunea antiqua* (Power and Keegan, 2001) and *C. cecillei* (Shi *et al*., 2005). Earlier studies have suggested that VDSP running into the ventral channel or other excrescent tissues would lead to sterilization. Aborted egg capsules were found only in imposex affected individuals with VDSP in *N. reticulatus* (Barroso *et al*., 2002; Huet *et al*., 1995). Shi *et al*. (2005) suggested that VDSP did not enter the ventral channel and was not the cause of the infertility of this type of imposex affected female.

Results of the present study showed that the gonads of imposex *Thais* sp. with VDSP were ovo-testis. Both seminiferous tubules and an ovary were side by side of each other. A lot of apoptotic type death in the ovarian structure and necrosis in testicular seemed to cause the infertile gonad. According to Napirei and Mannherz (2009), necrosis can be generally caused by ischemia, mechanical trauma, heat, chill, irradiation, and/or drug intoxication. Testicular necrosis in fish can also be induced by pollutants in the water (Takashima and Hibiya, 1995).

Therefore, the animals in Stage 4 imposex-Type C were diagnosed as sterile due to alteration of the gonad, even though the gonoduct pathway was in good condition, which has never been documented before. Previous studies on the gonad status of imposex affected snails showed that the ovary totally changed to testis full of normal spermatozoa in *T. clavigera* and *T. bronni* (Horiguchi *et al*., 1994) and in *B. japonica* (Horiguchi *et al*., 2006). However, whether or not gonad alteration has any relation with imposex incidence is still a big question. According to Horiguchi *et al*. (2006), imposition of male reproductive tract and spermatogenesis in female system is a separate phenomenon and represents responses to different hormone secretions activated by different exposure to the chemical induced imposex. The snails in imposex stage 4-Type C in the present study were collected from Marina Bay and showed a high TBT tissue burden, which suggests that these chemical compounds caused infertile gonad condition.

The OT tissue burdens of specimens from Miri were low in TBT and TPT as compared to the various degradation products (MBT, DBT and MPT, DPT), which suggested a low input of TBT's and TPT's into the environment of this region recently. The source of contamination might be from a previous event in this area before the global ban on the use of TBT as an antifouling agent on recreational boats and vessels < 25 m in length in 2003 (Senda, 2009). Castro *et* al. (2012) reported that there was a reduction of imposex and residual butyltin contamination in Southern Brazilian harbors. However, TBT is known to accumulate predominantly in sediment and biota and may have a half life of several decades before it breaks down into DBT and MBT (Chau *et al*., 1997). Dowson *et al*. (1996) reported rate of TBT degradation at different depths within various sediments ranging from 360 to 775 days (in surficial sediments) to tens of years (deeper and anaerobic sediments). Disturbing the surface and deeper parts of sediments could potentially resuspend any sediment-bound hazardous chemicals into water column (Ismail *et al*., 2007; Zulkifli *et al*., 2010b). Resuspension of such chemicals could potentially affect the population status and health of biota, as well as the aquatic ecosystem (Sow *et al*., 2012b, 2013b; Zulkifli *et al*., 2010a). TBT was only detected in imposex affected samples and it exceeded the concentration thought to induce imposex in laboratory control conditions as reported by Nishikawa *et al*. (2004) in *T. clavigera* and Mohamat-Yusuff *et al*. (2011) in *T. tuberosa*. Therefore, TBT and DBT might be the compounds inducing imposex in *Thais* sp.

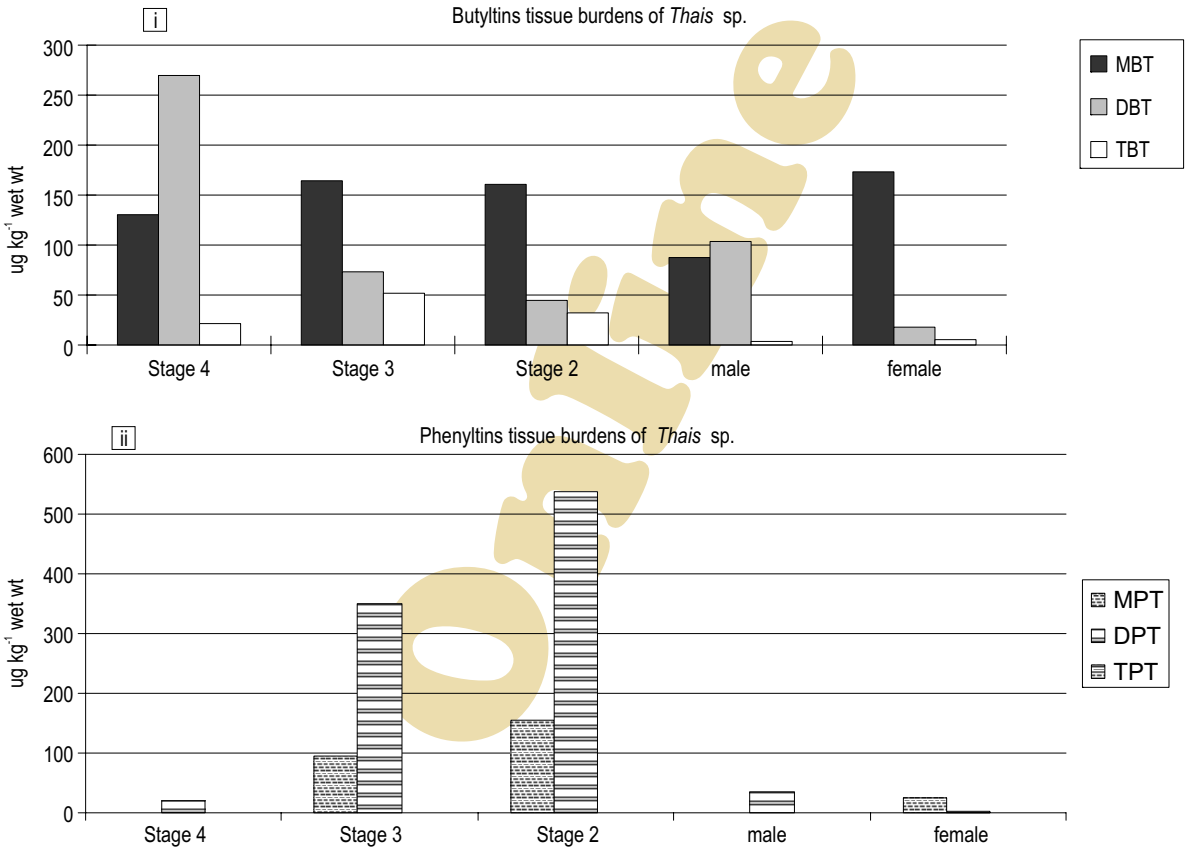


Fig. 4 : Concentration of butyltins (i) and phenyltins (ii) tissue burden of *Thais* sp. according to sexes and imposex stages

Online Copy

in the Miri area of East Malaysia that was observed in the present study as well as in other areas previously studied.

This study recommends a new imposex scheme for tropical marine neogastropod, *Thais* sp. Imposex in female individuals of this species could occur by three mechanisms that lead to sterilization. Imposex in female individuals of *Thais* sp. is also strongly correlated with TBT and DBT concentration in their tissue burdens. However, laboratory control analysis is needed to confirm the sterilization mechanisms associated with TBT and DBT exposure.

Acknowledgments

This work was jointly supported in part by Grants-in-Aid No. 18780141 and 20688008 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Japan Society for Promotion of Science (Asian CORE Program), the Research University Grant Scheme (Project No. 03-05-11-1549RU) from Universiti Putra Malaysia, the Fundamental Research Grant Scheme (FRGS) (Project No. FRGS/1/11/ST/UPM/02/12) and the FRGS for research collaboration between JSPS Asian CORE and Universiti Putra Malaysia (UPM) Matching Fund (Reference No. KPT.P.(S)400-7/2/29-4(65)) from the Ministry of Higher Education, Malaysia. We are also grateful to Mrs. Masako Hara for her considerable assistance during the SEM procedure and to Dr. Michael J. Miller for helping to improve the manuscript.

References

Arai, T., N. Chino, S.Z. Zulkifli and A. Ismail: Notes on the occurrence of the tropical eel *Anguilla bicolor bicolor* in Peninsular Malaysia, Malaysia. *J. Fish Biol.*, **80**, 692-697 (2012).

Axiak, V., A.J. Vella, D. Micallef, P. Chircop and B. Mintoff: Imposex in *Hexaplex trunculus* (Gastropoda: Muricidae): first results from biomonitoring of tributyltin contamination in the Mediterranean. *Mar. Biol.*, **121**, 685-691 (1995).

Barroso, C.M., M.A. Reis-Henriques, M.S. Ferriera and M.H. Moreira: The effectiveness of some compounds derived from antifouling paints in promoting imposex in *Nassarius reticulates*. *J. Mar. Biol. Ass. U. K.*, **82**, 249-255 (2002).

Castro, Í.B., M. Rossato and G. Fillmann: Imposex reduction and residual butyltin contamination in southern Brazilian harbors. *Environ. Toxicol. Chem.*, **31**, 947-954 (2012).

Chau, Y.K., R.J. Maguire, M. Brown, F. Yang and S.P. Batchelor: Occurrence of organotin compounds in the Canadian aquatic environment five years after the regulation of antifouling uses of tributyltin. *Water Qual. Res. J. Can.*, **32**, 453-521 (1997).

Dowson, P.H., J.M. Bubb and J.N. Lester: Persistence and degradation pathways of tributyltin in freshwater and estuarine sediments. *Estuarine Coastal Shelf Sci.*, **42**, 551-562 (1996).

Gibbs, P.E., G.W. Bryan, P.L. Pascoe and G.R. Burt: Reproductive abnormalities in female *Ocenebra erinacea* (Gastropoda) resulting from tributyltin induced imposex. *J. Mar. Biol. Assoc. U.K.*, **70**, 639-656 (1990).

Harino, H., N. Iwasaki, T. Arai, M. Ohji and N. Miyazaki: Accumulation of organotin compounds in the deep-sea environment of Nankai trough, Japan. *Arch. Environ. Contam. Toxicol.*, **49**, 497-503 (2005).

Harino, H., T. Arai, M. Ohji, A. Ismail and N. Miyazaki: Organotin contaminations in Malaysia. *Coast. Mar. Sci.*, **32**, 96-101 (2008).

Horiguchi, T., H. Shiraishi, M. Morita and M. Shimizu: Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan. *J. Mar. Biol. Assoc. U.K.*, **74**, 651-669 (1994).

Horiguchi, T., M. Kojima, F. Hamada, A. Kajikawa, H. Shiraishi, M. Morita and M. Shimizu: Impact of tributyltin and triphenyltin on ivory shell (*Babylonia japonica*) populations. *Environ. Hlth. Perspect.*, **114**, 13-19 (2006).

Horiguchi, T., J.H. Lee, J.C. Park, H.S. Cho, H. Shiraishi and M. Morita: Specific accumulation of organotin compounds in tissues of the rock shell, *Thais clavigera*. *Mar. Environ. Res.*, **76**, 56-62 (2012).

Huet, M., P. Fioroni, J. Oehlmann and E. Stroben: Comparison of imposex response in three prosobranch species. *Hydrobiologia*, **309**, 29-35 (1995).

Ismail, A., Z.Z. Syaizwan and M.Y. Ferdaus: Uptake of heavy metals in *Uca annulipes* (Brachyura:Ocypodidae) from Selangor Coastline.In: Aquatic Resources and Environmental Studies of the Straits of Malacca: Managing the Straits through Science and Technology. (Eds.: J.S. Bujang, A. Arshad, M.H. Zakaria and A. Kawamura). MASDEC Universiti Putra Malaysia, UPM Serdang, Malaysia. pp. 249-256 (2003).

Ismail, A. and R. Ramli: Trace metals in sediments and molluscs from an estuary receiving pig farms effluent. *Environ. Technol.*, **18**, 509-515 (1997).

Ismail, A. and A. Safahieh: Copper and zinc in intertidal surface sediment and *Telescopium telescopium* from Lukut River, Malaysia. *Coast. Mar. Sci.*, **29**, 111-115 (2005).

Ismail, A., S.Z. Zulkifli, F. Mohamat-Yusuff, Y. Usuki, S. Kitajima and K. Arizono: Assessment of the 17β-estradiol in the aquatic environments around Klang Valley, Malaysia. *Wetland Sci.*, **5**, 322-325 (2007).

Ismail, A., F. Mohamat-Yusuff, F. and S.Z. Zulkifli: Imposex in *Thais* sp. along the Straits of Malacca. In: Proceedings of the First Joint Seminar on Coastal Oceanography (Ed.: C. Nitithamyong). Chulalongkorn University, Bangkok. pp. 189-196 (2004).

Khodadoust, D., A. Ismail and S.Z. Zulkifli: Application of Java Medaka (*Oryzias javanicus*) fish and surface sediments to sssess Cd and Cu contamination in Linggi Estuary, Malaysia. *Acta Biol. Malays.*, **1**, 46-54 (2012).

Khodadoust, D., A. Ismail, S.Z. Zulkifli and F.H. Tayefeh: Short time effect of cadmium on juveniles and adults of Java medaka (*Oryzias javanicus*) fish as a bioindicator for ecotoxicological studies. *Life. Sci. J.*, **10**, 1857-1861 (2013).

McClanahan, T.R. and N.A. Muthiga: Comparative sampling methods for subtidal epibenthic gastropods. *J. Exp. Mar. Biol. Ecol.*, **164**, 87-101 (1992).

Mensink, B.P., J.M. Everarts, H. Kralt, C.C. Hallers and J.P. Boon: Tributyltin exposure in early life stages induces the development of male sexual characteristics in the common whelk, *Buccinum undatum*. *Mar. Environ. Res.*, **42**, 151-154 (1996).

Mohamat-Yusuff, F., S.Z. Zulkifli and A. Ismail: Imposex study on *Thais tuberosa* from port and non-port areas along the west coast of Peninsular Malaysia. *J. Trop. Mar. Ecosyst.*, **2**, 1-9 (2011).

Mohamat-Yusuff, F., S.Z. Zulkifli, A. Ismail, H. Harino, M.K. Yusoff and T. Arai: Imposex in *Thais gradata* as a biomarker for TBT

contamination on the southern coast of Peninsular Malaysia. *Water Air Soil Pollut*., **211**, 443-457 (2010).

Mohamat-Yusuff, F., S.Z. Zulkifli, A. Ismail, M.K. Yusof and T. Otake: Field survey and spatial distribution of tropical neogastropod, *Thais* spp., along Malaysian coastal area. *Acta Biol. Malays*., **1**, 9-17 (2012).

Nishikawa, J., S. Mamiya and T. Kanayama: Involvement of the retinoid X receptor in the development of imposex caused by organotins in gastropods. *Environ. Sci. Technol*., **38**, 6271-6276 (2004).

Napirei, M. and H.G. Mannherz: Molecules involved in recognition and clearance of apoptotic/necrotic cells and cell debris. In: Phagocytosis of dying cells (Eds.: D.V. Krysko and P. Vandenabeele). Springer, New York. (2009).

Nias, D.J., S.C. McKillup and K.S. Edyvane: Imposex in *Lepsiella vinosa* from Southern Australia. *Mar. Pollut. Bull*., **26**, 380-384 (1993).

Power, A.J. and B.F. Keegan: The significance of imposex levels and TBT contamination in the red whelk, *Neptunea antique* (L.), from the offshore Irish Sea. *Mar. Pollut. Bull*., **42**, 761-772 (2001).

Ramo´n, M. and M.J. Amor: Increasing imposex in populations of *Bolinus brandaris* (Gastropoda: Muricidae) in the north-western Mediterranean. *Mar. Environ. Res*., **52**, 463-475 (2001).

Reitsema, T.J. and J.T. Spickett: Imposex in *Morula granulata* as bioindicator of tributyltin (TBT) contamination in the Dampier Archipelago, Western Australia. *Mar. Pollut. Bull*., **39**, 280-284 (1999).

Shi, H.H., C.J. Huang, X. Yu and S. Zhu: An updated scheme of imposex for *Cantharus cecillei* (Gastropoda: Buccinidae) and a new mechanism leading to the sterilization of imposex-affected females. *Mar. Biol*., **146**, 717-723 (2005).

Sow, A.Y., A. Ismail and S.Z. Zulkifli: Heavy metals uptake by Asian swamp eel, *Monopterus albus* from paddy fields of Kelantan, Peninsular Malaysia: Preliminary study. *Trop. Life Sci. Res*., **23**, 27-38 (2012a).

Sow, A.Y., A. Ismail and S.Z. Zulkifli: Copper and zinc speciation in soils from paddy cultivation areas in Kelantan, Malaysia. *Acta Biol. Malays*., **1**, 26-35 (2012b).

Sow, A.Y., A. Ismail and S.Z. Zulkifli: An assessment of heavy metal bioaccumulation in Asian swamp eel, *Monopterus albus*, during plowing stages of a paddy cycle. *Bull. Environ. Contam. Toxicol*., **91**, 6-12 (2013).

Sow, A.Y., A. Ismail and S.Z. Zulkifli: Geofractionation of heavy metals and application of indices for pollution prediction in paddy field soil of Tumpat, Malaysia. *Environ. Sci. Poll. Res*. doi:10.1007/s11356-013-1857-9.

Takashima, F. and T. Hibiya: An atlas of fish histology – Normal and pathological features. 2nd Edn., Kondansha Ltd., Tokyo (1995).

Tan, K.S.: Imposex in three species of *Thais* from Singapore, with additional observations on *T. clavigera* (Kuster) from Japan. *Mar. Pollut. Bull*., **34**, 577-581 (1997).

Senda, T.: International trends in regulatory aspects. In: Ecotoxicology of antifouling biocides (Eds.: T. Arai, H. Harino, M. Ohji and W. J. Langston). Springer, Tokyo. pp. 23-34 (2009).

Uchida, D., M. Yamashita, T. Kitano and T. Iguchi: Oocyte apoptosis during the transition from ovary-like tissue to testes during sex differentiation of juvenile zebrafish. *J. Exp. Biol*., **205**, 711-718 (2002).

Zulkifli, S.Z., A. Ismail, F. Mohamat-Yusuff, T. Arai and N. Miyazaki: Johor Strait as a hotspot for trace elements contamination in Peninsular Malaysia. *Bull. Environ. Contam. Toxicol*., **84**, 568-573 (2010a).

Zulkifli, S.Z., F. Mohamat-Yusuff, T. Arai, A. Ismail and N. Miyazaki: An assessment of selected trace elements in intertidal surface sediments collected from the Peninsular Malaysia. *Environ. Monit. Assess*., **169**, 457-472 (2010b).

Zulkifli, S.Z., F. Mohamat-Yusuff and A. Ismail: Bioaccumulation of selected heavy metals in soldier crabs, *Dotilla myctiroides* (Decapoda: Ocypodidae) from Bagan Lalang, Selangor, Malaysia. *Acta Biol. Malays*., **1**, 94-100 (2012a).

Zulkifli, S.Z., F. Mohamat-Yusuff, A. Ismail and N. Miyazaki: Food preference of the giant mudskipper *Periophthalmodon schlosseri* (Teleostei: Gobiidae). *Knowl. Manag. Aquat. Ecosyst*., **405**, art. no. 07. (2012b). doi:10.1051/kmae/2012013

Online Copy