



UNIVERSITI PUTRA MALAYSIA

**IMPOSEX CASES IN SNAILS (*THAIS* SPP.) IN THE WEST COAST OF
PENINSULAR MALAYSIA**

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PENINSULAR MALAYSIA**

By

FERDIUS @ FERDAUS BINTI MOHAMAT YUSUFF

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

June 2006



DEDICATION

This thesis is dedicated to

My husband,

Syaizwan Zahmir bin Zulkifli

My Daughters,

Syazwina Yasmeeen binti Syaizwan Zahmir

Syazween Dahlia binti Syaizwan Zahmir

My parents, my brother and sister

Mohamat Yusuff bin Mat Judi

Aisah binti Abdullah

Faisalludin bin Mohamat Yusuff and wife

Soleha binti Mohamat Yusuff

My Parents in Law

Zulkifli bin Puteh

Saayah binti Din

All of you inspired me,

Without your prayers and encouragements,

It is impossible for me to be who I am today.

To all of you, your motivation, sacrifice, and support during the period of my academic mission are appreciated.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

IMPOSEX CASES IN SNAILS (*THAIS* SPP.) IN THE WEST COAST OF PENINSULAR MALAYSIA

By

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June 2006

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The objective of this research was to assess imposex cases in *Thais* spp. from the west coast of Peninsular Malaysia with respect of identification of imposex severity by using the vas deferens sequence (VDS) scheme and relative penis length (RPL), the relationship between imposex level and sex ratio of *Thais* spp. through statistical analysis and relationship between length of imposex organs (VDS and RPL) and length of shell size parameters (height, edge and aperture). The present study also observed the relationship between imposex cases and human activities in the sampling areas. Sampling activities were carried out at 27 sites from five states along the west coast of Peninsular Malaysia from November 2001 to April 2004. The sampling sites were chosen based on the occurrence of *Thais* spp.

In the present study, four species of *Thais* were chosen and they were *Thais gradata*, *Thais tuberosa*, *Thais hippocastanum* and *Thais bitubercularis*. These snails are well distributed along the rocky shore of the west coast of Peninsular Malaysia. *T. gradata* was found as the most abundant species, followed by *T. tuberosa*, *T. hippocastanum* and *T. bitubercularis*.



The presence and development of imposex were observed in four species of *Thais* collected from the 27 sites along the west coast of Peninsular Malaysia. Almost 100% of female snails showed imposex characters in various degrees. The observed imposex developments in this study included a pseudopenis and / or a vas deferens sequence (VDS) and could be classified as stage 1, 2, 3, 4, 5 and 6 according to the VDS classification scheme. Most of the imposex cases occurred at stage 2 (60%), followed by stage 1 (19%) then stage 4 (8 %). While the rest were stage 3, 5 and 6. The serious level of imposex that is stage 6 formed a penis and VDS size grew 100% similar with the size of male's sexual organs. The highest relative penis length (RPL) recorded in this study was 84.62%.

The sex ratio of affected populations was deviated from normal ratio. There was also a significant correlation showing the increment of RPL caused the decrease on female population. From Pearson's correlation coefficient, negative correlation coefficients found between imposex severity (which represent as RPL) with percentages of female population in *T. gradata* ($r = - 0.0485$, $p > 0.05$), *T. hippocastanum* ($r = - 0.6034$, $p > 0.05$) and *T. bitubercularis* ($r = - 0.0707$, $p > 0.05$). Furthermore, there was no obvious relationship between length of imposex organs (penis and VDS) and length of shell size parameters (height, edge and aperture). The incidence of imposex and the degree of imposex development in individuals were higher in samples collected from marinas, ports and areas with high boating and shipping activities.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KES IMPOSEX DI DALAM SIPUT (*THAIS* SPP.) DI SEPANJANG PANTAI
BARAT SEMENANJUNG MALAYSIA**

Oleh

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Objektif penyelidikan ini adalah untuk menilai kes-kes imposex pada *Thais* spp. dari pantai barat Semenanjung Malaysia melalui pengecaman tahap teruk imposex menggunakan skema jujukan vas deferens (VDS) dan panjang penis relatif (RPL), hubungan diantara tahap imposex dan nisbah jantina *Thais* spp. berdasarkan analisa statistik serta kaitan diantara panjang organ-organ imposex (VDS dan RPL) dengan panjang parameter saiz cengkerang (tinggi, lebar dan apertur). Kajian ini juga memperhatikan hubungan diantara kes-kes imposex dan aktiviti manusia di kawasan persampelan. Aktiviti-aktiviti pengambilan sampel telah dijalankan di 27 tempat dari 5 negeri di sepanjang pantai barat Semenanjung Malaysia bermula November 2001 hingga April 2004. Pemilihan tempat pengutipan sampel adalah berdasarkan kehadiran *Thais* spp.

Dalam kajian ini, empat spesies *Thais* telah dipilih dan siput-siput tersebut adalah *Thais gradata*, *T. tuberosa*, *T. hippocastanum* dan *T. bitubercularis*. Siput-siput ini adalah tersebar dengan banyak di sepanjang pantai berbatu di pantai barat



Semenanjung Malaysia. *T. gradata* telah ditemui sebagai spesies paling banyak, diikuti dengan *T. tuberosa*, *T. hippocastanum* dan *T. bitubercularis*.

Kehadiran dan pembentukan imposex telah dilihat pada empat spesies *Thais* yang telah dikutip dari 27 tempat di sepanjang pantai barat Semenanjung Malaysia. Hampir 100% siput betina menunjukkan ciri-ciri imposex pada pelbagai tahap. Perkembangan imposex yang telah diperhatikan dalam kajian ini merangkumi pseudopenis dan / atau jujukan vas deferens (VDS) serta boleh dikelaskan sebagai peringkat 1, 2, 3, 4, 5, dan 6 berdasarkan skema pengkelasan VDS. Kebanyakan kes-kes imposex berlaku pada peringkat 2 (60%), diikuti peringkat 1 (19%) dan kemudian peringkat 4 (8%). Manakala yang selebihnya adalah pada peringkat 3, 5, dan 6. Tahap serius imposex, iaitu peringkat 6, menunjukkan saiz penis dan VDS telah bertumbuh 100% menyerupai saiz organ-organ seks jantan. Nilai tertinggi panjang penis relatif (RPL) yang telah direkodkan dalam kajian ini adalah 84.62%

Nisbah jantina bagi populasi-populasi yang terlibat adalah menyimpang dari nisbah yang normal. Terdapat juga hubungan nyata yang menunjukkan peningkatan RPL mengakibatkan penurunan populasi betina. Daripada pekali korelasi Pearson, pekali korelasi negatif telah diperolehi diantara tahap teruk imposex (yang diwakili RPL) dengan peratusan populasi betina pada *T. gradata* ($r = -0.0485$, $p > 0.05$), *T. hippocastanum* ($r = -0.6034$, $p > 0.05$) dan *T. bitubercularis* ($r = -0.0707$, $p > 0.05$). Tambahan pula, tidak terdapat hubungan yang jelas antara kepanjangan organ-organ imposex (penis dan VDS) dengan kepanjangan parameter saiz cengkerang (tinggi, lebar dan apertur). Kejadian imposex dan tahap pembentukan imposex di dalam individu-

individu telah didapati tinggi didalam sampel-sampel yang telah dikutip dari marina, pelabuhan dan kawasan yang tinggi aktivit-aktiviti bot dan perkapalan.

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I certify that an Examination Committee has met on 6th June 2006 to conduct the final examination of Ferdius @ Ferdaus Binti Mohamat Yusuff on her Master of Science thesis entitled “Imposex Cases in Snails (*Thais* spp.) in the West Coast of Peninsular Malaysia” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

FERDIUS @ FERDAUS BINTI MOHAMAT YUSUFF

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LIST OF ABBREVIATIONS

| | |
|-------------------|--|
| mg tin/kg | milligram tin per kilogram |
| mm | millimeter |
| MRM | River Mouth, Malacca |
| MSSBt | Sungai Sebatu, Malacca |
| MTG | Teluk Gong, Malacca |
| MTK | Tanjung Keling, Malacca |
| MTM | Tanjung Meriam Patah, Malacca |
| ng/g | nanogram per gram |
| ngl ⁻¹ | nanogram per liter |
| N | sample size |
| NOEL | No effect level |
| NPDJ | Port Dickson Jetty, Negeri Sembilan |
| NPP | Pasir Panjang, Negeri Sembilan |
| NRCEJ | Royal Custom and Excise Jetty, Negeri Sembilan |
| NTK | Teluk Kemang, Negeri Sembilan |
| NTP | Teluk Pelanduk, Negeri Sembilan |
| PkBgSgT | Bagan Sungai Tiang, Perak |
| PkTLDM | Naval Base, Lumut, Perak |
| PkTgM | Tanjung Mengkudu, Perak |
| PkTgP | Tanjung Piandang, Perak |
| PkTkB | Teluk Batik, Perak |
| PPB | Butterworth, Penang |
| PPBA | Bagan Ajam, Penang |
| PPBtM | Batu Maung, Penang |
| PPC | Custom Jetty, Penang |
| RPL | relative penis length |
| RPS | relative penis size |
| SBL | Bagan Lalang, Selangor |
| SBP | Bagan Pasir, Selangor |
| SBT | Batu Laut, Selangor |
| SKS | Kuala Sekinchan, Selangor |
| SPR | Pantai Remis, Selangor |

| | |
|--------------------------|---------------------------------------|
| SSJ | Sungai Janggut, Selangor |
| TBT | tributyltin |
| $\mu\text{g TBTO/liter}$ | microgram tributyltin oxide per liter |
| $\mu\text{g/liter}$ | microgram per liter |
| UV light | Ultra Violet light |
| VDS | vas deferens sequence |

CHAPTER I

INTRODUCTION

General Introduction

The term "imposex" is used to describe the "superimposition of male characters on to females". According to Smith (1971), "imposex" word usually refers to the formation of male genital organs, such as penis and vas deferent duct in females of dioecious snails. The unusual symptom of imposex is the masculinization of female snails (Matthiessen and Gibbs (1998). In specific, one or both of male genital organs (penis or deferent duct) are formed and developed, causing lower function of ovary (incomplete oogenesis) or transformation of ovary into testis, or transforming oviduct into prostate gland (swollen deferent duct, an organ found in male) in some cases (Smith, 1971; Gibbs *et al.*, 1987; Gibbs *et al.*, 1988). However, the reverse phenomenon to imposex, that is feminization of male snails, has never been observed in the fields.

Imposex is known to be induced by some kinds of organic tin compounds, such as tributyltin (TBT), even in a low concentration of 1ng/L or so, and independently from growth stage (age), when exposed to such organic tin compounds (Gibbs *et al.*, 1987; Bryan *et al.*, 1988; Horiguchi *et al.*, 1995; Horiguchi *et al.*, 1997a; Horiguchi *et al.*, 1997b). In a series of studies of the intertidal mud snails, *Nassarius obsoletus* (*Ilyanassa obsoleta*), the imposex condition was first linked to pollution in marinas, then antifouling bottom paints, and finally the chemical tributyltin (TBT), a major component of the antifouling paints (Smith, 1981). This was confirmed by long-term



field and laboratory experiments with *Nucella lapillus* (Gibbs *et al.*, 1986; Bryan *et al.*, 1987), which showed that the bioaccumulation of tin within the female correlated with an increase in the development of imposex.

Serious imposex would cause a lowering or loss of spawning ability. Such a lowering or loss is known to occur in three patterns: i) blockade of vulva (opening for spawning) due to increased peripheral tissues following the formation of deferent duct (Gibbs and Bryan, 1986), ii) lowering or loss of oogenesis ability, including transformation of ovary into testis (Gibbs *et al.*, 1988); and (iii) cleavage of oviduct (incomplete closing in the process of growth) or impediment to copulation and spawning due to transformation of oviduct into prostate gland (Gibbs *et al.*, 1990).

The blocking of the pallial oviduct in the imposex snails will prevent the release of egg capsules and rendering the female sterile. A high incidence of females carrying aborted capsules was found in declining populations close to sources of TBT. The build-up of aborted capsules seemed, eventually, to be lethal to the females; resulting fewer females than expected in affected areas (Gibbs and Bryan, 1986). The same authors reported that the gross morphological changes occurring in late imposex in the dogwhelk seem to be irreversible, since animals transferred from a moderately contaminated site to a "clean" site showed no resorption of the penis. If this has happened, recovery is impossible at the level of individuals (Gibbs *et al.*, 1987; Bryan *et al.*, 1986). Symptoms such as formed penis or vulva blockade will never be resolved. At the population level, however, it is thought that following reduction of organic tin pollution in an area, imposex symptom in a population will be relieved,



and the situation will be "recovered" with an increase in population (Gibbs and Bryan, 1996).

Gibbs and Bryan (1987) stated that imposex in the dogwhelk was seen in sea water with TBT concentrations of less than 1ng/L. They reported that the reproductive failure, along with a lack of juvenile recruitments, had led to population declines, almost to the point of extinction, in areas of high TBT contamination. Gibbs *et al.* (1988) showed that dogwhelks transferred from an uncontaminated area to sea water with 9-19ng/L of tin (TBT fraction) demonstrated the development of imposex within 18 months. These transferred adults were able to spawn for much of this period. The author has compared these results with results for juveniles, which developed imposex earlier and were sterile before reaching maturity. They discussed the implications for the recolonization of areas where reproduction in the dogwhelk has been eliminated. Adults are irreversibly affected by imposex. Recolonization is unlikely until the TBT levels in sea water fall to around 2 ng/L of tin, a concentration at which the juveniles are not sterilized before they reach sexual maturity. The extent of recovery of populations would, therefore, depend on the success of control measures and the water concentrations resulting from bigger ships exempt from the ban on TBT-containing antifouling paints.

So far, some hypotheses have been presented on the mechanism of inducing imposex (Crofton *et al.*, 1989). There are three types of hypotheses: (i) aromatase inhibition theory (Bettin *et al.*, 1996); (ii) cerebral ganglion inhibition theory (Feral and Le Gal, 1983), and (iii) androgen excretion inhibition theory (Ronis and Mason, 1996). Of them, the first (i) and the third (iii) theories are paying attention to steroid hormone,



and the second (ii) to neurohormone, respectively. According to the first theory, TBT will inhibit aromatase, causing an increase in concentration of internal androgen (testosterone) significantly out of proportion to estrogen (estradiol), and the excessive androgen (testosterone) will be combined to its receptor, triggering the masculinization of female (Bettin *et al.*, 1996).

In the androgen excretion inhibition theory, TBT will promote imposex by inhibiting sulfate conjugation ability, restrain androgen (testosterone) and its metabolic product from being excreted as sulfate conjugator outside the body, causing an increase in concentration of internal androgen (testosterone) to trigger the masculinization of female (Ronis and Mason, 1996). As for the second theory, naturally in female, penis formation inhibiting factor is excreted from cerebral ganglion against the penis formation accelerating factor from foot ganglion, but TBT will inhibit the excretion of penis formation inhibiting factor, causing the formation and growth of penis in female (Feral and Le Gall, 1983).

Imposex has now been observed in at least 72 species of gastropods, belonging to 49 genera (Oehlman *et al.*, 1991), and several whelks, other than *N. lapillus*, have been recommended as suitable bioindicators of TBT pollution. They include: *N. lamellosa*, *N. canaliculata* and *N. emarginata* (Bright and Ellis, 1989; Alvarez and Ellis, 1990), *Cronia margariticola*, *Drupella rugosa*, *Morula musiva* and *Thais luteostoma* (Ellis and Pattisina, 1990), *T. haemastoma* (Spence *et al.*, 1990b), *Lepsiella scobina* and *L. albomarginata* (Smith and Mc Veagh, 1991; Stewart *et al.*, 1992), *T. orbita* (Foale, 1993), *T. orbita* and *M. marginata* (Wilson *et al.*, 1993) and *L. vinosa* (Nias *et al.*,



1993). According to Horiguchi *et al.* (2001), several marine gastropods in Japan have been observed to show imposex occurrence as shown in Table 1.1.

Table 1.1: Japanese marine gastropods which have been observed to be affected by imposex (up to July 1999) (Horiguchi *et al.*, 2001).

| Mesogastropoda | |
|-----------------------|--|
| Strombidae | <i>Strombus luhuanus</i> |
| Naticidae | <i>Neverita didyma</i> |
| Oocorythidae | <i>Galeocorys leucodoma</i> |
| Cymatoodae | <i>Fusitriton oregonensis</i> , <i>Monoplex echo</i> , <i>Charonia sauliae sauliae</i> |
| Tonnidae | <i>Tonna luteostoma</i> |
| Neogastropoda | |
| Muricidae | <i>Rapana venosa</i> , <i>Ergalatax contractus</i> , <i>Ceratostoma burnetti</i> , <i>Cronia pothuauii</i> , <i>Morula musiva</i> , <i>Morula granulata</i> , <i>Morula marginatra</i> , <i>Drupella fragum</i> , <i>Thais clavigera</i> , <i>Thais bronni</i> , <i>Thais luteostoma</i> , <i>Nucella freycineti heyseana</i> , <i>Nucella freycieti alabaster</i> , <i>Nucella emarginata</i> , <i>Nucella lima</i> |
| Buccinidae | <i>Japeuthria ferra</i> , <i>Searlesia fuscolabiata</i> , <i>Pusiostoma mendicaria</i> , <i>Kelletia lischkie</i> , <i>Babylonia japonica</i> , <i>Volutharpa ampullaceal perryi</i> , <i>Buccinum middendorffi</i> , <i>Buccinum opisthoplectum</i> , <i>Neptunea arthritica arthritica</i> |
| Melongenidae | <i>Hemifusus tuba</i> |
| Nassariidae | |
| Fasciolariidae | <i>Reticunassa festiva</i> |
| Cancellariidae | <i>Fusinus perflexus perflexus</i> |
| Conidae | <i>Sydaphera spengleriana</i> |
| | <i>Virroconus ebraeus</i> , <i>Virroconus fulgetrum</i> , <i>Virgiconus lividus</i> , <i>Conus marmoreus bandanus</i> |

Importance of Study

Imposex cases have been reported as a result of TBT toxicity in gastropods. Imposex is used world wide as bioindicator for TBT contamination. TBT is an added antifouling agent in paints. It can leach out in to the environment, especially seawater, and cause TBT contamination. So far, only few information on TBT contamination (e.g. Tong *et al.*, 1996; Hashimoto *et al.*, 1998; Sudaryanto *et al.*, 2002; Sudaryanto *et al.*, 2004). Hence this study is important, by using imposex as an indicator to assess TBT contamination; therefore study on record of imposex cases in *Thais* spp. along the west coast of Peninsular Malaysia is necessary.

Moreover Malaysia is surrounded by the busiest waterways such as Malacca Straits and South China Sea. Malacca Straits is one of the oldest and busiest shipping lanes in the world. The Straits serves as a primary conduit for the movement of cargo and human traffics between Indo-European region and the rest of Asia and Australia. Strategically located to serve nearer trades within South-East Asia, Myanmar, Bangladesh, India, Sri Lanka, Pakistan, and farther trades with the Far East region, Australia/Pacific region, Africa/Mid East region and EU region, compared to the Indonesia's Macassar and Lombok Straits. As well, there are a few main ports located along the Straits of Malacca. Every year about RM3.8 trillion worth of goods and services plying through the region formed by the Straits of Malacca and other associated shipping routes. With the increase of economic activities in the East Asia region and the Pacific, the traffic in the Malacca Straits becomes busier. Therefore, these situations may lead to heavy contamination of organotins in the environment as well as their potential pollution sources.