Chapter 1

Introduction

1.1 Background and rationale

Macrobenthic fauna plays a significant role in the food chain in marine and aquatic ecosystems, acting as a link between the detritus deposited on the bottom, and the higher trophic level. These animals have limited mobility and many of them are unable to avoid adverse conditions brought about by natural stressors or human impacts. With their life span exceeding two years, they are useful indicators of environmental factors. They are sensitive indicators of environmental conditions (Bergen et al 2000, Dauer et al 2000, Summers 2001, Hyland et al 2003) and are used in integrated environmental monitoring where chemical data, toxicity testing and detailed ecology are measured, to provide a complete summary of the health of the ecosystem being investigated (Hagger and Galloway 2009).

Detection of imposex in gastropods is one such indicator. The occurrence of imposex in gastropods has been recognized as a very important biomarker of tributyltin (TBT) contamination used for monitoring on a global scale (Fent 2005). This is a serious issue in many countries world-wide because over 200 gastropods species have been shown to be affected by imposex (Shi et al 2005).

The aim of the study of imposex in gastropods in the Gulf of Thailand is to see how the prevalence of imposex varies by location in the Gulf, and also the sensitivity of each gastropod species. But in this survey, no species were found in every study area.

This made it difficult to use simple data analysis tools to compare the data. With respect to this limitation, a contamination model was used for analysis. The results indicated the current status of imposex in gastropods for each area in the Gulf, including which species were most prone to imposex.

For ecosystem management purposes, it is very important to know the environmental status in order to be able to detect any changes. Benthic animal activity has a wide influence on the physico-chemical conditions in the sediment. In addition, its diversity has a corollary for the functioning of the aquatic ecosystem. In our second study, macrobenthic fauna abundance in the Middle Songkhla Lake was studied with respect to both spatial and seasonal patterns.

In the Songkhla Lake, quantitative studies have been conducted including abundance, diversity, distributions and variations (Predalumpaburt and La-ongsiriwong 1997, Angsupanich and Siripech 2001, Ruensirikul et al 2007). However, due attention has not been given to the environmental factors affecting organism abundance. So this work has been done to study how efficient data analysis methods can be used to extract relevant new information and thus better understand the main processes affecting the current condition and possible future developments as well.

1.2 Definition of terms

Gastropods: Wallace (2009) gave the following definition, “A gastropod is a single valve (shell), soft-bodied animal belonging to the mollusk phylum. Gastropods, which are also known as univalves or gasteropods, are the largest class belonging to the mollusk family. Estimates of how many species of gastropod are alive today range from 65,000 to 90,000.”

Macrobenthic fauna: Vitaliano et al (2006) defined macrobenthic fauna as "invertebrates living in or on the sediments that are quantitatively sampled by a 0.1 m^2 Smith McIntyre grab and are retained on a 1.0 mm or 0.5 mm sieve."

1.3 Review of literature

1.3.1 Imposex in gastropods

The phenomenon of induced male sex characteristics superimposed on normal female gastropods was first named "Masculinization" or "Pseudo-hermaphroditism" and soon became commonly known as "Imposex" (Swennen et al 2009). The first observation was in the early 1970s for *Nucella lapillus* found along the coastline of the United Kingdom (Gibbs et al 1987). The occurrence of imposex correlated significantly with TBT levels (Hagger et al 2006) which are elevated close to harbour (Blaber 1970) and shipping traffic (Vos et al 2000).

Additional research showed that these morphological changes were caused by TBT (Smith 1981, Gibbs and Bryan 1986, Oehlmann et al 1993, Mensink et al 1996), and was confirmed by long-term field and laboratory experiments (Nishikawa 2006).

TBT is defined as an endocrine-disrupting or neurotoxic chemical reagent (Nakatsu et al 2006). It has been used in marine paints in order to control fouling by organisms on ships' hulls. It is leached from the surface of the paint and thus prevents attachment (Law et al 2005).

de Mora et al (1995) indicated that TBT degradation occurred with first order kinetics and that TBT in marina sediments had a half-life of about 2.5 years. In addition, TBT degradation in sediment is very slow under anaerobic conditions and remobilization occurs via mixing and dredging (Hagger et al 2006).

Bryan et al (1989) studied effects of TBT pollution on the mud snail, *llyanassa obsoleta*, from the York River and Sarah Creek, Chesapeake Bay and reported that the exposure to trace levels of 1-2 ng l^{-1} induce imposex.

Gibbs et al (1987) described six successive stages of imposex in *Nucella lapillus*. Stage 1 is formation of a deferens between the penis and the prostate, penis development stage 2 to 4, block-age of the oviduct stage 5 and 6.

Several mechanisms have been suggested to explain imposex induction. One, that TBT increases androgen levels by inhibiting the enzyme activity that metabolizes testosterone. Two, that TBT acts as a neurotoxin to abnormally release the peptide hormone penis morphogenic factor (Féral and Le Gall 1983). However, this has not yet been confirmed. There must be something else that directly interacts with TBT in the initial step of imposex induction.

Determining the occurrence of TBT in seawater is a complicated chemical procedure. However, if TBT pollution has occurred it can easily been shown by biological indicator. Even in very low concentrations, TBT creates a male/female hormonal imbalance in many organisms, which generates abnormalities in the reproductive organs, and induces change in behavior, growth, mortality, and sex ratio shifts in favour of males (Blaber 1970, Bryan et al 1987, Swennen et al 2001, Hagger et al 2006).

Imposex has been investigated in Southern Thailand, along both the western and eastern coast, since 1996 (Bech 1999, Swennen et al 1997). Several species had a high imposex incidence at several areas in the Gulf of Thailand (Swennen et al 1997).

Bech (2002) reported that imposex in *Thais distinguenda* from the east coast of Phuket Island increased significantly from 2002 to 2004 (p-value < 0.001: Chi Square test for trend). Increasing intensity of imposex was also observed for *Thais bitubercularis*. These two species are recommended as indicators of TBT in Southeast Asia because of their sensitivity and wide distribution in the region.

Kan-Atireklap et al (1997) surveyed butyltin compounds (BTs) including TBT, dibutyltin (DBT) and monobutyltin (MBT) in sediments from coastal water of Thailand in 1995. The finding suggested that the major TBT pollution in Thailand probably originated from antifouling paints used mainly on the hulls of far seas commercial vessels.

1.3.2 Macrobenthic fauna variation

Macrobenthic fauna are recognized as sensitive indicators of environmental disturbance (Pearson and Rosenberg 1978, Rygg 1985, Engle et al 1994, Weisberg et al 1997, Borja et al 2000, Ranasinghe et al 2004). The observed distribution of macrobenthic fauna has been useful in diagnostic studies and environmental monitoring (Warwick 1986).

Tropical areas of the world have some features that differ from temperate regions, where most of the techniques for assessing the biological effects of pollution on communities have been developed (Warwick and Clarke 1995).

Nizzoli et al (2002) studied the sediment characteristics and evaluated the effect of the dominant macrobenthos on sediment respiration, benthic nitrogen exchanges and nitrification rates in two sheltered sites located at opposite poles of the Sacca di Goro, a coastal lagoon of the Pole River delta. The results showed that dissolved inorganic nitrogen fluxes correlated with the biomass of the dominant species. Ammonium fluxes were significantly correlated with the biomass of macrofauna.

Armah et al (2005) studied macrofauna variation in the south-western part of the Keta Lagoon, Ghana. The physico-chemical parameter distributions were found to exhibit a high degree of similarity, but their spatio-temporal spread was mainly influenced by salinity and turbidity, which again are mainly under the influence of water depth, which by itself is probably a function of water discharge into the lagoon from streams feeding the lagoon.

Various statistical methods are useful for clustering according to patterns of variation in space and time (Hawkins et al 2000, Joy and Death 2000, Frédou et al 2006). To describe patterns of species abundance, multivariate analytical techniques such as cluster analysis and dendrograms based on similarity matrices, multidimensional scaling, and correspondence analysis, have been used extensively. Numerous benthic metrics are commonly used in assessments, and impacts to any single metric could be due to combinations of chemical and physical water characteristic parameters, such as the gradient of salinity from the sea to the river as well as the often associated sedimentary changes or variations in turbidity, including sediment physico-chemical features, making their identification complicated (Melwani and Thompson 2007).

Clarke and Warwick (1994) outlined the basic methods now commonly used by biological scientists for analysis of their data. For descriptive studies, these methods include data transformation using square roots, fourth roots, or logarithms (after adding 1 to cell counts or densities to handle zeros) to remove skewness, principal components analysis of covariance matrices, and ordination procedures to cluster taxa in space and time, as well as more complex multivariate analytical techniques such as dendrograms based on similarity matrices and multidimensional scaling. Measures of association in assemblage data such as the Bray-Curtis similarity index are preferred to Pearson correlation coefficients “for sound biological reasons” (Clarke et al 2006), but such measures do not satisfy the positive-definiteness assumptions that underpin conventional multivariate statistical analysis. Furthermore, the traditional methods used by biologists to correlate taxa abundances with environmental determinants do not generally provide standard errors for estimated parameters.

1.3.3 The Gulf of Thailand

The Gulf of Thailand is a semi-enclosed tropical sea located between UTM 520000E and 1130000E in the west-east direction and between UTM 1500000N and 680000N in the north-south direction (Figure 1.1).

The Gulf of Thailand is a gulf that borders, but is not part of the South China Sea. The average depth is 45 m and the maximum depth is 80 m. The overall area is approximately 320,000 km^2. The mouth of the Gulf is defined by a line connecting the Cape of Camau (the southernmost tip of Vietnam) and the coastal town of Tumpat in north Malaysia - near the Thailand-Malaysia border (Johnston 1998).

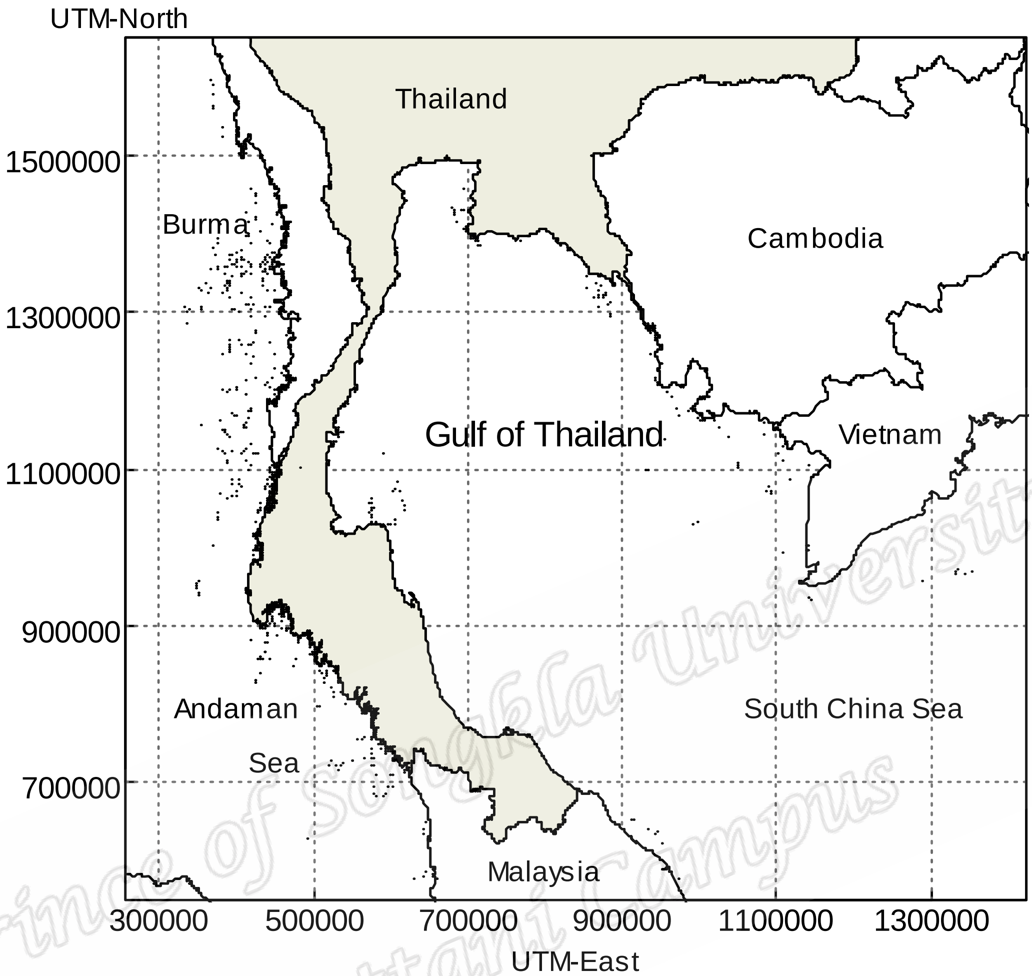

Figure 1.1: The Gulf of Thailand

The Gulf of Thailand is one of the most productive regions of the world and thus marine fisheries are an important industry for the Gulf's coastal countries. Marine fishery products have long been a significant percentage of the diet for people in this region (UNESCO 1997).

The pollutants in the Gulf come from either land-based or sea-based activities and from both point sources, such as industrial discharge, oil spill incidents, and domestic sewage, and non-point sources like agriculture runoff, all of which affect coastal water quality, marine sediment conditions, and particular organisms, mangrove, seagrass and coral reefs (Chongprasith and Praekulvanich 2003).

Using the Gulf of Thailand as a shipping route is of immense value to the coastal states. For example, the shipping volume was about 140 million tons of cargo and 2.4 million TEUs of container by over 5,000 vessels called at Thai ports in 1997. These numbers were predicted to increase by about 12% annually (UNESCO 1997). In 2004, the Gulf ports handled more than 100 million tons of cargo. The largest port (Laem Chabang on the Eastern Seaboard) handled 63 million tons. Port operations and marine transportation are sources of pollution that have impacts on marine environments and animals (Bhatt et al 2006).

1.3.4 The Songkhla Lake

The Songkhla Lake (Thai name: Thale Sap Songkhla) is the largest lake in Thailand. It is located in the southern region, on the western coast of the Gulf of Thailand. The lake covers an area of 1,040 km^2, with a width of 20 km and length 75 km approximately. It is divided into three parts: the Upper Songkhla Lake in Phatthalung, the Middle Songkhla Lake between borders of Songkhla and Phatthalung, and the Lower Songkhla Lake around Songkhla being a harbor, which is the mouth of the lake (Figure 1.2).

Some canals pour fresh water into the lake. The water around the outlet of those canals is fresh water. The salinity slowly increases where the fresh water and the sea water met. Thus, the water in the central area of the lake is brackish, and become saltier in the area near the lake’s mouth.

The Songkhla Lake is known as an important source of aquatic animals in Thailand. Several hundred species of fish have been found in the lake. Benthic fauna affect the biodiversity of aquatic animals (Angsupanich 2007). Over 160 species of macrobenthos have been recorded (Angsupanich and Kuwabura 1995). The abundance and species composition are generally higher during the late southwest monsoon (October) than during the mid-northeast monsoon (December).

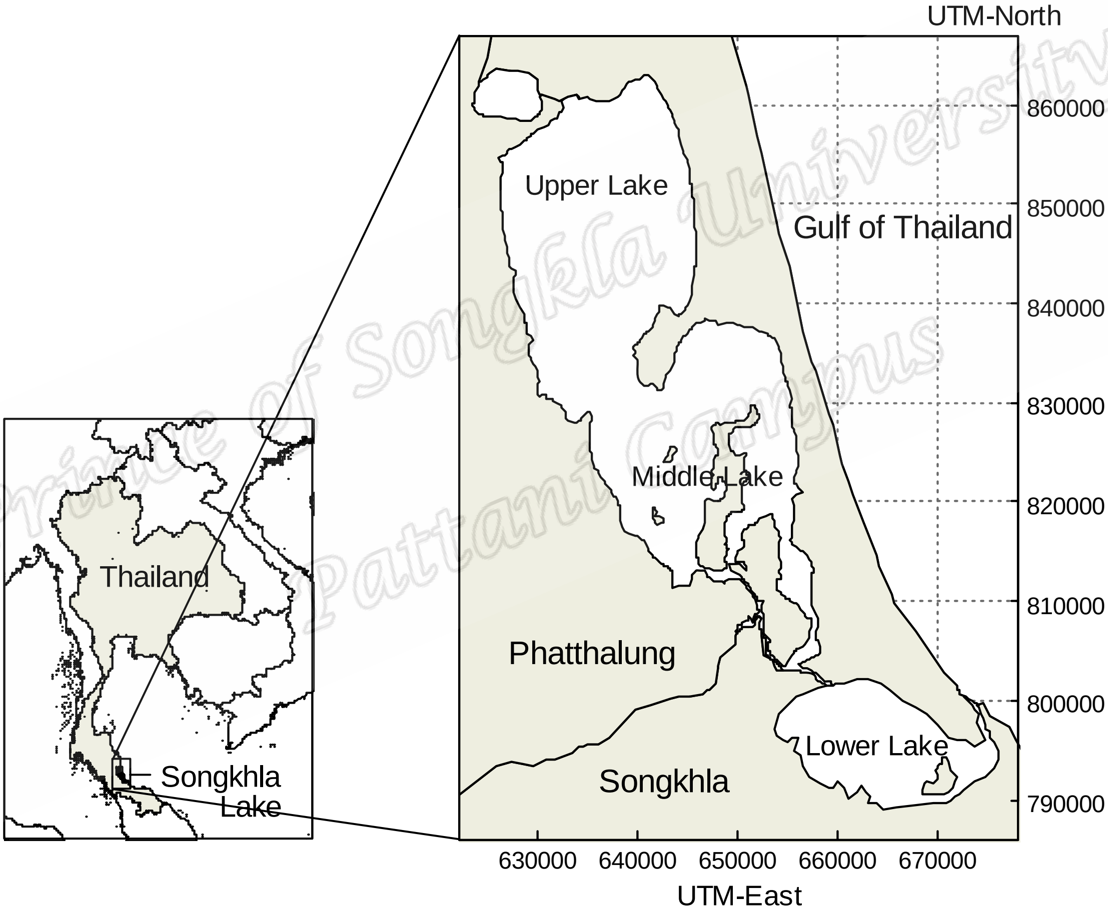

Figure 1.2: The Songkhla Lake and three zones of the lake

The lake has combined freshwater and estuarine complex of high productivity representing an extraordinary combination of environmental resources. Conflicting land use changes have degraded the watershed area and have changed the rainfall pattern and runoff, which eventually impacted the salinity patterns and water quality of the lake (Chufamanee et al 2003).

1.4 Objective

The aim of these studies was to investigate methods for modeling the health and abundance of coastal and estuarine animals in Thailand. Such statistical analysis can provide a better understanding of the effect of environmental change on such living organisms in their environment. The statistical methods apply to two outcomes: (1) the prevalence of imposex in gastropods of a size greater than 10 mm in coastal environments, and (2) the density of general macrobenthic fauna (polychates, crustacean, pelecypods and gastropods) in estuarine environments.

1.5 Publications of the thesis

Two peer-reviewed original publications are as follows:

Publication 1 (Appendix 1): Cornelis Swennen, Uraiwan Sampantarak and Nukul Ruttanadakul. 2009. TBT-pollution in the Gulf of Thailand: a re-inspection of imposex incidence after 10 years. *Marine Pollution Bulletin*, 58 (4): 526-532.

Publication 2 (Appendix 2): Uraiwan Sampantarak, Noodchanath Kongchouy and Saowapa Angsupanich. 2009. Regression-based modeling of macrobenthic fauna density in Middle Songkhla Lake, Thailand. (Manuscript)

Publication 3 (Appendix 3): Noodchanath Kongchouy and Uraiwan Sampantarak. 2010. Confidence Intervals for Adjusted Proportions Using Logistic Regression. *Modern Applied Science*, 4 (6).

1.6 Expected advantages of the thesis

The major scientific findings presented in this thesis are as follows:

(1) To provide analytical techniques in order to show how to analyse biological data collected from a large area, each with a different assemblage of species.

(2) To analyse and describe community structure of macrobenthic fauna and their relation with environmental variables.

Publication 1 identifies the prevalence of imposex in gastropods in the Gulf of Thailand. The main objectives were to (1) establish the present levels of imposex in the area; and (2) clarify possible differences in sensitivity between species. Logistic regression was used to model the effects of multiple determinants on the prevalence of imposex and also for adjusting a frequency that varies with a determinant of interest for a covariate determinant.

Publication 2 uses a multivariate multiple regression modeling to describe the variation in density over space and time of the 24 families of macrobenthic fauna. For model fitting, the response variable was taken as log $(1 + c \times \text{density})$ with the multiplier c chosen to approximate symmetry of error distributions. The environmental variables were defined as environmental factors based on factor analysis, which were then used as predictor variables in the model.

Publication 3 presents confidence intervals for adjusted proportions using logistic regression with weighted sum contrasts. The methods are applied to data from two studies, (1) imposex percentages among female gastropods at different locations in the Gulf of Thailand adjusted for different species, and (2) complication-based neonatal morbidity risk for births at a major hospital adjusted for demographic factors.

1.7 Structure of the thesis

The chapters following the introduction are organized as follows. Reviews of the methodology in the both publications are presented in Chapter 2. The application of the logistic regression model to imposex gastropods prevalence is presented in Chapter 3. Chapter 4 presents the linear regression model with application to macrobenthic fauna density. Finally, Chapter 5 summarizes the work and includes discussion and conclusions.