# Population Characteristics and Diet of Sailfin Molly *Poecilia velifer*a (Regan, 1914) (Poeciliidae: Cyprinodontiformes): an Alien Fish Invading Songkhla Lake Basin, South Thailand

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| **ชื่อวิทยานิพนธ์** | ลักษณะประชากรและอาหารของปลาสอดกระโดง *Poecilia velifer*a (Regan, 1914) (Poeciliidae: Cyprinodontiformes): ปลาต่างถิ่นที่กำลังรุกรานลุ่มน้ำทะเลสาบสงขลา |
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## บทคัดย่อ

จากการศึกษาการแพร่กระจายของปลาสอดกระโดงต่างถิ่น *Poecilia velifera* (Regan, 1914) (Cyprinodontiformes: Poeciliidae) ในลุ่มน้ำทะเลสาบสงขลา พบว่าปลาชนิดนี้แพร่ระบาดเฉพาะในหาดแก้วลากูนและทะเลสาบสงขลา (ธันวาคม 2550) เก็บตัวอย่างปลาโดยใช้อวนทับตลิ่งตาถี่จับปลาจากทะเลสาบสงขลาและหาดแก้วลากูนเดือนละ 1 ครั้ง เป็นเวลา 13 เดือนแล้วจึงนำปลาที่จับได้มาศึกษาลักษณะประชากรและอาหารในกระเพาะ ผลปรากฏว่าสัดส่วนเพศผู้ต่อเพศเมียเป็น 1.0:1.8 เพศผู้และเพศเมียถึงวัยเจริญพันธุ์เมื่อมีความยาวมาตรฐาน 16.8 และ17.1 มม. ตามลำดับ (ความยาวสูงสุด 69.8 ม.ม.) ประชากรปลาชนิดนี้สามารถสืบพันธุ์ได้ต่อเนื่องตลอดทั้งปี โดยตัวเมียแต่ละตัวสามารถให้กำเนิดลูกปลาได้ตั้งแต่ 3-252 ตัวต่อครั้ง ปลาสอดกระโดงเป็นปลาที่โตเร็ว มีช่วงชีวิตค่อนข้างสั้น อัตราการตายส่วนใหญ่เป็นผลมาจากธรรมชาติ จากการศึกษาอาหารในกระเพาะ พบว่า *P. velifera* กินอาหารหลากหลายประเภท แต่ที่พบมากได้แก่ พืช, สาหร่ายขนาดเล็ก, ครัสเตเชียน และแมลง

ผลการศึกษาบ่งชี้ว่าปลาสอดกระโดงมีความสามารถในการเพิ่มขนาดประชากรได้ดี จึงมีความเป็นไปได้ที่ปลาชนิดนี้จะเพิ่มขนาดประชากรและแพร่กระจายยึดครองพื้นที่ชายฝั่งซึ่งเป็นแหล่งอนุบาลสัตว์น้ำท้องถิ่นวัยอ่อนในลุ่มน้ำทะเลสาบสงขลาต่อไป

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## ABSTRACT


Yucatan Molly *Poecilia velifer*a (Regan, 1914) has been observed for the first time in the Haad-kaew Lagoon and the Thale Sap Songkhla, part of the Songkhla Lake Basin, south Thailand. The information on its population characteristics and diet were investigated by collecting the fish monthly for 13 months, from January 2007 – January 2008 using fine-mesh-size beach seine. Standard length, weight, sex, maturity, number of fry in gravid females and age of the specimens were determined. Age of the specimens was examined by using otolith microstructure analysis since it was proved that their otoliths exhibited daily increments. In this study, *P. velifera* began to mature when male and female reach 16.8 and 17.1 mm SL respectively. Male to female sex ratio is 1.0:1.8. *P. velifera* reproduced continuously throughout the year with two modes present in June-August 2007 and in November-December 2007. It appeared that males and females Yucatan molly displayed no different age-length relationship. In contrast, there is a significant difference in the length-weight relationship. Other population parameters were estimated as: $L_\infty$=73.58 mm SL, K=0.82 year$^{-1}$, Z=3.8 year$^{-1}$, F=1.6 year$^{-1}$ and M=2.2 year$^{-1}$. In addition, the diet of *P. velifera* was assessed by investigating stomach content analysis, using the Modified Index of Relative Importance. It revealed that this fish feed on a wide variety of food. They feed mainly on vascular plant and microalgae, and a relatively small proportion of small aquatic and terrestrial animals. Moreover, the differences of food composition between sexes, body sizes and seasons were observed. The results of this study indicate that *P. velifera* in the Songkhla Lake Basin exhibits an ability to extend their population size, adapt to fluctuated

environment, and expand their distribution range to the other rest un-invaded areas of the basin.

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Suebpong Sa-nguansil

# CONTENT

| | Page |
|---|---|
| **Chapter 1 Introduction** | **1** |
| 1.1 Background and rationale | 1 |
| 1.2 Review of Literatures | 3 |
| 1.2.1 Alien species | 3 |
| 1.2.2 Reasons for introductions | 4 |
| 1.2.3 Impact of exotic fishes | 5 |
| 1.2.4 Poeciliids | 5 |
| 1.2.5 Yucatan molly *Poecilia velifera* (Regan, 1914) | 7 |
| 1.3 Goal | 8 |
| 1.4 Structure of the thesis | 8 |
| **Chapter 2 Research methodology** | **10** |
| 2.1 Study site | 10 |
| 2.1.1 The Songkhla Lake Basin | 10 |
| 2.1.2 The Songkhla Lagoon | 11 |
| 2.1.3 The Haad-kaew Lagoon | 13 |
| 2.2 Sampling methods | 14 |
| 2.2.1 Distribution survey | 14 |
| 2.2.2 Sampling methods and data collection | 15 |
| 2.2.3 Laboratory works | 16 |
| **Chapter 3 The occurrence and distribution of Yucatan molly *Poecilia velifera* in the Songkhla Lake Basin** | **19** |
| 3.1 Introduction | 19 |
| 3.2 Materials and methods | 21 |
| 3.3 Results | 21 |
| 3.3.1 Brief identity of the *Poecilia* in the Songkhla Lake Basin | 21 |

# CONTENT (continued)


| | Page |
|---|---|
| 3.3.2 Distribution range | 22 |
| 3.4 Discussion | 26 |
| **Chapter 4 Reproductive status of Yucatan molly *Poecilia velifera* in the Songkhla Lake Basin** | **28** |
| 4.1 Introduction | 28 |
| 4.2 Materials and methods | 29 |
| 4.2.1 Reproductive traits assessment | 29 |
| 4.2.2 Data analysis | 29 |
| 4.3 Results | 30 |
| 4.4 Discussion | 32 |
| **Chapter 5 Growth and population parameters estimation of Yucatan molly *Poecilia velifera* in the Songkhla Lake Basin** | **36** |
| 5.1 Introduction | 36 |
| 5.2 Materials and methods | 37 |
| 5.2.1 Otolith validation | 37 |
| 5.2.2 Age determination | 38 |
| 5.2.3 Growth, recruitment and mortality parameters assessment | 39 |
| 5.2.4 Data analysis | 40 |
| 5.3 Results | 41 |
| 5.3.1 Otolith validation | 41 |
| 5.3.2 Age and age-length relationship | 42 |
| 5.3.3 Length-weight relationship | 47 |
| 5.3.4 Population parameters and recruitment pattern | 48 |
| 5.4 Disscussion | 51 |

# CONTENT (continued)

| | Page |
|---|---|
| **Chapter 6  Diet of Yucatan molly *Poecilia velifera* in the Songkhla Lake Basin** | **54** |
| 6.1 Introduction | 54 |
| 6.2 Materials and methods | 55 |
| 6.2.1 Stomach content analysis | 55 |
| 6.2.2 Data analysis | 57 |
| 6.3 Results | 58 |
| 6.4 Discussion | 65 |
| **Chapter 7  General discussion** | **68** |
| 7.1 *Poecilia velifera* (Regan, 1914): a potential invasive species in the Songkhla Lake Basin | 68 |
| 7.2 Trend of population status of *Poecilia velifera* to the Songkhla Lake Basin | 69 |
| 7.3 Possible impacts of *Poecilia velifera* to the Songkhla Lake Basin | 70 |
| 7.3.1 Positive impacts | 71 |
| 7.3.2 Negative impacts | 71 |
| 7.4  Suggested implication to manage *Poecilia velifera* in the Songkhla Lake Basin | 73 |
| 7.5 Future directions | 74 |
| **References** | **76** |
| **Appendix** | **85** |
| Appendix 1  Published record of *Poecilia velifera* (Regan, 1914) | 85 |
| Appendix 2  The distribution survey of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand | 86 |

# CONTENT (continued)

**Page**

Appendix 3 Morphological counts and measurement of 31 *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, Thailand 89

Appendix 4 Length frequency distribution of *Poecilia velifera* (Regan, 1914) collected from Songkhla Lake Basin, from January 2007 to January 2008 90

Appendix 5 Stomach content of *Poecilia velifera* (Regan, 1914) collected from Songkhla Lake Basin, from January 2007 to January 2008 defined by $\%IRI_{mod}$ for pool specimens and that grouped by sexes (M= male; F= female), size classes (1: SL ≤ 30.0mm; 2: 30.0mm < SL ≤ 50.0mm; 3: SL > 50.0mm) and seasons (SE= Southeast monsoon season; SW= Southwest monsoon season; NE= Northeast monsoon season) 91

**Vitae** **92**

# LIST OF TABLES


| | Page |
|---|---|
| Table 2.1 Six fixed sampling stations located in the Thale Sap Songkhla (a part of the Songkhla Lagoon) and the Haad-kaew Lagoon | 16 |
| Table 5.1 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008, fitted by von Bertalanffy and Logistic growth models | 46 |
| Table 5.2 Relative Recruitment Values of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008 | 50 |
| Table 6.1 The composition of each food category found in the stomach of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from Jan. 2007-Jan. 2008 | 59 |
| Table 6.2 MANOVA table testing the difference between diet compositions of *Poecilia velifera* (Regan, 1914) exhibited different sexes, size classes and seasons of collection (using Pillai's Trace). The specimens were collected from the Songkhla Lake Basin, from Jan. 2007-Jan. 2008 | 61 |

# LIST OF FIGURES

**Page**

Figure 1.1 Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin: (a) male, (b) female 2

Figure 2.1 Map of the Songkhla Lagoon, the main water of the Songkhla Lake Basin, south Thailand 12

Figure 2.2 Map of the Haad-kaew Lagoon, Songkhla Province, south Thailand 14

Figure 2.3 Variation in shape of Sagittal otolith dissected form Yucatan mollies *Poecilia velifera* (Regan, 1914) in different standard length. These otoliths, arranged from the smallest size to the biggest size, belong to the fish with 7.8, 15.5, 26.5, 35.4, 45.5, 55.5 and 61.0 mm standard length respectively. The smallest otolith is of a fry in a gravid female. 17

Figure 2.4 Position of otolith in bony fish (adapted from Maine In-situ Sound & Color Lab, 2003) 18

Figure 3.1 The result of distribution survey of Yucatan molly *Poecilia velifera* (Regan, 1914) in the Songkhla Lagoon and the Haad-kaew Lagoon, in December 2007 23

Figure 3.2 Habitats of Yucatan molly *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand; A: mangrove, B: flooded grassland, C: irrigation canal in shrimp farm. 25

# LIST OF FIGURES (continued)

**Page**

Figure 4.1 Sex ratios of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, in January 2007 to January 2008, divided into size classes 30

Figure 4.2 Fecundity of female Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008) 31

Figure 4.3 Proportions of female Yucatan molly *Poecilia velifera* (Regan, 1914) in each of the 3 reproductive stages collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008) 32

Figure 5.1 Two illuminated margin rings marked by Oxytetracycline, in the otolith of *Poecilia velifera* 42

Figure 5.2 Daily rings in the otolith of *Poecilia velifera* (Regan, 1914) taken from Songkhla Lake Basin. The pictures show pattern of daily ring of the fish in different body size; A: 12.2 mm standard length, B: 58.4 mm standard length 43

Figure 5.3 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008; $r^2$ = 0.9181; fitted by simple linear regression 44

Figure 5.4 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008; fitted by von Bertalanffy Growth model 45

## LIST OF FIGURES (continued)


| | Page |
|---|---|
| Figure 5.5 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008; fitted by Logistic Growth model | 46 |
| Figure 5.6 Length-weight relationship of male and female *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008, $n_{Male}$ = 1,569; $n_{Female}$ = 2125 | 48 |
| Figure 5.7 von Bertalanffy Growth Function (VBGF) and length frequency plot of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008 | 49 |
| Fig 5.8 Recruitment pattern of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008 | 49 |
| Figure 6.1 Proportion of food (%IRI) in stomachs of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008) | 60 |
| Figure 6.2 Proportion of food composition ($\%IRI_{mod}$) in stomachs of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan. 2008) comparing between sexes | 62 |
| Figure 6.3 Proportion of food composition ($\%IRI_{mod}$) in stomachs of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan. 2008) comparing between body size classes (mm standard length) | 63 |

## LIST OF FIGURES (continued)


**Page**

Figure 6.4 Proportion of food composition (*%*$IRI_{mod}$) in stomachs of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan. 2008) comparing between sampling seasons 64

# TERMINOLOGY USED IN THIS THESIS

| Terms | Definitions |
|---|---|
| brood size | the number of offspring the fish produced each time |
| cenote | a sinkhole with exposed rocky edges containing groundwater, typically found in the Yucatán Peninsula and some nearby Caribbean islands |
| fecundity | the average number of offspring containing in the reproductive track of *Poecilia velifera* |
| phenotypic plasticity | the change in phenotype of an organism in response to chances in the environment |
| sex ratio | Sex ratio is the ratio of males to females in a population (Lawrence, 2000). Sex ratio generally divided into 4 types (Wikipedia, 2010):<br>- primary sex ratio = ratio at fertilization<br>- secondary sex ratio = ratio at birth<br>- tertiary sex ratio = ratio at sexually active organisms<br>- quaternary sex ratio = ratio in post reproductive organisms |
| sexual characteristic | It is separated to be primary and secondary sexual characteristics. Primary sexual characteristics refer to the sex organ. Another one, secondary sexual characteristics are features that distinguish the two sexes of a species, but that are not directly part of the reproductive system. They are believed to be the product of sexual selection for traits which give an individual an advantage over its rivals in courtship and aggressive interactions. |

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND AND RATIONALE

Nowadays the introductions of fishes become virtually worldwide (Lever, 1998). The introductions of these exotic fishes have been done to serve several aims including aquaculture, sport, improvement of wild stocks, ornament, biological control of pest species, and also accidental introduction (Welcomme, 1988).

Yucatan molly *Poecilia velifera* (Regan, 1914) (Figure 1.1), is one of the fishes introduced worldwide. It is in the Family Poeciliidae (Order Cyprinodontiformes). Although its natural distributed is only in Yucatan Peninsula, Mexico (Hankison *et al*., 2006), it was introduced to many countries as an aquarium fish and also as a biological control agent of insects, especially mosquitoes (Courtenay and Meffe, 1989; Lever, 1996). Now, it was observed in natural water of many countries around the world.

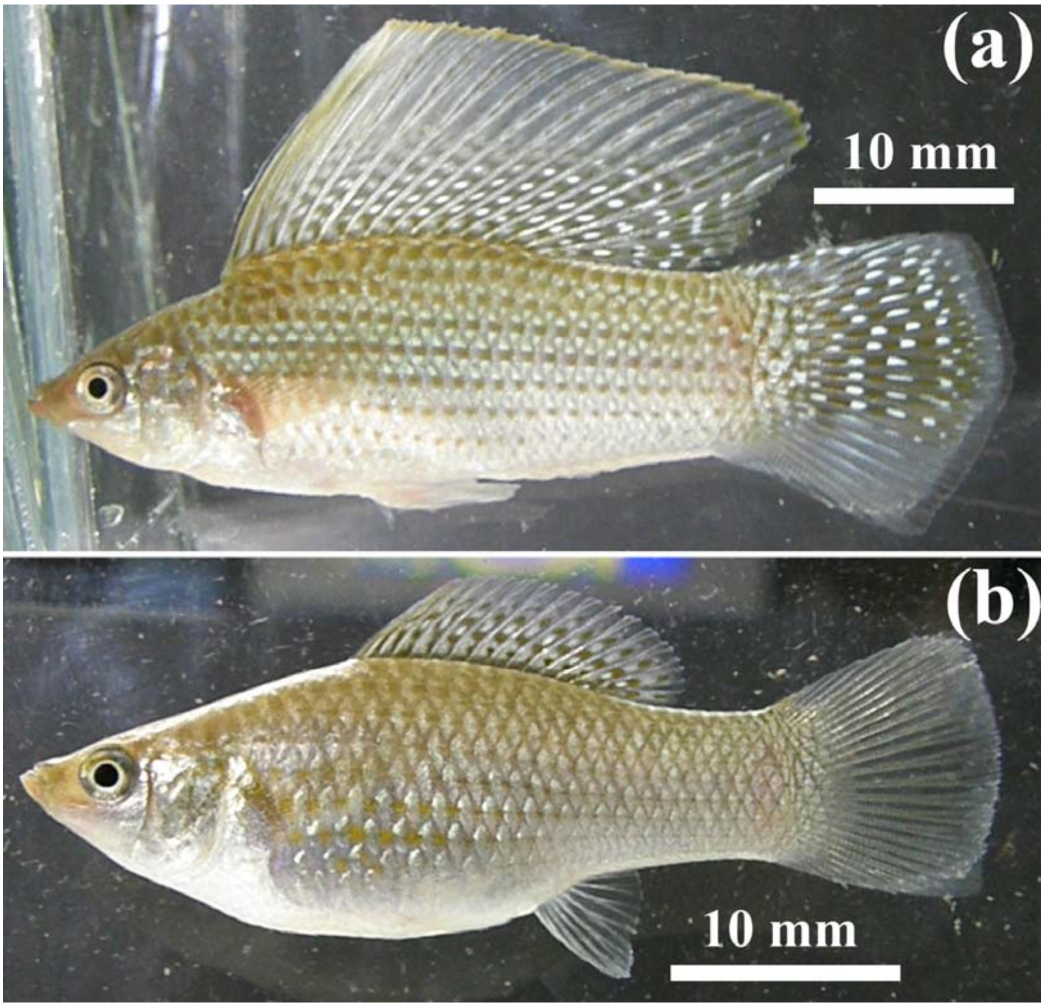


Figure 1.1 Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin: (a) male, (b) female

Although *Poecilia velifera* has been introduced worldwide, information on the population characteristics of the fish for example growth, sex ratio, size at maturity, fecundity, *etc.* is not available (see also www.fishbase.org), not even of those introduced populations but also of native ones. This information is important because Yucatan molly has great phenotypic plasticity: the environment having a big influence on its phenotype (Reznick and Miles, 1989a; Trexler, 1989a). Therefore, the life history traits of this fish are expected to differ from place to place.

Moreover, the information on feeding biology, especially diet, is also important. In the study of exotic species, information on its diet is crucial to picture the impact of its introduction (Garcia-Berthou, 1999). However, our knowledge on the diet of *Poecilia velifera* is also scarce. The relevant information on diet available is that of *Poecilia latipinna* (Lesueur, 1821), a closely related species to *P. velifera*, and those of other guppies.

This is the first known report of its presence in the Songkhla Lake Basin, south Thailand. In this study, information on the distribution range, population characteristics and diet of *Poecilia velifera* in the Songkhla Lake Basin are reported. This is the attempt to fulfill the gap of knowledge on biology of *P. velifera*. Furthermore, this information is necessary for the authorities of the Songkhla Lake Basin because it could play a significant role in planning any management program to prevent the further spread of *P. velifera* in the area effectively. Besides, in order to assess or control the impact of the fish in the Songkhla Lake Basin, this information could be useful.

## 1.2 REVIEW OF LITERATURES

### 1.2.1 Alien species

Lincoln *et al.* (1998) define "alien species" as the species occurring in an area to which is not native. In addition, there are a number of terms used in the same meaning: exotic species, introduced species, non-native species, non-indigenous species, *etc.* (Lincoln *et al.*, 1998; Welcomme and Vidthayanon, 2003). Although the definitions of these words do not mention the root of introduction, Welcomme and Vidthayanon (2003) claimed it normally is the consequence of human activity, both intentionally and accidentally.

For the introduction of fishes, although major introductions are a relatively recent phenomenon (Lever, 1998), it is believed that introduction of fishes occurred since Roman times (Balon, 1995). Since then, the transfers of many species of fishes to new localities, outside their natural range, turn out to be a worldwide phenomenon.

### 1.2.2 Reasons for introductions

Throughout the world, the introduction of fish was manipulated for a variety of purposes. The reasons of the fish introduction can be classified into 6 cases (Lever, 1996, 1998):

*Aquaculture*

Many exotic fishes play an important role in the development of aquaculture, for example, the culture of tilapias in South America and Southeast Asia and of Chinese carps worldwide. Many of these species have escaped or have been released into the wild, where in some countries they have become naturalised.

*Sport*

Many fishes were introduced to provide game fish for fishing activity. Normally, the valuable alien fishes for anglers are carnivorous fishes, for example, salmonids, trout, largemouth bass, pikeperch, *Sander lucioperca* (Linnaeus, 1758), *etc*.

*Improvement of wild stocks*

The principal objective for introducing exotic fishes for improvement of wild stocks is to establish a new commercial or subsistence fisheries. Basically, the introduction of one species usually follows by the need of introduction of another species. When piscivorous fishes, for example, are introduced to the new fish communities which have not evolved in the presence of those predators, the decline or extinction of native fishes exhibited as preys or competitors of the predators normally occur. In order to avoid this problem, the introduction of another exotic prey is required to be the replacement food fishes.

*Ornamental*

There are many fish species introduced for ornamental reason, for example, goldfish, guppy, swordtail, sucker, pompadour, arowana, cichlid, *etc.* (Axelrod *et al*., 1981). These fishes are commonly distributed outside its natural range for aquarium trade: breeding in ornamental fish ponds or keeping in aquaria. Many of these fishes have frequently escaped into the wild, or else, they are released to natural water for merit making.

*Biological control*

There were many attempts using exotic fishes as the tools for biological control. Early attempts were in the 1920s in the use of alien species to control unwanted aquatic organisms. One example is the use of the mosquitofish *Gambusia affinis* (Baird & Girard, 1853) to eliminate mosquito larvae, the vector of Malaria. Some molluscivorous fishes have been used to control of the aquatic snail vector of Schistosomiasis (bilharzia). Several species have been introduced to control excessive growths of aquatic vegetation, of which the grass carp is the prime example.

*Accident*

Many exotic fishes have become accidentally naturalised outside their natural range by escaping from captivity, natural diffusion, accidental transportation with other species, and as the stowaways in the ballast-water tanks of trans-oceanic vessels.

### 1.2.3 Impact of exotic fishes

Although Lever (1998) acknowledged that alien fishes could cause both adverse consequences and benefits, introduction is predicted to pose special problems because exotic species, which is a totally new element, is inserted into the specific ecosystem without co-evolution. For example, introductions of the fishes in genus *Tilapia* of family Cichlidae could destroy many native fauna and macrophytes of those waters in which they have become established (Lever, 1998). However, the actual consequences of introduction are difficult to predict, but they commonly include environmental disturbance, predation, competition, bringing about new disease, genetic contamination/hybridization and co-introduction of pest species (Welcomme and Vidthayanon, 2003).

### 1.2.4 Poeciliids

Poeciliids (Family Poeciliidae) are the fishes classified into the Order Cyprinodontiformes. It is a medium sized family (ca. 200 species) which is composed of killifishes, livebearer, guppies, mollies, swordtails and platyfishes. This family is a very interesting group of fishes for biologists working in a high diversity of biological fields of research ranging from sex determination and genetics of melanomas to vicariance biogeography.

As a result of their attractive and variable color, many fishes in this family become one of the most popular aquarium fishes. In addition, many countries use mosquitofish to control mosquito larvae in urban and suburban drainage and natural water. *Gambusia affinis* and *Poecilia reticulata* Peters 1859 are usually used to serve this objective.

At least, nineteen species of poeciliids have been reported as introduced species beyond their previously known historical ranges, either as the result of escapes or releases from aquaria or through introductions for controlling mosquitoes (Lever, 1996). Poeciliids, as the role of alien species, could cause many adverse impacts. It could eradicate most or all of the smaller indigenous species, both fishes and invertebrates, and could seriously affect the young of game or food fishes which is commercially important, via predation and competition (Lever, 1996). For example, in coastal Queensland streams around Brisbane where *Gambusia* , *Poecilia* and *Xiphophorus* are predominant, nine indigenous fishes in genera *Melanotaenia, Pseudomugil, Craterocephalus* and *Retropinna* are often rare or absent. In contrast, these indigenous fishes are normally abundant in the places those introduced species do not occur (Arthington *et al.*, 1981 *cited in* Lever, 1996).

Poeciliids have fascinating ability to survive and successfully reproduce in exotic warm water ecosystems. This ability is probably paralleled with cichlids (particularly tilapines), some cyprinids, and salmonids in colder waters. There was a report suggested that poeciliids were able to survive and establish their populations in new environment even in the presence of predators (Courtenay and Meffe, 1989). This probably due to the fact that poeciliids are excellent colonizers. A single gravid female can produce a new population due to superfetation (the simultaneous development of several broods within the ovary where they are nourished) enabled by the entrance and storage of sperm in the ovary. Furthermore, poeciliids typically inhabit in refuge, shallow water often partially or heavily vegetated, which could effectively protect them from most predations.

### 1.2.5 Yucatan molly *Poecilia velifera* (Regan, 1914)

Yucatan molly, *Poecilia velifera* is a valid species in *P. latipinna* complex and closely related to *P. latipinna* (Miller, 1983; Breden *et al.*, 1999) belonging to the family Poeciliidae of the order Cyprinodontiformes. The body of *P. velifera* is small to moderate, short and deep, largely compressed. Female is usually larger than male. Eyes are moderately large. Mouth is supraterminal, small and protrusible. Pectoral fins are falcate. Caudal fin is truncate or slightly lanceolate (Carpenter and Niem, 1999). It is highly sexually dimorphic; males possess an enlarged dorsal fin for female courtship display (Figure 1.1a). Dorsal-fin position is far forward, its origin is in advance of pelvic fin, consisting of 16-19 (rarely 15) typically 17 or 18 soft rays. Length of dorsal-fin base is 0.5-1.7 times of predorsal distance. There are 9 or fewer predorsal, 20 circumpeduncular and typically 26 or 27 lateral scales. Adult male with a submarginal row of elongate darkblotches in the interradial membranes of dorsal fin, and round pale spots in the proximal two-thirds of the fin. Inner teeth are conic (Miller, 1983).

Parenti (1994) stated that there is no larval stage in poeciliids. Their individuals are sexually mature in just over a month after hatching.

Mollies are constant feeder. They can feed on prey at the water surface and algae on the bottom surface (McInerny and Gerard, 1989). Their habits vary. Some are peaceful and some, especially large male, are bullies (Axelrod *et al.*, 1981)

Size distribution is also important in the population study of poeciliids. The amount of size variation within a population is unrelated to any complex of abiotic factors (salinity, conductivity, water temperature, degree-days for the region, habitat type, abundance of submerged macrophytes and degree of tidal influence) (Travis and Trexler, 1987). However, size variation in females appears to be a good index of fecundity variation. Populations with larger females have higher per-capita fecundity rates. Thus female size distribution can reveal much about the status of a molly population (Travis and Trexler, 1987).

## 1.3 GOAL

The goal of the present study is to assess the population characteristics of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin. The information obtained from the present study will be used as baseline data for gaining the insight to interpret future ecological changes correctly, to distinguish the potential impact of *P. velifera* invasion and to judge what should be done to control its population and/or its adverse impact.

## 1.4 STRUCTURE OF THE THESIS

This thesis is an attempt to provide the information on the current distribution, reproductive status, population status and diet of the *Poecilia velifera* invading the Songkhla Lake Basin. The current distribution range is shown as the map of the occurrence. Reproductive status of the fish is indicated by assessing their length at first maturity, sex ratio, fecundity and reproductive season. In order to age the fish using otolith microstructure, otolith validation was performed to prove that the otolith of *P. velifera* deposit daily increments. Then a set of population parameters including age-length and length-weight relationship, growth parameter (asymptotic length-$L_\infty$; growth coefficient-K), mortality and recruitment pattern were investigated.

This thesis is organized into chapters in the way that is easy to understand. Chapter 2 shows sampling methods and some laboratory works that its results were mentioned in the next chapters (standard length, body weight, sex, maturity, pregnancy stage, brood size). Other specific procedures were mentioned in Chapter 3 – Chapter 6 which contained the results and discussions divided into sub-topics. Chapter 3 discusses the occurrence and distribution of *Poecilia velifera* in Songkhla Lake Basin. Chapter 4 focuses on the reproductive aspect. Chapter 5 deals with growth and population parameters estimation. And Chapter 6 talks about its diet.

This thesis ends up with general discussion in Chapter 7 talking about overall results and their implication.

# CHAPTER 2

# RESEARCH METHODOLOGY

## 2.1 STUDY SITE

### 2.1.1 The Songkhla Lake Basin

The Songkhla Lake Basin is located in southern Thailand. It lies in 3 provinces which are Nakorn Si Thammarat Province (2 districts), Phattalung Province (11 districts) and Songkhla Province (12 districts). The basin covers approximately 8,729 sq km consisting of land area (7,687 sq km) and lagoon area (1,082 sq km) (International Lake Environment Committee, 2009). The basin spans about 150 km from north to south, and about 65 km from east to west.

The Songkhla Lake Basin is influenced by the tropical monsoon climate, with an average water temperature is 29.7 °C (Angsupanich, 1997). Average annual rainfall is over 2,000 mm. There are 3 seasons in a year according to seasonal wind directions. The first one is the intermediate rainy period influenced by strong south-west monsoon (May-September). Next is the rainy period which brings in more than 60% of the rain, influenced by north-east monsoon (October-December). The last one is the relatively dry period influenced by south-east predominant wind (January-April) (Evenson, 1983).

In this study, *Poecilia velifera* (Regan, 1914) was collected from the Songkhla Lagoon (the main water in the Songkhla Lake Basin), and Haad-kaew Lagoon (a small lagoon in the basin).

### 2.1.2 The Songkhla Lagoon

The Songkhla Lagoon is the main water of the Songkhla Lake Basin. It is huge, approximately 1,082 sq km in area (International Lake Environment Committee, 2009), situated between 7° 8' N and 7° 48' N, and between 100° 7' E and 100° 35' E. It is a shallow lagoon opening to the Gulf of Thailand with three water regimes: fresh water, brackish water and salt water. The lagoon is usually divided into four distinct parts including Thale Noi, Thale Luang, Thale Sap and Thale Sap Songkhla, arranged respectively from north to south (Figure 2.1)

The Songkhla Lagoon receives fresh water from precipitation, surface water run-offs from land mixing with salt water from the sea. This feature makes the Songkhla Lagoon a large lagoon system. The mixing of fresh water and saline water in the Songkhla Lagoon causes the water salinity to vary spatially and temporally. Understandably, in rainy seasons, the area of fresh water extend while in the dry season more saline water intrudes further north into the lagoon.

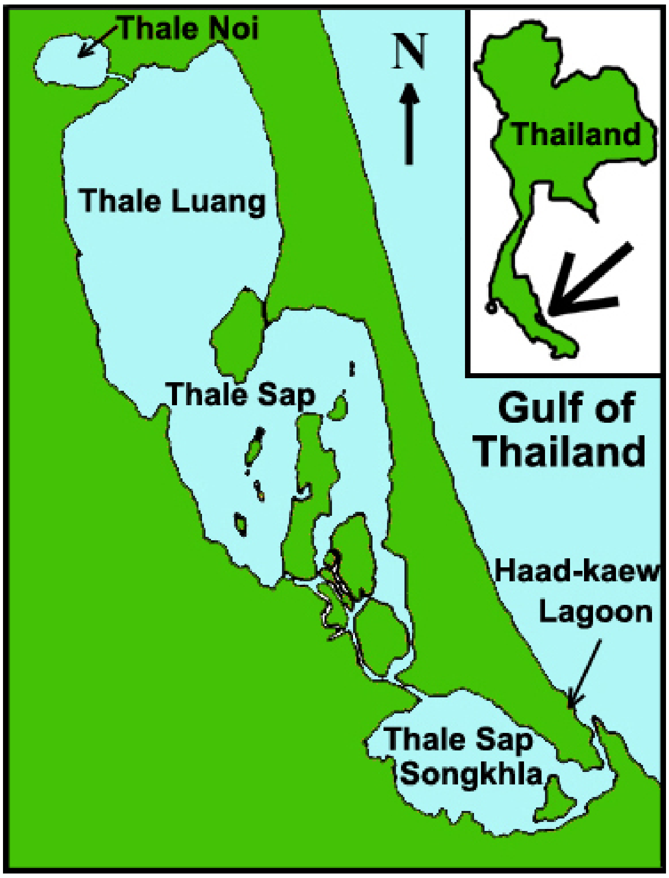


Figure 2.1 Map of the Songkhla Lagoon, the main water of the Songkhla Lake Basin, south Thailand

Water salinity varies greatly throughout the different seasons and different parts of the lagoon. The fluctuation of rain fall throughout a year plays an important role in temporal variation of salinity in a year. Furthermore, spatial variation of water salinity also occurs. Water salinity ranges from 0-32 ppt in the outermost part (Thale Sap Songkhla), and 0-3.5 ppt in the innermost part (Thale Noi) (Angsupanich and Rakkheaw, 1997; Asean Regional Centre for Biodiversity Conservation, 2010).

The average depth of the lagoon is 1.2 m, over the most areas, with the deepest parts approximately 8.0-8.8 m in channels and in the mouth of the lagoon (Angsupanich and Rakkheaw, 1997). There are, however, considerable fluctuations in water level. At the peak of northeast monsoon season, at the Thale Sap, the average depth is 1.5 m. Water levels will retain like this and decline to around 0.5 m within two months.  The average tidal ranges at the mouth of the lake is approximately 0.5 m, with maximum 1.3 m, at the Thale Sap, and the tidal ranges can be 0.03-0.10 m, and nil at Thale Luang and Thale Noi.

The Songkhla Lagoon is surrounded by a mixture of urban areas, fishing villages, shrimp ponds, seasonally flooded forests, paddy fields and mangroves.

### 2.1.3 The Haad-kaew Lagoon

The Haad-kaew Lagoon is a lagoon located on the eastern coast of Sathing Phra Peninsula, opening to the Gulf of Thailand at the mouth of Songkhla Lagoon (Figure 2.2).  It is much smaller comparing with Songkhla Lagoon, located between 7° 14' N and 7° 15' N, and between 100° 32' E and 100° 34' E.  The Haad-kaew Lagoon is a relatively small, opened coastal lagoon, about 2.7 km in length and 300 m maximum width.  This lagoon was splitted by sand bar into two parts: the seasonally-closed part in the north and the open part in the south. Geomorphologically, the lagoon is a type of bar build estuary, with a sand dune extending from part of the Sathing Phra Peninsula.  The estuary is mostly shallow, average water depth is about 1.9 m, except the main channel depth ranging 2-5 m in the navigational portion of the system.  During the period of northeast monsoon season, water level in the lagoon is consistently high, approximately +2.0 m above the lowest water level, and persisted for at least 3-4 months (December-March), due to strong wind and wave on the seaward and water runoff from the innermost.  However, following southwest and southeast predominately wind, the lagoon subjects to the tidal cycle, which is diurnal and/or mixed tide, ranging from 0.7-1.7 m above the lowest water level, which varied from time to time due to water runoff and wind.  The Haad-kaew Lagoon is surrounded by fishery villages, Songkhla Deepsea Port and small degraded mangroves.  *Cassurina, Barringtonia*, *Anona grabra,* and some mangrove swamp species such as *Aviciennia marina* and *Rhizophora apiculata etc.* are the predominant fringing species along the inner shoreline.

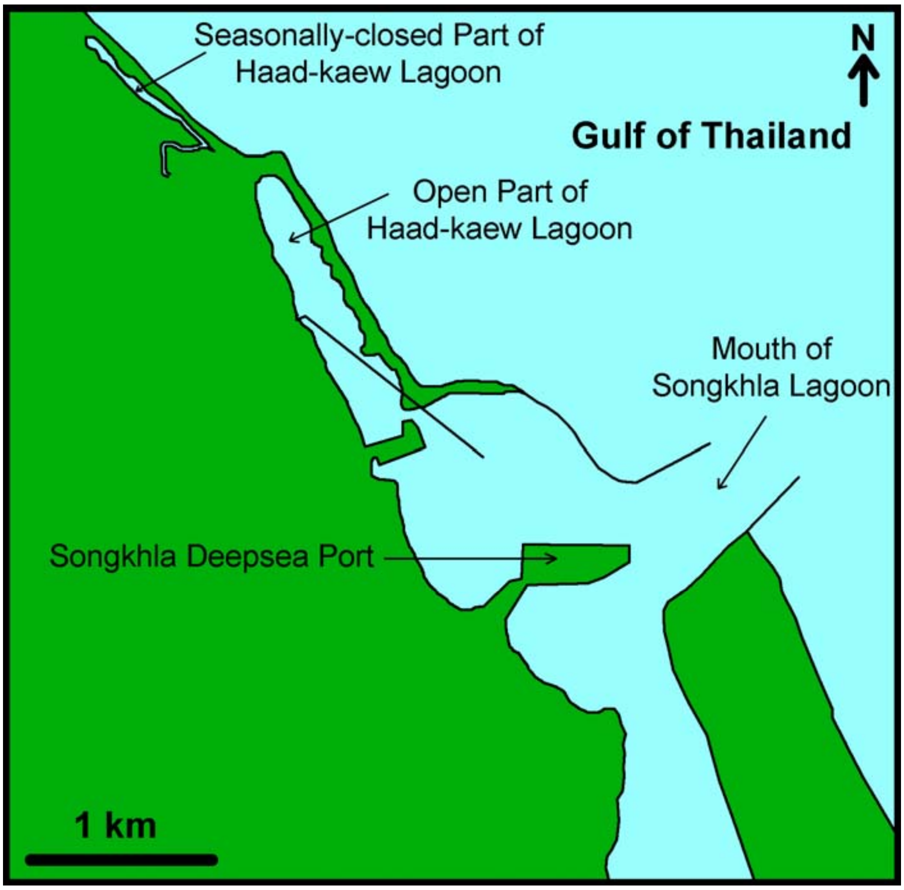


Figure 2.2 Map of the Haad-kaew Lagoon, Songkhla Province, south Thailand

## 2.2 SAMPLING METHODS

### 2.2.1 Distribution survey

A preliminary survey of the distribution range of *Poecilia velifera* in the Songkhla Lake Basin was conducted in December 2007 by traveling around the Songkhla Lagoon and the Haad-kaew Lagoon by land and by boat. To determine presence/absence of *P. velifera*, multiple sites in the two lagoons were scanned by visual observation where the water was clear, or sampled with a beach seine (4.2 m×1.3 m with 0.5 cm mesh size) and a cast-net (2.4 m diameter with 2.5 cm mesh size) where it was turbid water. In a site with clear water, the fish can be easily detected when present especially in high number because it is typically surface-dwelling fish that tend to congregate. For the site deeper than 1 m, a cast net was operated to confirm the existence of the fish. This survey was performed not only to specify current distribution range but also to find fixed sampling stations for the study of population characteristics and diet (the criteria used to consider appropriate sampling stations were mentioned in section 2.2.2).

### 2.2.2 Sampling methods and data collection

Bag beach seines (5.0 m×1.2 m with 0.5 cm mesh size) was used to collect *Poecilia velifera* from 6 fixed stations (table 2.1; see also station 2,3,4,7,10 and 11 in Fig 3.1B). These 6 sites were where the fish was known to be abundant and the collecting gear was considered appropriate for the particular habitat, coastal shallow water near vegetated areas. The samples were collected monthly for 13 months (January 2007 to January 2008). A sub-sample, varying in body sizes, was kept immediately in 10% formalin each station each month. These fish samples were kept for the stomach content analysis (Chapter 6). The remaining fish were kept in an ice box while traveling and later frozen in a refrigerator in laboratory. These samples were prepared for the study of reproductive status and population parameters (Chapter 3 – Chapter 5).

Table 2.1  Six fixed sampling stations located in the Thale Sap Songkhla (a part of the Songkhla Lagoon) and the Haad-kaew Lagoon

| Station | Station Name | Record coordinates | |
|---|---|---|---|
| | | Latitude | Longitude |
| 1 | Klong Pa-wong | 7º08'31"N | 100º33'26"E |
| 2 | Klong Khwang | 7º09'04"N | 100º34'03"E |
| 3 | Ban Kok Rai | 7º09'57"N | 100º35'02"E |
| 4 | Ban Bor Ang | 7º13'01"N | 100º32'03"E |
| 5 | Ban Khao Nui | 7º11'49"N | 100º33'34"E |
| 6 | Seasonally-closed Haadkaew Lagoon | 7º15'12"N | 100º33'00"E |

### 2.2.3 Laboratory works

In the laboratory, every frozen specimen was given an individual code indicating the month and the station it was collected, and specific number. Then body size was measured as standard length (SL) to the nearest 0.1 mm.

Fish in each station each month were randomly selected and then body weight, sex, maturity and brood size were determined. Body weight was the weight without viscera, to the nearest 0.01 g. Sex and maturity were determined. Mature males were the fish gonopodium completely developed, and *vise versa* were immature males. Mature females were the fish eggs or embryos present in the enlarged ovary. Females that standard length were 30.0 mm or longer but there was no embryos in the ovary were assumed as not pregnant mature females. The rest immature fish were sexed by gonad identification. To evaluate brood size of gravid females, the numbers of embryos present were counted since it is live-bearing fish: fertilization and development occur within ovaries.

A pair of the Sagittal otoliths (Figure 2.3, 2.4) were dissected and removed from each selected specimen. These otoliths are called Sagittae (because of the arrow-head shape). They then were cleaned in tap water, submerged in ethanol for a few seconds and left dry on towel paper. Finally, these otoliths were kept separately in small zip bag for further age determination process.


Figure 2.3 Variation in shape of Sagittal otolith dissected form Yucatan mollies *Poecilia velifera* (Regan, 1914) in different standard length. These otoliths, arranged from the smallest size to the biggest size, belong to the fish with 7.8, 15.5, 26.5, 35.4, 45.5, 55.5 and 61.0 mm standard length respectively. The smallest otolith is of a fry in a gravid female.

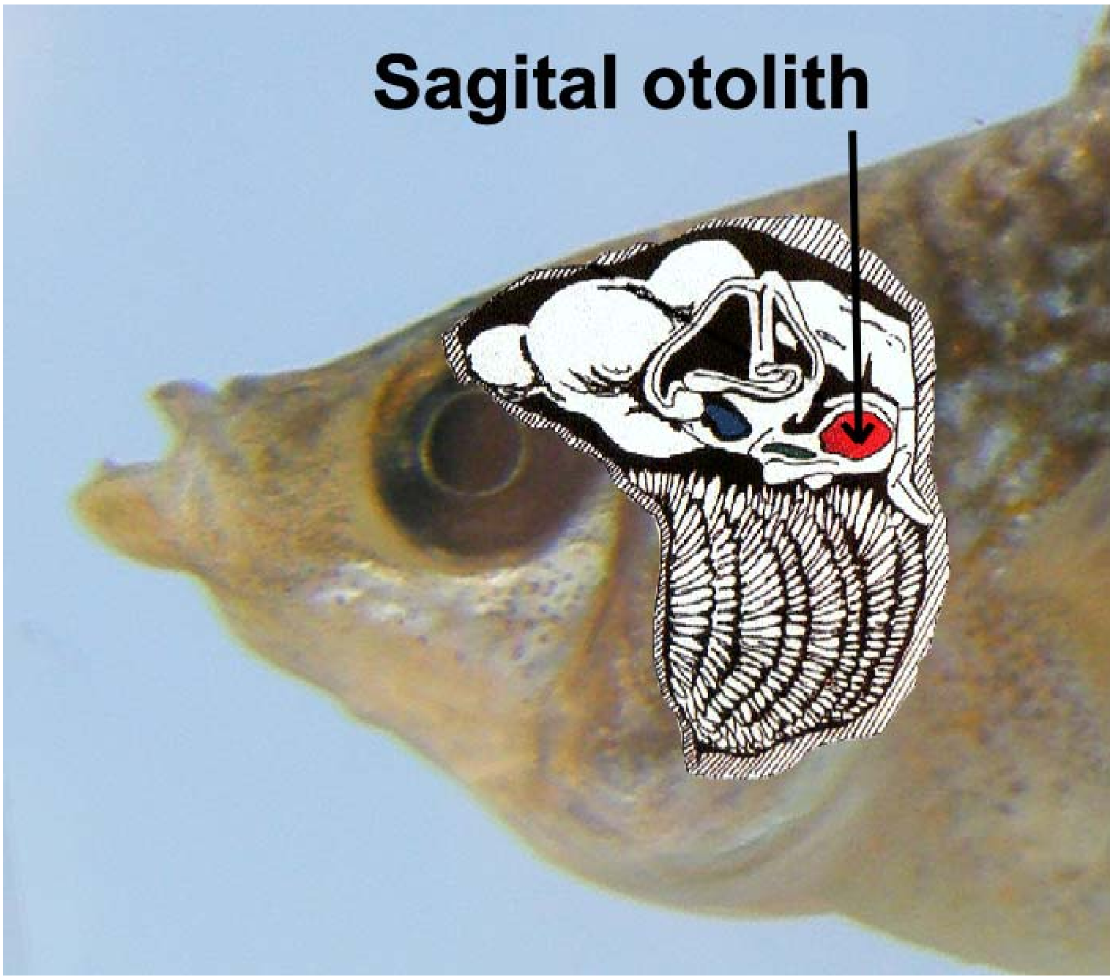


Figure 2.4  Position of otolith in bony fish (adapted from Maine In-situ Sound & Color Lab, 2003)

# CHAPTER 3

# THE OCCURRENCE AND DISTRIBUTION OF YUCATAN MOLLY *POECILIA VELIFERA* (REGAN, 1914) IN THE SONGKHLA LAKE BASIN

## 3.1 INTRODUCTION

The Yucatan molly, *Poecilia velifera* (Regan, 1914), is a member of the Poeciliidae, a large family of small-bodied fishes native to fresh and brackish waters of the New World and parts of Africa.

*Poecilia velifera* is from Central America and native to the Yucatan Peninsula, Mexico (Hankison *et al.*, 2006) where it typically occurs in coastal brackish waters of Campeche, Yucatan and Quintana Roo City, and the nearby islands of Mujeres and Cozumel (Miller, 1983). It has been introduced to many countries around the world via the aquarium fish trade and also as a biological control of insects, especially mosquitoes (Courtenay and Meffe, 1989; Lever, 1996). There are confirmed and unconfirmed reports of non-native *P. velifera*, or possible hybrids, as collected or observed at one or more sites in North America: Florida; South America: Brazil, Colombia and Peru; Asia: Israel, Singapore, Taiwan, Vietnam and Thailand (Welcomme, 1988; Ng *et al.*, 1993; Fuller *et al.*, 1999; Shen, 1993; Kuo *et. al.,* 1999; Golani, 2000; Magalhães *et al.*, 2002; Vidthayanon and Premcharoen, 2002; Welcomme and Vidthayanon, 2003; Ferriter *et al.*, 2006; Ortega *et al.*, 2007). Some, not all, represent established populations.

*Poecilia velifera* was first introduced into Thailand in approximately 1960 for sale in the aquarium trade (Welcomme and Vidthayanon, 2003; Nico *et al*., 2007). Later, it was reintroduced from Taiwan in 1987 for controlling algal flocs in shrimp ponds in the inner Gulf of Thailand (Welcomme and Vidthayanon, 2003).

After that, its nourishing population has been reported in 2002 (as *P. sphenops*) in the estuary mouth of the Chao Phraya River, inner Gulf of Thailand (Vidthayanon and Premcharoen, 2002; Nico *et al*., 2007).

Poeciliids normally live in small shallow bodies of water (springs, marshes, and ponds), or shallow marginal areas of larger water (rivers and lakes) (Meffe and Snelson, 1989). These areas are typically slow-water habitats, often partially or heavily vegetated, or with other cover.

In case of *Poecilia velifera*, it is restricted to coastal habitats, such as anchialine cenotes, tidal pools and salt marshes, never higher than *ca.* 20 m above sea level (Hankison *et al*., 2006). Their preferred habitats is high vegetated area which can help them not to be hunted easily (Chick and Mlvor, 1997) Populations of *P. velifera* are found in substantially lower flow regimes where they experience a wider range of piscine predators (not only cichlids, but also many marine fishes, e.g. *Megalops atlanticus* Valenciennes 1847, *Arius* spp., *Strongylura* spp., *Lutjanus* spp., *Gobiomorus dormitor* Lacepede 1800 and *Centropomus* spp.) and waders, such as storks, herons and egrets as well as crocodiles such as *Crocodylus moreletii* and *C. acutus* (Schmitter-Soto *et al*., 2002).

From the preferred habitats of *Poecilia velifera* mentioned above, these habitats seem to be typical in marginal area of the Haad-kaew Lagoon and the Songkhla Lagoon. Therefore, *P. velifera* is expected to be able to invade the entire lagoons.

Since the information on distribution of *Poecilia velifera* which is important for managing a plan to prevent further dispersion is not available, in this chapter, the information on the occurrence and current distribution range of *P. velifera* in the Songkhla Lake Basin was investigated. The occurrence and the information on its profitability were conducted by interviews. In addition, the preliminary survey for the distribution of *P. velifera* was conducted along coastal area of Songkhla Lagoon and Haad-kaew Lagoon.

## 3.2 MATERIALS AND METHODS

Some male Yucatan molly were collected from the Thale Sap Songkhla for describing its external characteristics to confirm that this fish is actually *Poecilia velifera*. Later, a preliminary survey of the distribution range of *Poecilia velifera* in the Songkhla Lake Basin was performed (see section 2.2.1).

Furthermore, in order to assess more information on the occurrence and benefit of the fish to local people, the interviews were conducted. There were 35 interviews had been carried out. A few *P. velifera* including both males and females were caught and kept in a clear bottle. This sample was used to show the interviewees who were local fishermen fishing in the area, or some villagers lived in the area. Then a set of questions was used to gain the information from the interviewees. Those set of questions included as following:

- Have the interviewees ever seen *P. velifera* (the fish in the bottle) in their area?
- When was the first time the interviewee recognized this fish in the area?
- Is this fish of any use or benefit?

## 3.3 RESULTS

### 3.3.1 Brief identity of the *Poecilia* in the Songkhla Lake Basin

A total of 31 mature males of *Poecilia*, with lengths ranging from 31.5 to 56.7 mm SL, were captured from the Thale Sap Songkhla in the SLB for a brief examination of identity. These were registered and deposited at Prince of Songkla University Zoological Collection (PSUZC-20100204.01). They are relatively small fish. Body slender, laterally compressed; body depth 2.5-2.7 in standard length.

Mouth is normal, relatively small, supraterminal and protrusible. The origin of dorsal fin ray is well in advance of pelvic-fin insertion, 16-19 dorsal fin rays. Males have very large dorsal. Length of dorsal fin base is 0.8-1.6 times of predorsal distance. The pelvic fins well developed, tips of pelvic fins are swollen, smooth and approaches roundness. Gonopodium, from ventral surface of ray 3, large, fleshy palp usually arising that may envelop ventral half of gonopodium. Subdistal segments of gonopodial ray with spines and processes. Scales small, cycloid and relatively deciduous 27-29 in lateral series, 9 predorsal and 20 circumpeduncular scales. Mature males develop brilliantly colored fin ornaments, a very large dorsal. Body is marmorated velvet white and grey in color, dorsal and upper half of caudal fins are marked with small, round light dots. Based on meristic data, these specimens were identified as Yucatan molly, *Poecilia velifera* (Regan, 1914).

### 3.3.2 Distribution range

From the survey along the coast of Haad-kaew Lagoon and Songkhla Lagoon, It was found that *Poecilia velifera* was presented in both parts of Haad-kaew Lagoon (Figure 3.1: Station 7, 8) and only in the Thale Sap Songkhla - Songkhla Lake Basin. For the Thale Sap Songkhla, the distribution of the fish was found ranging from Ban Hua Khao Daeng (Station 9: 7° 13′ 04″N 100° 34′ 28″E) to Ban Bor Ang (Station 11: 7° 13′ 01″N 100° 32′ 03″E), and Ban Tha Sa Arn (Station 1: 7° 10′ 57″N 100° 35′ 37″E) to Ban Pak Ror (Station 17: 7° 15′ 03″N 100° 26′ 03″E). The fish was also observed around Koh Yor (Station 12) , a big island located in the southeast part of the Thale Sap Songkhla (Figure 3.1; Appendix 2). Very dense populations of *P. velifera* were observed in the seasonally-closed part of the Haad-kaew Lagoon (Station 7), the stations near fishery villages (Station 10 and 11), and the stations near Songkhla City (Station 1, 2 and 3). In the west coast of the Thale Sap Songkhla, on the other hand, the fish was observed only in Station 17 with a very low density.

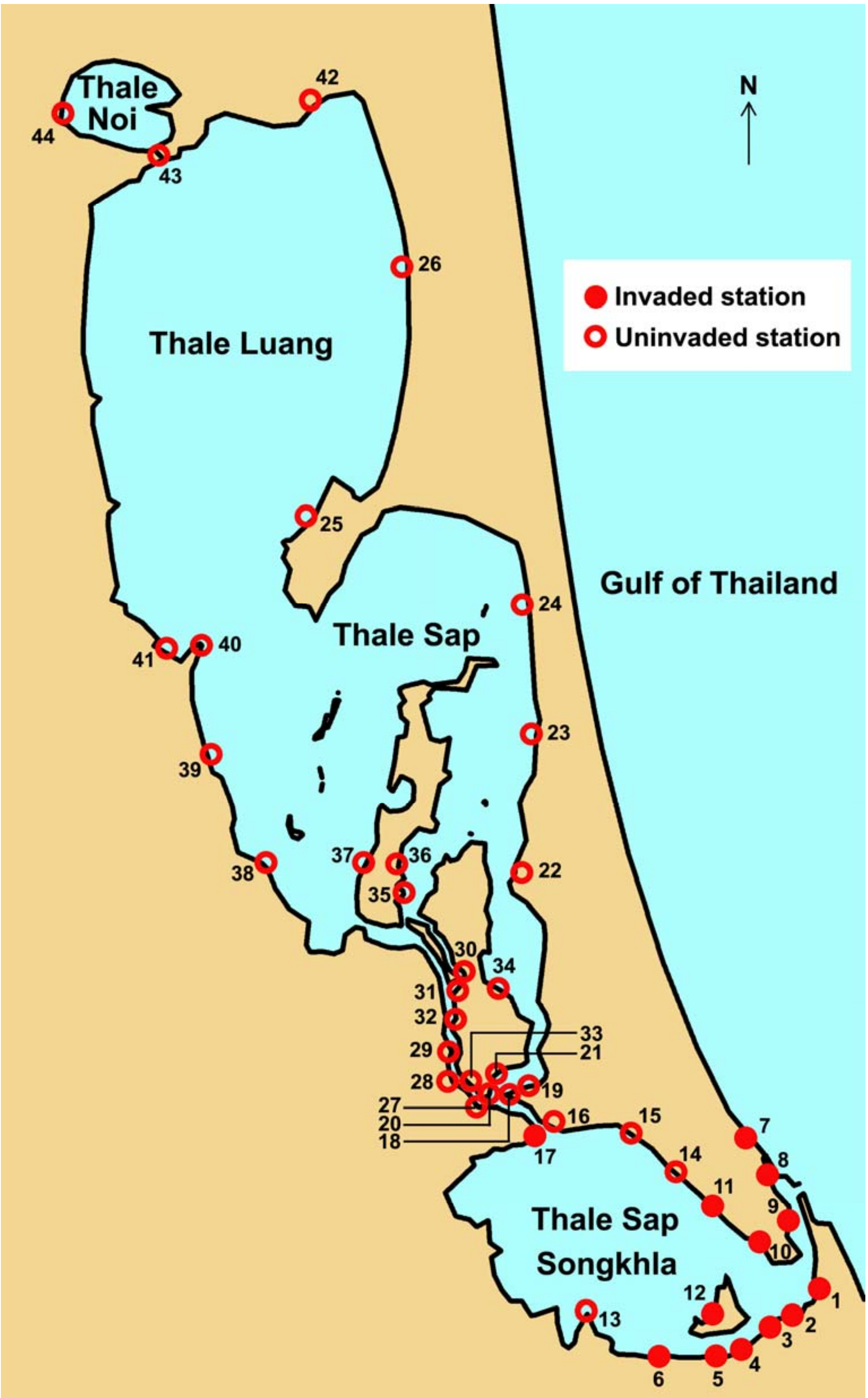


Figure 3.1 The result of distribution survey of Yucatan molly *Poecilia velifera* (Regan, 1914) in the Songkhla Lagoon and the Haad-kaew Lagoon in December, 2007 (see Appendix 2)

*Poecilia velifera* established in near-shore shallow water, not more than 1 m deep (personal observation). The preferred habitats were sheltered areas such as mangroves, flooded grassland and shallow lakesides with garbage (Figure 3.2). However, they were also found in un-sheltered habitats such as the very shallow near-shore habitats or irrigation canals in shrimp farms.

This fish usually congregated into several small schools dwelled near the water surface. Each school composed of one or a few males and several females. This was very easy to notice if it was clear water. In turbid water, the movement of their schools caused the disturbed water surface especially in the place with dense population.

From the interviews, it was found that local fishermen did not know when *Poecilia velifera* was first introduced to the Songkhla Lake Basin. In 2002, however, some interviewees experienced a large numbers of *P. velifera* in most of their fishing traps placed in the lakeside area. In addition, the interviewees said that this fish was not being used to control algae in shrimp ponds and mosquito larva in the area.

The interviewees claimed that *Poecilia velifera* had no commercial importance for local people. Fishermen did not intend to catch this fish for food or for other benefit. However, a fisherman had caught them for selling in fresh market but there was no one interested in buying them as food. Another fisherman had tried this fish as well but he mentioned that its taste was not attractive.

Some fishermen stated that the occurrence of this fish in the Thale Sap Songkhla was troubling their job. When their gill nets were being placed in the near-shore area to catch mullets *Liza* spp. and mud crabs *Scylla* spp., a lot of *Poecilia velifera* were caught. The vibration of the gill nets caused by that *P. velifera* could let mullets aware the position of gill nets. When the fishermen took that gill nets back home, in addition, it took times to untie them from the gill nets.

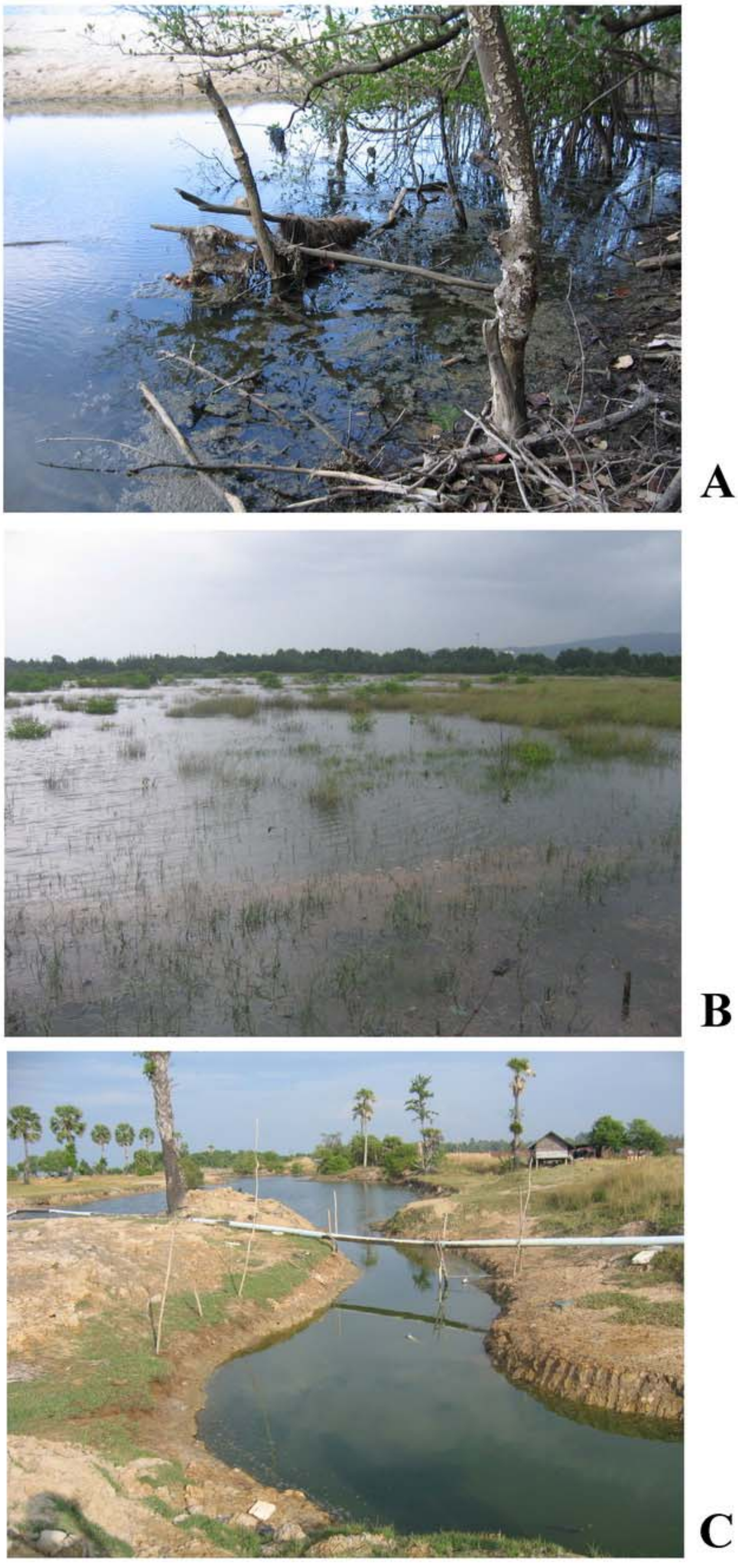


Figure 3.2  Habitats of Yucatan molly *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand; A: mangrove, B: flooded grassland, C: irrigation canal in shrimp farm.

## 3.4 DISCUSSION

On the basis of the morphology of the gonopodium, the number of circumpeduncular scales and fin ray counts, it was determined that the introduced molly in the Songkhla Lake Basin belong to the species *Poecilia velifera* (Regan, 1914).

The occurrence of *Poecilia velifera* in the Songkhla Lake Basin had been observed by local fishermen for several years. They knew that this fish was not native species. However, they did not realize alien species may cause adverse consequences to ecosystem.

In the Songkhla Lake Basin, *Poecilia velifera* was not being used to control mosquito larvae or control algae in shrimp ponds as in estuary mouth of Chao Phraya River (central Thailand) mentioned in Welcomme and Vidthayanon (2003). In addition since this fish established in the Songkhla Lake Basin, local people did not tend to eat this fish. This might be the result of that the poeciliid fishes did not the native species in Thailand and these fish were not imported into Thailand as being food, therefore, local people did not familiar with eating it.

There is no documentation to ascertain when and how *Poecilia velifera* was introduced to the Songkhla Lake Basin. From the information obtained by interviewing local fishermen in the present study, however, it suggests that *P. velifera* was first introduced to the Songkhla Lake Basin before the year 2002, the year they experienced its nourishing populations. It is possible that the source of *P. velifera* invading the Songkhla Lake Basin was household aquarium. Mollies are ones of the most popular aquarium fishes in Thailand. They are sold not only in professional aquarium and pet shops but also in market fair. These fishes become popular because they exhibit high variety of attractive color patterns. In addition, some mollies that have not favored colors were sold for being lived food for other carnivorous aquarium fishes such as Arowana for example. This suggested that the first introduction of *P. velifera* into this area might be that escaped from household aquarium or that were released to do a merit making or that were release because the owner wanted to stop keeping them. Possibly, the places that this fish was first introduced into the area were the seasonally-closed part of the Haad-kaew Lagoon or the urban area of Songkhla City where dense populations of *P. velifera* were observed, or the adjacent of that mentioned areas.

Interestingly, in the Songkhla Lake Basin *Poecilia velifera* did not distribute in only shallow habitats exhibiting high complexity mentioned in Meffe and Snelson (1989) and Hankison *et al.* (2006), but also in the irrigation canals in shrimp farms which is clear habitats (Fig. 3.2 C). Although mollies need sheltered area to prevent them form being hunted by piscivorous fishes and other predators (Chick and Mlvor, 1997), the shallowness of water in the canals and the disconnection of the canals and the Songkhla Lagoon may restrict the predation by larger fishes Meffe and Snelson (1989).

Although the current distribution range of *Poecilia velifera* in the Songkhla Lake Basin is limited in the entire Haad-kaew Lagoon and only in the Thale Sap Songkhla of the Songkhla Lagoon (Fig. 3.1), this range could be extended. Suitable habitats for *P. velifera* are the near-shore, highly vegetated shallow habitats and with a rather slow or no water current (Meffe and Snelson, 1989; Hankison *et al.*, 2006). From the survey along the coast of the Songkhla Lagoon, these habitats were found in all uninvaded parts of Songkhla Lagoon: Thale Sap, Thale Luang and Thale Noi. Therefore, there are many preferred habitats that could potentially support the establishment of the fish in those parts of the Songkhla Lagoon. In addition, *Poecilia* can tolerate a wide range of salinities (Meffe and Snelson, 1989) and it can stand a high degree of salinity fluctuations: *P. velifera* can survive in hypersaline water (45 ppt) even after it is transplanted immediately from freshwater (0 ppt) (Wannachote, 2008). As a result, they can stand salinity fluctuations in the Songkhla Lake Basin that range from 0-34 ppt. This information supports the view that the fish will probably extend their distribution range effectively throughout the Songkhla Lake Basin. However, the fish is supposed not to invade outer sea areas because near-shore marine currents act as an effective barrier against its dispersal as found with other mollies (Schlupp *et al.*, 2002).

# CHAPTER 4

# REPRODUCTIVE STATUS OF YUCATAN MOLLY *POECILIA VELIFERA* (REGAN, 1914) IN THE SONGKHLA LAKE BASIN

## 4.1 INTRODUCTION

Poeciliids display an interesting set of reproductive adaptation, making them obviously apart from most other fishes. Males reform their anal fin to be gonopodium, the mating organ transmitted the sperms to females. Females can keep and maybe nourish the sperms gained from males in their reproductive tract for several months (Constantz, 1989). Fertilization and development occur within ovarian follicles, therefore, birth coincides with ovulation. Poeciliids display a great diversity of maternal-fetal nutrient transfer, from almost none in the species that produce large egg to complete nutrient provisioning in the species with tiny ova. In some species, a female may carry broods of two or more different ages, called superfetation. And almost all species deliver precocious neonates.

Not only reproductive adaptations making poeciliids interesting in biological aspect, but also the phenotypic plasticity in their life histories. Poeciliids normally exhibit high phenotypic plasticity (Reznick and Miles, 1989a; Trexler, 1989a). The poeciliids exposed to different environmental factors exhibit different life span and size at maturity, sex ratio, fecundity and so on (Trexler, 1989a). Those environmental factors including, temperature, salinity, food availability, photoperiod, social context and oxygen level (Trexler, 1989b). Therefore, the reproductive traits of poeciliids living in different environmental conditions are expected to be different from each other.

For the *Poecilia velifera* (Regan, 1914) invading the Songkhla Lake Basin, as a poeciliid fish, their reproductive traits are possibly different from the populations living in other sites of the world. Since the reproductive traits of a fish play an important role in indicating its population characteristics and this information of the fish in the area on is not available, they needs to be investigated. In this study, it is an attempt to collect the information on the sex ratio, maturity, fecundity and reproductive period of the *Poecilia velifera* living in the Songkhla Lake Basin.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Reproductive traits assessment

The data needed (from each specimen) in this chapter including standard length, sex, maturity, number of offspring in gravid females. The methods gathering this information was provided in section 2.2.

### 4.2.2 Data analysis

Size at first maturity was defined as the size of the smallest individual of sexually mature males and females. Then the average standard length of sexually mature females was calculated. The overall sex ratio was calculated from total samples and from each 10.0 mm size class. The overall sex ratio and sex ratio of the size class >10.0-20.0 mm SL were tested for equality by using the Chi-square goodness of fit test. The relationship between batch size and body length was assessed by using a simple linear regression, with the independent and dependent variables were female standard length and batch size respectively. Then the predicted fecundity, fecundity at the average sexually mature female length, was calculated using Length-fecundity relationship equation. All statistical analyses were performed following the guidance of Zar (1996).

**4.3 RESULTS**

*Poecilia velifera* started to mature when the males and females body lengths reached 16.8 and 17.1 mm SL respectively (minimum length of males and females are 7.5 and 7.8 mm SL respectively; maximum length of males and females are 62.3 and 69.8 mm SL respectively). The average standard length of sexually mature females was 39.0 ±0.2 mm (mean ±S.E.; n = 1655). The overall sex ratio (male: female) was 1.0:1.8 not 1:1 (n = 6,033; $\chi^2$ = 3.841; d.f. = 1; $p$ <0.0001). The sex ratio of the smallest size class (>10.0-20.0 mm SL) was 1.0:1.3, not 1:1 (n = 666; $\chi^2$ = 8.2222; d.f. = 1; $p$ = 0.0041). Surprisingly, the sex ratio changed continuously through the size classes from 1.0:1.3 in the smallest size class (>10-20mm SL) to 1.0:7.7 in the biggest size class (>60-70mm SL) (Figure 4.1).

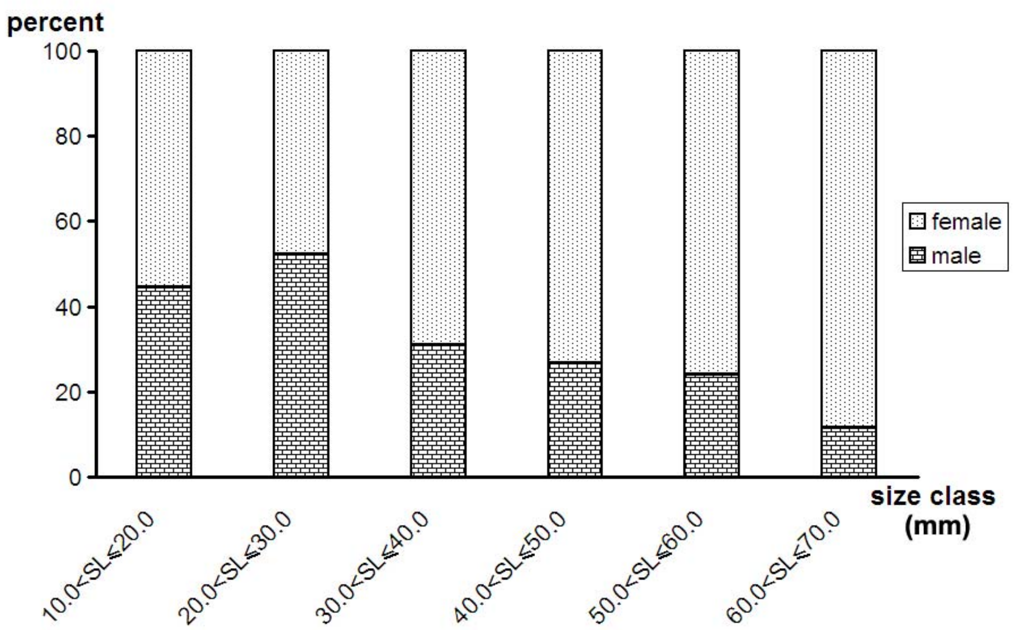


Figure 4.1 Sex ratios of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, in January 2007 to January 2008, divided into size classes

Female *Poecilia velifera* produced varied numbers of offspring in each batch, ranging from 5–252 embryos (n = 876). The trend of the fecundity appeared to be as shown in Equation 4.1 (see also Figure 4.2):

Equation 4.1: Batch size = $3.7182e^{0.0531(\text{female body length})}$

(Length in mm SL, $r^2 = 0.4831$; n = 876, $p < 0.05$)

The predicted fecundity of the average sexually mature female length (SL), was 29.5 offspring.

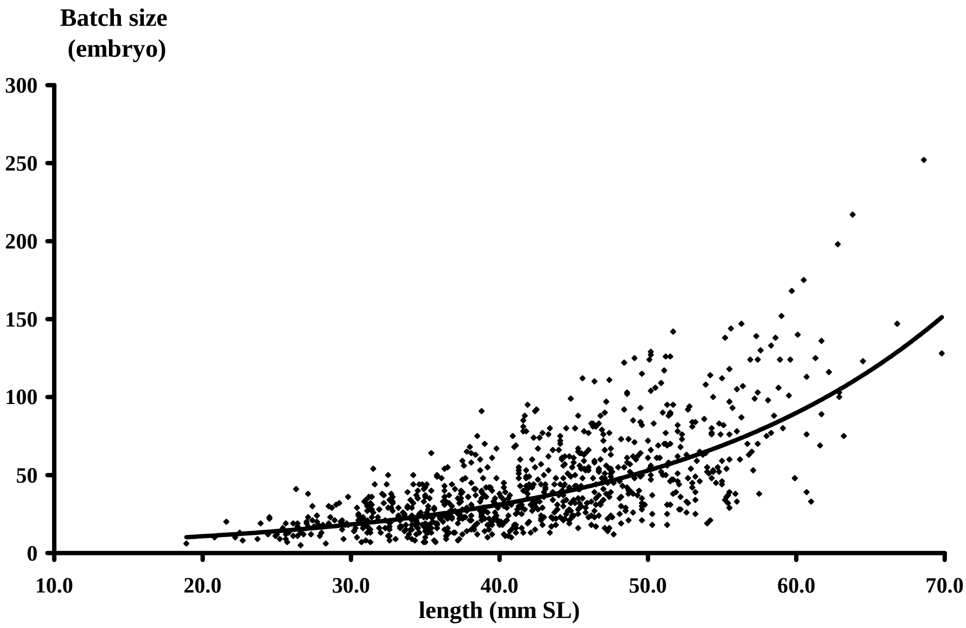


Figure 4.2 Fecundity of female Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008)

From January 2007 to January 2008, at least 30 percent of all mature females were pregnant each month. Although it was evident that *Poecilia velifera* were reproducing throughout the year, two periods of high reproductive activity were observed. The first was from March 2007 to May 2007, the other from August 2007 to October 2007. As expected, each peak of reproduction was followed by a peak of immature fish present in the samples, one in June-August 2007 and the other in November-December 2007 (Figure 4.3).

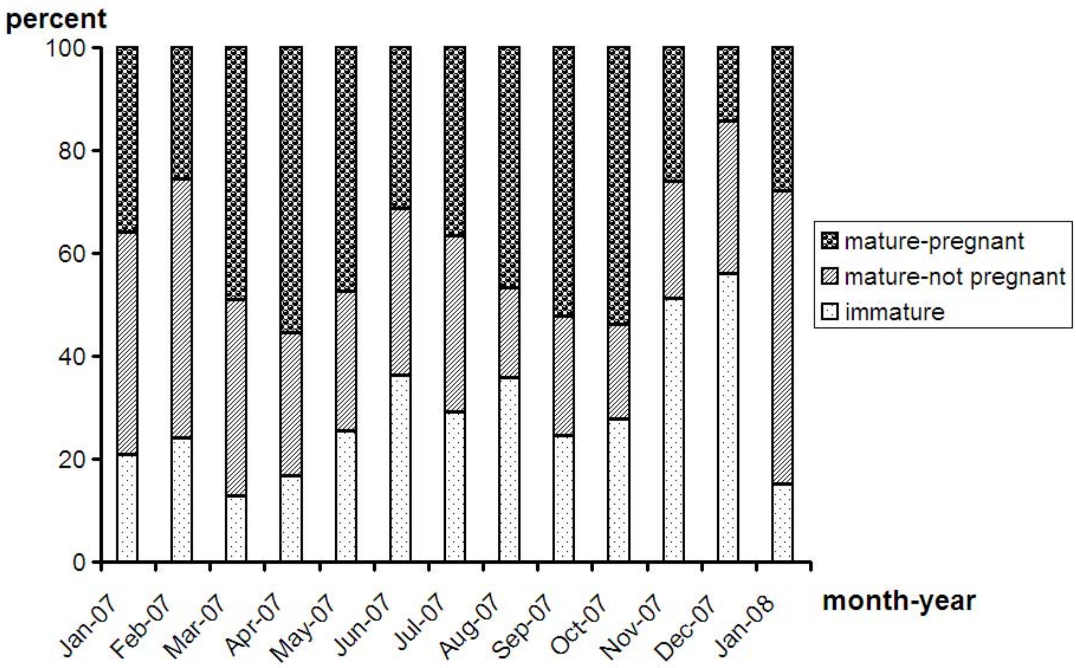


Figure 4.3 Proportions of female Yucatan molly *Poecilia velifera* (Regan, 1914) in each of the 3 reproductive stages collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008)

**4.4 DISCUSSION**

For the study on the reproductive traits of *Poecilia velifera* in the Songkhla Lake Basin, it will be informative to compare the results with other study of the same species living in a native area or in a different infected area. Unfortunately, that information is not available. Therefore, the results were compared with those for *Poecilia latipinna* (Lesueur, 1821) which is closely related to *P. velifera*, as both are in the same species complex (Breden *et al.*, 1999; Miller, 1983), and they have similar life history traits (Constantz, 1989; Reznick and Miles, 1989a; Snelson, 1989).

In this study, it was found that the *Poecilia velifera* living in the Songkhla Lake Basin reached sexual maturity rapidly. The fish are getting mature while their body sizes are small, only 16.8 and 17.1 mm SL for males and females respectively. That mentioned body sizes (the sizes of smallest sexually mature male and female) are about two months of age (from birth) or a months after parturition (see section 5.3, figure 5.4).

Rapid maturation in the present study may result from predators preferring large sexually-mature Yucatan molly, or because of the unstable environment in the Thale Sap Songkhla and Haad-kaew Lagoon, such as changes in salinity and fluctuations of water level, that could induce rapid maturation of mollies (Reznick and Miles, 1989a).

It is interesting to note that, in the study of the sex ratio of *Poecilia velifera* in the Songkhla Lake Basin, although the overall sex ratio skewed to female like most of other poeciliids (Snelson, 1989), the smallest size class that is supposed to be similar to the secondary sex ratio (sex ratio at birth) does not depart much from 1:1. Snelson and Wetherington (1980) have also found similar information for *P. latipinna*.

Considering the sex ratio divided by the size class (Figure 4.1), however, the proportion of males was less within specimens belonging to the larger size classes. This is not supposed to be the result of sexual dimorphism, females larger than males, because *P. velifera* in the SLB display not different age-length relationship (figure 5.3). The skewness towards females of *P. velifera* in the present study was possibly due to the consequence of higher death rate of males than females as suggested in Snelson (1989). Males suffer higher mortality because males have bigger lateral projections: lateral areas include the dorsal fin, body and anal fin. Although bigger lateral projection gives more advantage in the female's mate choice (MacLaren *et al.*, 2004), it increases the risk of being seen and hunted by predators. In addition, it is generally accepted that male poeciliids are more susceptible to a variety of stressors such as extreme temperature, over crowding and starvation (Snelson, 1989). As a result, these stresses could reduce their fitness and increase the mortality of male Yucatan molly. In conclusion, a higher mortality of male *P. velifera* could reduce the proportion of males to females and result in the sex ratio of *P. velifera* in the Songkhla Lake Basin to be female-biased.

Fecundity of the average sexually mature female length of *Poecilia velifera* caught from the Songkhla Lake Basin in this study is greater than that of the wild-caught *P. latipinna* as reported by Reznick and Miles (1989b), 22.1 ±2.6 offspring (mean ±S.E.) at body length 37.3 mm. The average sexually mature female size is an effective index of the variation of their fecundity. Populations with a higher average mature female size have a greater per-capita fecundity rate (Travis and Trexler, 1987; Reznick and Miles, 1989a). Furthermore, considering fecundity of female molly at the 37.3 mm SL (average body size of mature female mollies mentioned in Reznick and Miles, (1989b)), fecundity of *P. velifera* caught from the Songkhla Lake Basin (26.9 offspring) is greater than those *P. latipinna*. It means that *P. velifera* in the Songkhla Lake Basin has a higher reproduction ability than *P. latipinna* reported elsewhere. One factor that could explain this difference is daylength. Constantz (1989) have reported that daylength interacts in an incompletely understood way to control female reproduction. Burns (1985) also found that *Poeciliopsis gracilis* (Heckel, 1848) and *P. sphenops*, two closely related species to *P. velifera*, had higher gonadosomatic indices at longer photoperiods. The Songkhla Lake Basin has a long photoperiod (average 6.5 hours in the study period; the amount of time when it was direct sunlight) (Songkhla Meteorological Center, unpublished data). Therefore, it is reasonable that *P. velifera* in the Songkhla Lake Basin had a high fecundity.

From the monthly sampling of *Poecilia velifera* in the Songkhla Lake Basin since January 2007 to January 2008, gravid females were found every month (Figure 4.3). It indicates that the population of *P. velifera* in the Songkhla Lake Basin reproduces continuously. This shows the different life history traits of poeciliids between temperate and tropical zones. Poeciliids in temperate zones reproduce seasonally whereas those in tropical zone reproduce throughout the year (Billard, 1986; Constantz, 1989). In tropical zone, food for *P. velifera* is available throughout the year (Axelrod *et al.*, 1981; Mills and Vevers, 1989; Angsupanich and Rakkheaw, 1997) and this could support continuous reproduction of the fish.

Interestingly, there were two modes of gravid females found in the study period. The first and the second modes of gravid females occurred 1-2 months before the south-west monsoon (May-September) and north-east monsoon (October-December) (Figure 4.3). The seasonality trend in the present study is approximates to the spawning trends in the coastal fish in the tropical Indo-Pacific region, as noted in Tiews *et al.* (1975). There is a possible relationship between reproduction of *Poecilia velifera* in the Songkhla Lake Basin and monsoon winds as suggested by Pauly and Navaluna (1983). It has been suggested that the established population of *P. velifera* in the Songkhla Lake Basin have adapted their reproductive tactics to this new atmosphere and it is possible that alterations of the weather conditions between the seasons induce reproduction of *P. velifera* in the Songkhla Lake Basin.

# CHAPTER 5

# GROWTH AND POPULATION PARAMETERS ESTIMATION OF YUCATAN MOLLY *POECILIA VELIFERA* (REGAN, 1914) IN THE SONGKHLA LAKE BASIN

## 5.1 INTRODUCTION

Population parameters assessment is the operation to assess population status and provide advice to manage or advice on the optimum exploitation of aquatic living resources (Sparre and Venema, 1992). For the commercially important species, this is the approach to search for the exploitation level which gives the maximum profit in the long term. For the case of alien species, this is the method to indicate the status of the species in order to control their population size and dispersion.

For *Poecilia velifera* (Regan, 1914) this information could detail the dynamics of their population since it was expected to display no migration as its close relatives, *P. latipinna* (Lesueur, 1821) (Froese and Pauly, 2009). Therefore, in this chapter, it is an attempt to evaluate the population parameters of *P. velifera* invading the Songkhla Lake Basin. The population parameters include recruitment, growth and death. Growth was performed in both age-length relationship and length-weight relationship. Death was evaluated in the form of death rate, divided into natural mortality, fishing mortality and total mortality. Recruitment was revealed as growth function and recruitment pattern.

In order to assess growth of poeciliids, length-frequency analysis is generally unsatisfactory because of the protracted reproductive season of most poeciliids (Snelson, 1989). Therefore, assessing age-length relationship is more appropriate. Since Panella (1971) discovered otolith microstructures and proved it to be a daily settlement, various scientists have proved that daily increments occur in many fishes. For *Poecilia velifera* although its no available report dealing with otolith validation, age estimation using otolith can be operate after it was proven that the increments appeared in the otolith of the fish were produced daily. The age gained from otolith microstructure analysis is the number of days since birth which occurred in the mother's ovarian follicles, not since parturition.

## 5.2 MATERIALS AND METHODS

In this chapter, the information on body length, body weight, sex and age of the specimens were required. The first three parameters of information had been investigated in section 2.2.3. The last one, age, needs to be specified from the otolith.

### 5.2.1 Otolith validation

In order to prove that the rings existing in otolith of *Poecilia velifera* are daily deposition, otolith validation was performed using double oxytetracycline (OTC) staining method (applied from Morales-Nin, 1992). Using this method, double illuminated margin rings were created by treating the live fish with OTC solution twice with a specific number of days interval.

Processing this method, 30 size-varied lived *Poecilia velifera* were collected from the Songkhla Lake Basin and brought back to the laboratory. In the laboratory, the fish were transferred to 3 10×20×12 inch$^3$ aquariums containing 25 liters of fresh water, 10 fish for each. The fish were fed commercial aquarium fish food twice a day.

After the fish have been acclimated in the aquariums for 14 days, the first OTC treatment began. The fish in each aquarium were transferred to a black bucket containing 5 liters of 250 mg/l OTC solution (Schmitt, 1984) and kept in darkness, to avoid the degradation of OTC solution, for 24 hours. After that, the fish were transferred back to the aquariums and kept for 20 days.

When it was the due date, the second OTC treatment began. The process of the second treatment was exactly as the first one. After finishing the second treatment, the fish were transferred back to the aquariums and kept there for 3 weeks. Then the fish were killed by frozen in a refrigerator. On the way to prove daily ring, the Sagittal otoliths were dissected from the fish and cleaned by washing with fresh water. Next, the slide of thin section through nucleus of the otolith was prepared following the method given by Secor *et al.* (1991). Later, the number of increments between two illuminated rings (OTC stained ring) were counted using compound microscope under UV light and compared to the number of days between the first and the second OTC treatment (20 days) whether it was 1:1.

### 5.2.2 Age determination

Age of each specimen can be assessed by operating an otolith microstructure analysis, specified the number of daily rings appeared in the otolith. A sub-sample of the otoliths dissected in the section 2.2.3 was selected randomly varying in body length. The otoliths of the specimens collected from specific month were regrouped by sexes. Within each sex, otoliths of 3 specimens were selected from each 10 mm size class to represent the age of that specific range of body length.

Each was prepared a slide of a thin section through nucleus following the method applied from Secor *et al.* (1991). Next, the numbers of daily rings in the otoliths were counted under compound microscope. Three independent counts of daily rings were performed for each otolith. The second and the third counts were carried out in the next 3 and 6 days respectively from the first one to let the later counts independent. The numbers of daily rings gained from these three counts were averaged, and then represented as the age of each specimen.

### 5.2.3 Growth, Recruitment and Mortality Parameters Assessment

The scatter plots of age-length and length-weight relationship were constructed. The results of males and females were plotted separately in case that there was a different relationship between sexes. On the other hand, if there was no different in the relationship, it was no need to plot separately.

Age-length relationship were fitted with two models, von Bertalanffy growth model (Equation 5.1) and logistic growth model (Equation 5.2). These two models were selected because it seem to be the representative models suggested by Pauly (1984), and the softwares analyzing these two models were available.

Equation 5.1: $L(t) = L_\infty (1 - be^{-kt})$

where: 1. $L_o$ (y-axis intercept) is the length at birth (t = 0),

2. $L_\infty$ is the maximum length (t = infinity),

3. k is a rate constant with units of reciprocal time (e.g. year-1).

4. $b = (L_\infty - L_o) / L_\infty$

Equation 5.2: $L(t) = (L_o \cdot L_\infty) / \; L_o + (L_\infty - L_o)e^{-rt}$

where 1. $L_o$ (y-axis intercept) is the length at birth (t = 0),

2. $L_\infty$ is the maximum length (t = infinity),

3. r is a rate constant with units of reciprocal time (e.g. year-1).

The FISAT II software (Gayalino *et al.*, 1996) was used with the standard length-frequency distributions, 2.5 mm size classes, to estimate growth parameters: asymptotic length ($L_\infty$) and growth coefficient (K). $L_\infty$ was assessed using Powell-Wetherall plot (Gayanilo *et al.*, 1996). This method allows the estimation of $L_\infty$ when sufficient samples are available. Next, its result was used to calculate K using Shepherd's method which is best applied when $L_\infty$ is already provided. K represents the growth rate of a population. The population exhibiting greater K grows faster than the one exhibiting smaller K.

In addition, length frequency plot, and recruitment pattern (backward projection along a trajectory defined by VBGF) were investigated. These were provided using The FISAT II software (Gayalino *et al.*, 1996) as well.

Furthermore, total mortality coefficient (Z), natural mortality (M) and fishing mortality coefficient (F) were estimated. Z was determined using length-converted catch curve method. Natural mortality (M) of *Poecilia velifera* collected from the Songkhla Lake Basin was also calculated using the FISAT II software. This parameter was assessed using Pauly's M equation. $L_\infty$ , K gained from previous calculation, and mean annual habitat temperature (water temperature: T) were used as the input parameters. Performing the equation, it needed to transform $L_\infty$ from standard length (mm) into total length (cm) using the relationship that total length was about 1.273 times of standard length (the relationship between total length and standard length gained from the present study), and then converted the unit. T was 29.7 °C (Angsupanich, 1997). Finally, fishing mortality coefficient (F) can be estimated by the relationship showing in Equation 5.3.

Equation 5.3: $$Z = F + M$$

**5.2.4 Data analysis**

Simple linear regressions of age-length and length-weight relationships were analyzed, separately by sexes, using the GRAPHER software version 1.22 (Golden Software, 1993) and PAST software version 1.97 (Hammer *et al.*, 2001). For age-length relationship, age (day) and standard length (mm) were set as the independent and the dependent variables respectively. For length-weight relationship, standard length (mm) and weight (g) were set as the independent and the dependent variables respectively.

The difference between regression lines of males and females were tested for both age-length and length-weight relationships using t-test (Zar, 1996). In case that it was no difference between the regression line of males and females, the simple linear regression of pool data was analyzed. Least sum of square method wes used to identify the most appropriate model of age-length relationship. Length-weight relationship was also be tested whether it was isometric or allometric growth pattern, following (Zar, 1996).

## 5.3 RESULTS

### 5.3.1 Otolith validation

There were 30 *Poecilia velifera* collected to do otolith validation. Their standard length ranged from 20.5 – 42.3 mm. All over the experimental period, the fish showed no abnormal character or behavior, or any illness.

The result of otolith validation showed that every specimen exhibited 20 rings between two illuminated margin rings (Figure 5.1) marked by OTC. It was exactly the same number of days interval between the first and the second OTC treatments. In another word, the ratio of the days between the OTC treatments and the number of rings between illuminated rings is 1:1.

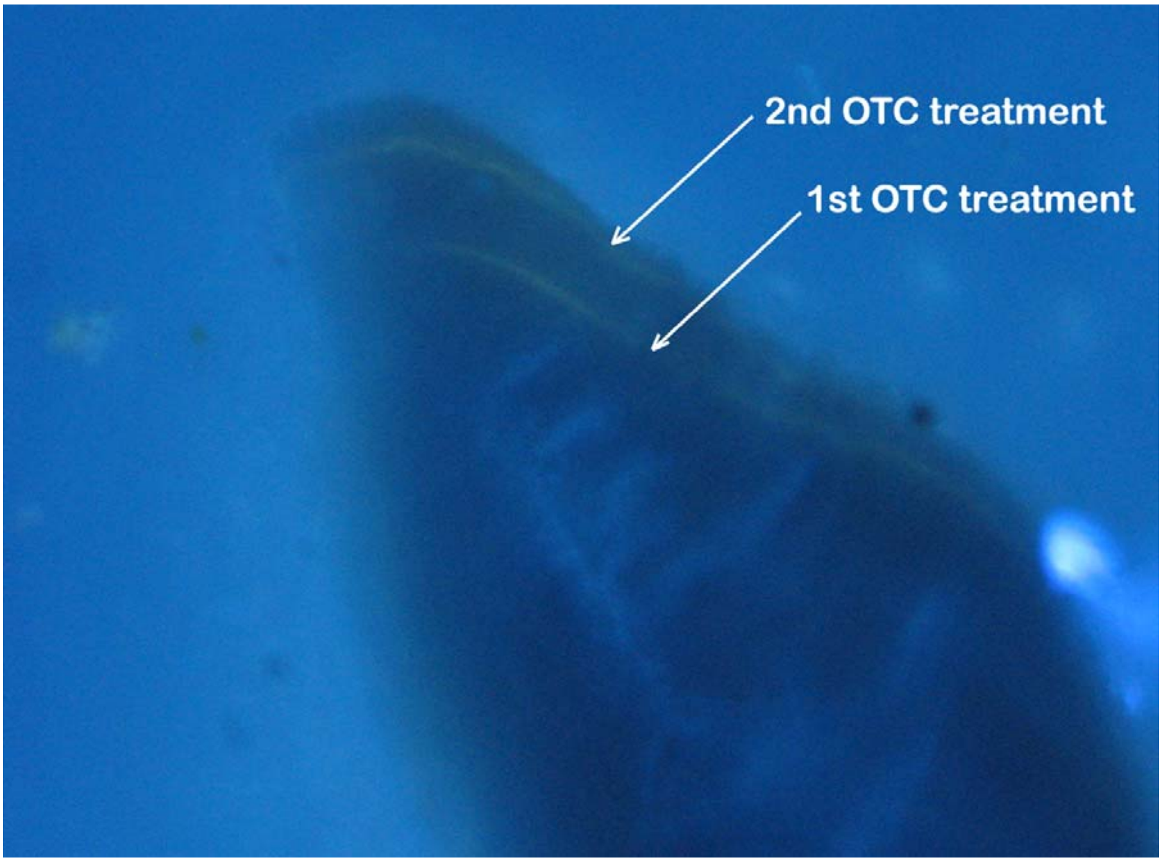


Figure 5.1 Two illuminated margin rings marked by Oxytetracycline, in the otolith of *Poecilia velifera*

### 5.3.2 Age and age-length relationship

The otoliths dissected from *Poecilia velifera* taken from the Songkhla Lake Basin exhibited clear increments (Figure 5.2 A and B). The increments widths were relatively wide in the region around the nucleus. It was relatively narrower in the outer ones.

The age of smallest sample (7.5 mm standard length) was 31 days. This sample is expected to be caught soon after parturition.

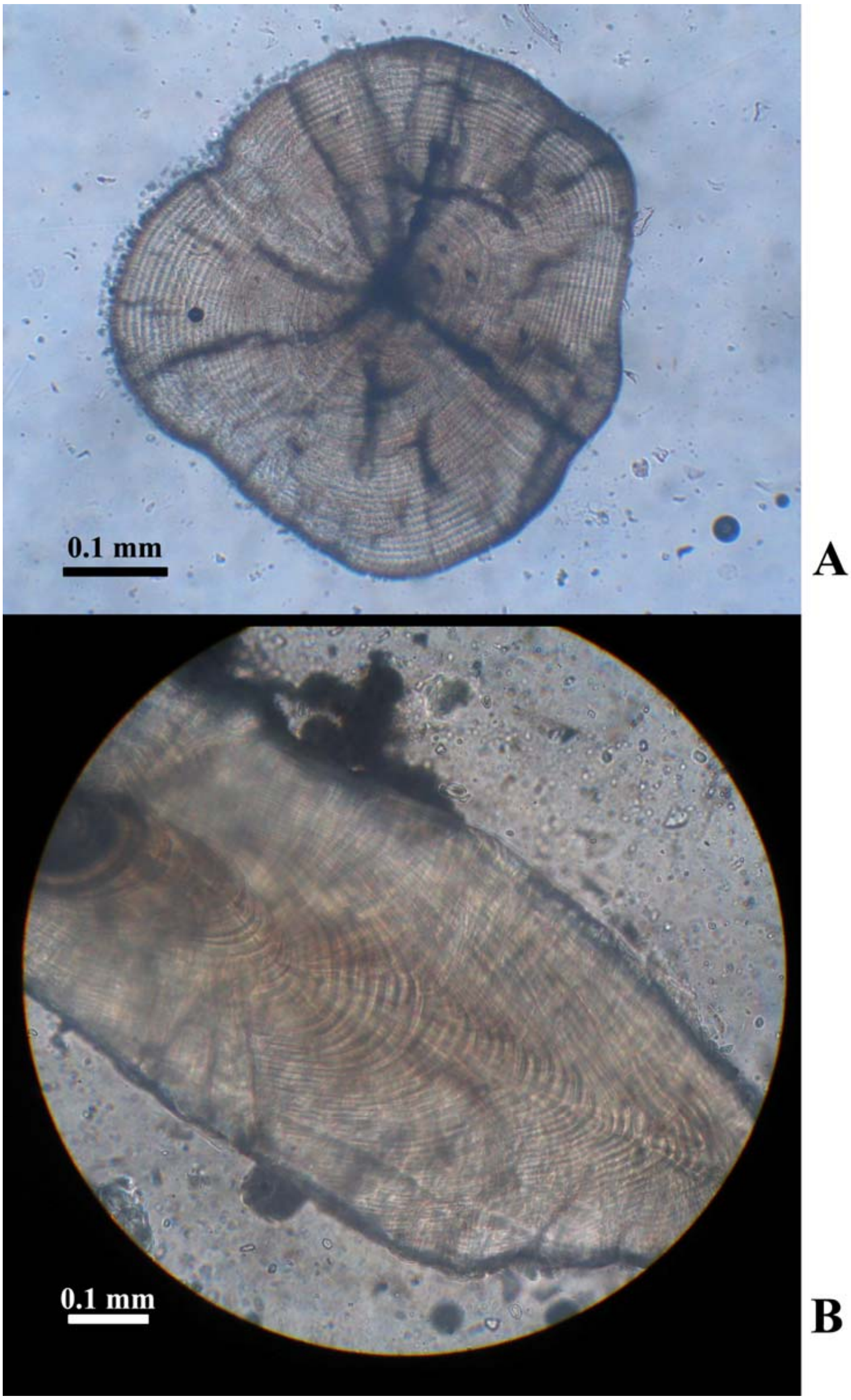


Figure 5.2 Daily rings in the otolith of *Poecilia velifera* (Regan, 1914) taken from Songkhla Lake Basin. The pictures show pattern of daily ring of the fish in different body size; A: 12.2 mm standard length, B: 58.4 mm standard length

Performing simple linear regression, males and females Yucatan mollies displayed not different age-length relationship (test for different between two regression coefficients: $|t|$ = 1.188, d.f. = 384, $p > 0.05$; test for different between two elevation: $|t|$ = 1.354, d.f. = 384, $p > 0.05$) (Figure 5.3). Growth curve of pool specimens was indicated as Equation 5.4.

Equation 5.4: standard length = 0.2631(age)+1.5828 ; $r^2 = 0.9181$
Standard length displayed in mm, age displayed in day.

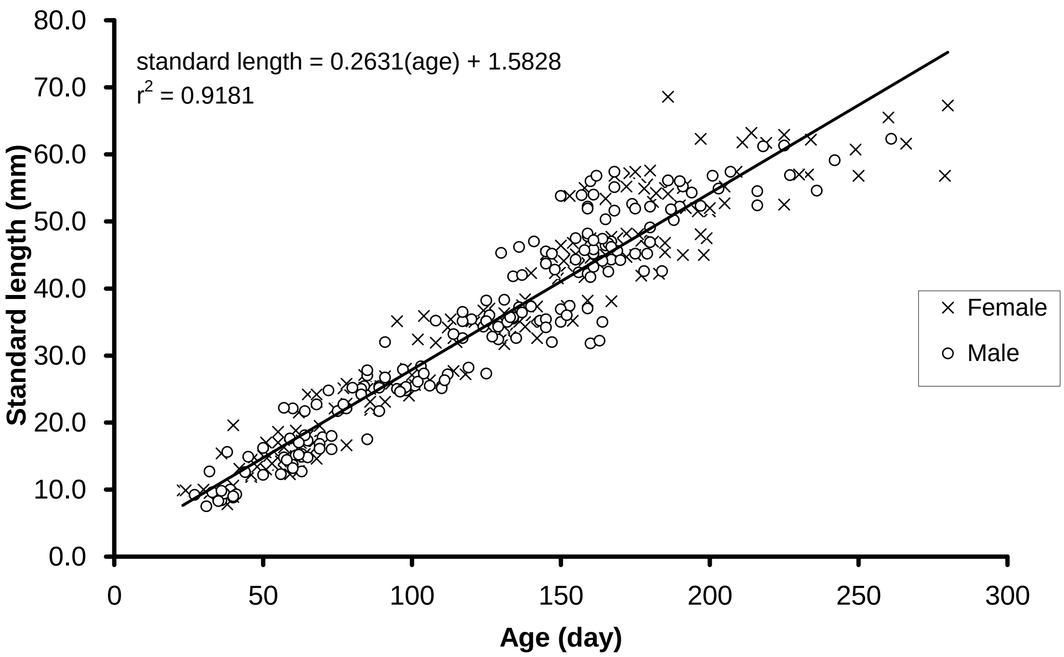


Figure 5.3 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008; $r^2 = 0.9181$; fitted by simple linear regression

Von Bertalanffy Growth model of the age-length relationship of *Poecilia velifera* indicated as Equation 5.5 (see also Figure 5.4, Table 5.1).

Equation 5.5: $SL = 69.286(1-1.1345e^{-0.0090296 * Age})$

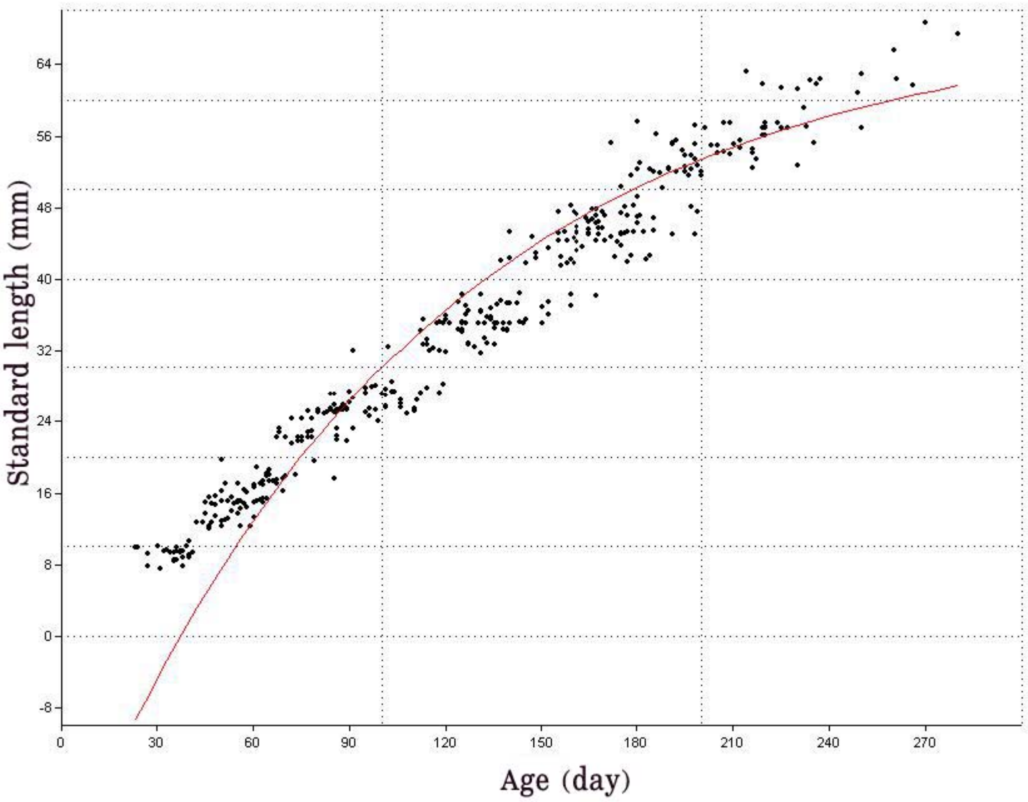


Figure 5.4 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008; fitted by von Bertalanffy Growth model

Logistic Growth model of the age-length relationship of *Poecilia velifera* indicated as Equation 5.6 (see also Figure 5.5, Table 5.1).

Equation 5.6: $SL = 69.286/(1+6.4272e^{-0.017653 * Age})$

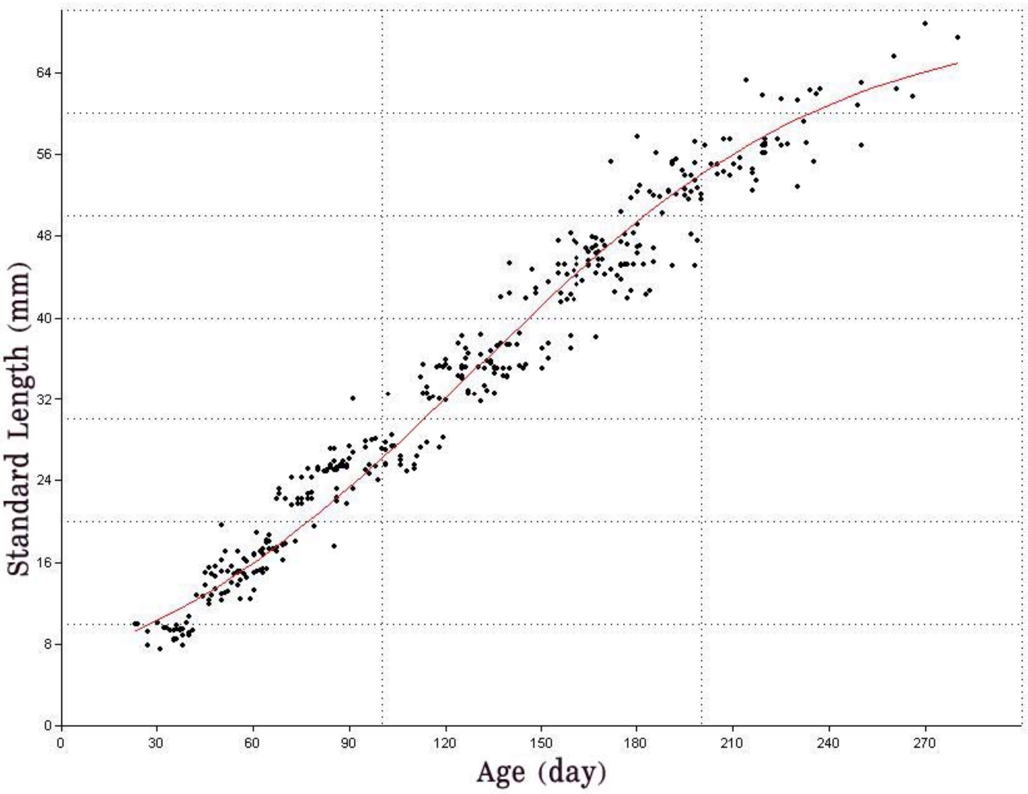


Figure 5.5 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008; fitted by Logistic Growth model

Table 5.1 Age-length relationship of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008, fitted by von Bertalanffy and Logistic growth models

| **Growth model** | **Equation** | **Sum of square** |
|---|---|---|
| von Bertalanffy | $SL = 69.286(1-1.1345e^{-0.0090296 * Age})$ | 22,607 |
| Logistic | $SL = 69.286/(1+6.4272e^{-0.017653 * Age})$ | 15,281 |

### 5.3.3 Length-weight relationship

In contrast, male and female Yucatan mollies displayed different length-weight relationship (test for different between two regression coefficients: $|t|$ = 7.241, d.f. = 3677, $p<0.05$) (Figure 5.6). Growth curve of each sex were described as Equation 5.7 and Equation 5.8. Length-weight relationship of male *P. velifera* showed allometric growth pattern ($t$ = 213.6992, d.f. = 1567, $p<0.0001$), as well as female ($t$ = 211.1945, d.f. = 2123, $p<0.0001$).

Equation 5.7:

Male: $\text{Weight} = (\text{Standard length})^{3.23713} \times 1.3241\times10^{-5}$; $r^2 = 0.9862$

Equation 5.8:

Female: $\text{Weight} = (\text{Standard length})^{3.19357} \times 1.45833\times10^{-5}$ ; $r^2 = 0.9873$

(Standard length displayed in mm, weight without viscera displayed in g.)

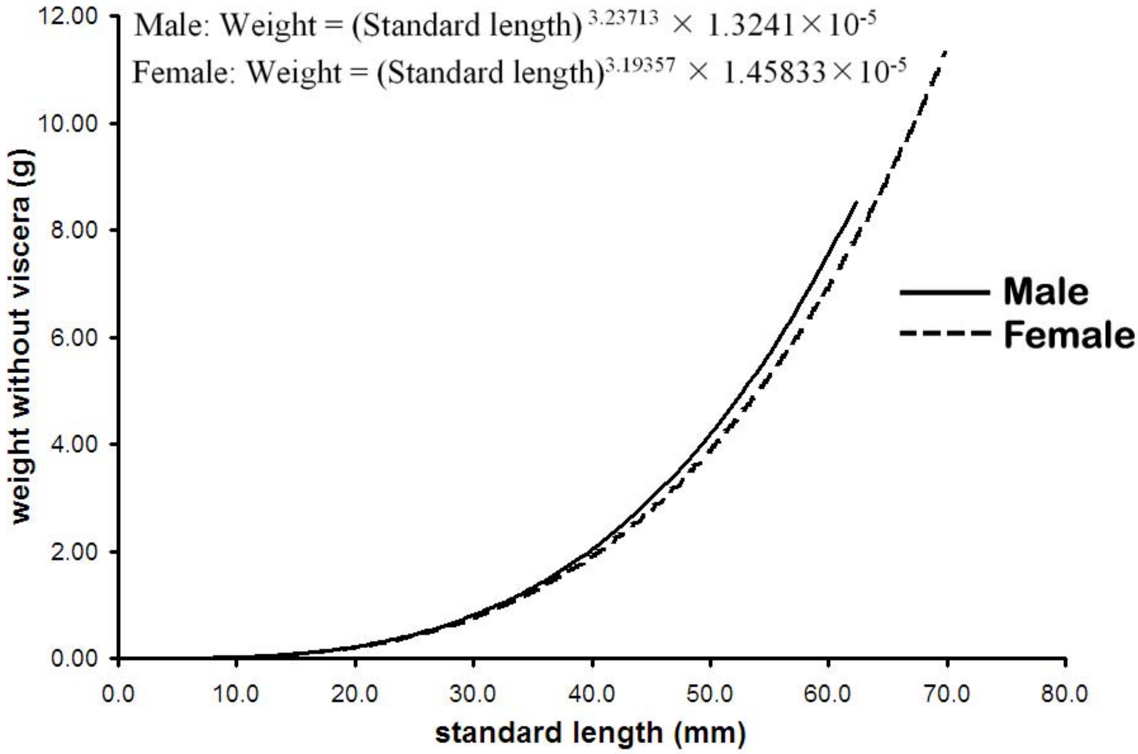


Figure 5.6 Length-weight relationship of male and female *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008, $n_{Male}$ = 1,569; $n_{Female}$ = 2125

### 5.3.4 Population parameters and recruitment pattern

Length frequency distribution of *Poecilia velifera* collected from Songkhla Lake Basin showed as Table 5.2. Using FISAT II software, it indicated that population parameters of *P. velifera* in the Songkhla Lake Basin were as: $L_\infty$ = 73.58 mm, K = 0.82 year $^{-1}$, total mortality coefficient Z= 3.8, natural mortality M = 2.2, and fishing mortality coefficient (F) = 1.6. Moreover, VBGF and length frequency plot (Figure 5.7), and recruitment pattern (Figure 5.8) were pictured.

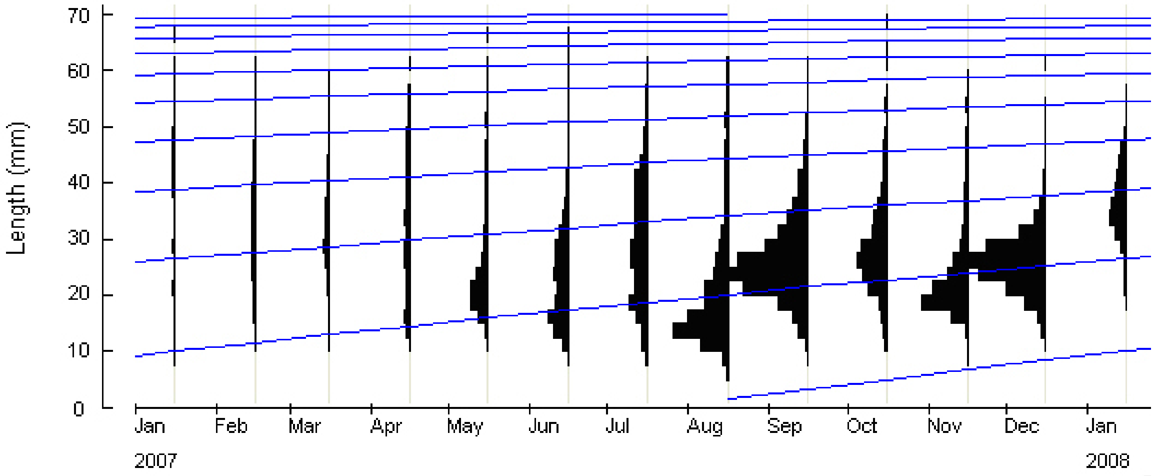


Figure 5.7  von Bertalanffy Growth Function (VBGF) and length frequency plot of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008

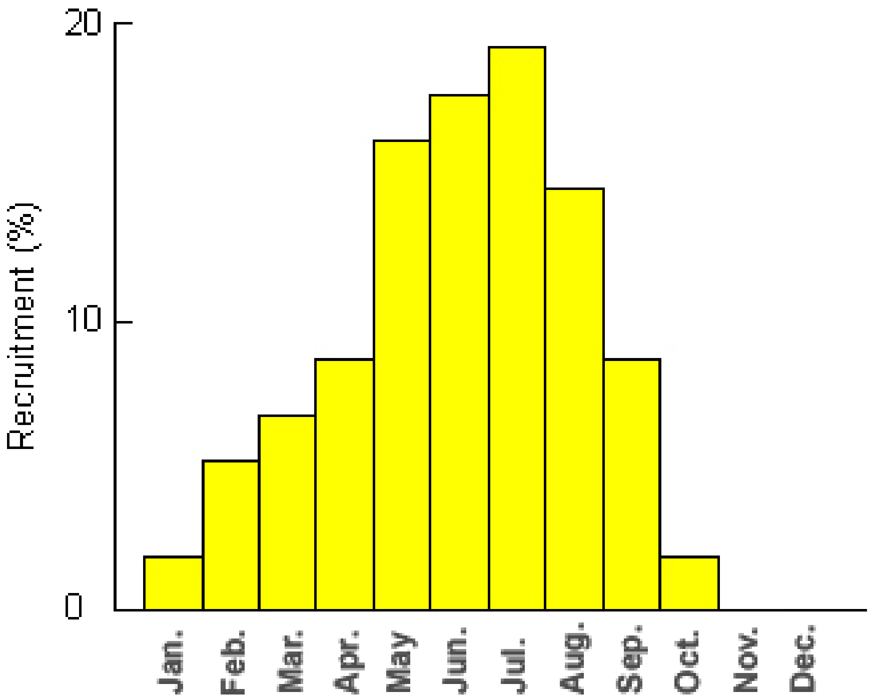


Figure 5.8  Recruitment pattern of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008

Table 5.2 Relative Recruitment Values of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from January 2007 to January 2008

| **Relative Time** | **Percent Recruitment** |
|---|---|
| January | 1.76 |
| February | 5.13 |
| March | 6.63 |
| April | 8.59 |
| May | 16.02 |
| June | 17.71 |
| July | 19.17 |
| August | 14.49 |
| September | 8.58 |
| October | 1.88 |
| November | 0.04 |
| December | 0.00 |

## 5.4 DISSCUSSION

Otolith validation of the *Poecilia velifera* collected from the Songkhla Lake Basin indicates that this species deposits daily increments in their otoliths. The result from the otolith validation strongly recommended this since the number of days interval between the days the fish were treated by OTC (20 days) is equal to the number of increments between the marks in the otolith. Therefore, the numbers of the increments in the otoliths of *P. velifera* can be interpreted as the age of each fish (days old) since it was born. This study supports Panella (1971) who indicated that otolith microstructure analysis can be used to determine the ages of bony fishes. Otoliths are complex polycrystalline matters being a part of the organ of balance in the inner ear (Gauldie, 1988). Otoliths grow by surface deposition of calcium carbonate enriched material (appeared as light band) or fibrous collagen-liked protein enriched matter (appeared as dark band) influenced by daily fluctuation of metabolism (Morales-Nin, 1992). The result is the formation of daily growth increments in the otolith.

The result on age of the specimens can be used to estimate the time the offspring took in ovarian follicles before parturition. It was presumed to be about a month (equal to the age of the smallest specimen). The similar information was proposed by Parenti (1994). He claimed that, in killifishes, there is no laval period, and individuals are sexually mature in just over a month after hatching. Since mollies are included in killifishes, the information can support the age at parturition in this study.

Normally, poeciliids are fast growing fish and it was reported that female usually grow faster than male (Snelson, 1989). However this observation does not necessarily apply to all poeciliids. Assessing growth of *Poecilia velifera* in this study, it was found that males and females display no difference (age-length growth curve), or else, a slight difference (length-weight relationship) in growth pattern to each other. The possible factor promoted the growth of male to be comparable to female is the quality and quantity of food ingested (Trendall, 1983; Wurtsbaugh and Cech, 1983). Food composition of males composed of greater proportion of aquatic animals and other small insects (Figure 6.2). This difference may induce the growth of male *P. velifera* in the Songkhla Lake Basin.

Considering age-length relationship of *Poecilia velifera* invading the Songkhla Lake Basin, it is suggested that the von Bertalanffy growth model is more appropriate than Logistic growth model (Figure 5.4 and 5.5). This conclusion was performed by taking sum of square of each growth model into account. The model exhibits the least sum of square is assumed to be the most fitted model. Table 5.1 shows that the sum of square of Logistic growth model is less than of von Bertalanffy growth model. Therefore Logistic growth model is indicated to be the representative model for age-length relationship of the fish.

It was found that *Poecilia velifera* in the Songkhla Lake Basin facing relatively low mortality comparing to the study of Trexler *et al.* (1992) which studying the mortality rates of its close relatives, *P. latipinna*. Trexler *et al.* (1992) reported that *P. latipinna* faced high mortality in winter rather than summer. Therefore in the Songkhla Lake Basin which exhibits tropical climate, weather conditions could promote survival of *P. velifera* in the area. This low mortality, however, contrast to the information of Courtenay and Meffe (1989) which indicating that poeciliids normally exhibits high mortality.

Focusing on M and F, in addition, it was found that M is greater than F. This is what was expected since it reveals in the Chapter 3 that fishermen living in the infected area do not exploit this fish. However, F still appears in a noticeable value compared to M. Therefore, it may be concluded that this fish may be caught unintentionally by local fishermen as by-catch product, or was exploited for other benefits.

Unexpectedly, the result of recruitment pattern assessment shows that *Poecilia velifera* in the Songkhla Lake Basin exhibits a peak of recruitment a year, not conforming to the result in Chapter 4 which indicating 2 peaks of recruitment. As it was mentioned in Chapter 4 that this fish displays continuous reproduction through out a year with two peaks present, the difference in percent of reproduction between low and high reproductive months may be not enough contrast for the recruitment pattern analysis. Therefore, the analysis tends to conclude that it is only one peak in July – the month lies between two peaks of reproduction mentioned in chapter 4.

Another reason is that this method is based on von Bertalanffy Function was developed for the stock assessment of the organisms living in the temperate zone where the organisms normally recruit once a year. More over, von Bertalanffy Growth model does not fit with the growth of *P. velifera* living in the Songkhla Lake Basin, therefore in this case VBGF is not appropriate.

# CHAPTER 6

# DIET OF YUCATAN MOLLY *POECILIA VELIFERA* (REGAN, 1914) IN THE SONGKHLA LAKE BASIN

## 6.1 INTRODUCTION

In the studies of fish biology and ecology, information on the diet in stomach is one of central interests. This information is a basic one picturing trophic interactions within a community, trophic level of a species, feeding mode(s) and feeding preference of a species. By having this knowledge, the function(s) of a species to the community and the ecosystem could be indicated including relationship between the species and its preys and maybe its competitors as well. In addition, this information can benefit aquaculture, for example, preparing appropriate food for the species.

As an alien species, information on diet becomes very important. Feeding biology of an alien species affects food web of the ecosystem undoubtedly, classified into at least 3 different ways (Lever, 1998). First, a nourishing exotic population could provide more prey available to native predators. Second, in case the alien species having the overlap food categories with of other indigenous species, competition for food occurs. And the last, the presence of an alien species could affect population dynamic of their preys. In addition, food requirement as well as feeding behavior of an exotic species could initiate habitat alteration and socio-economic effects (Taylor *et al.*, 1984).

For poeciliids, they exhibit many trophic types, including carnivores, herbivores and omnivores (Greenfield *et al.*, 1982; Greenfield *et al.*, 1983; Turner and Snelson, 1984; Arthington, 1989; Alkahem *et al.*, 2007). However, most of them are omnivores, eat mixtures of many food including terrestrial and aquatic invertebrates, detritus, algae and vascular plant (Meffe and Snelson, 1989).

Unfortunately, the information on diet of *Poecilia velifera* (Regan, 1914) invading the Songkhla Lake Basin as well as other introduced and native populations is not available. Therefore, this is the effort to investigate the diet of *P. velifera* in the Songkhla Lake Basin to fulfill this gap of knowledge.

This chapter is the attempt to find out what are the compositions and proportion of diet of *Poecilia velifera* taken form the Songkhla Lake Basin. In addition, it is also being tested whether it was any different food preference between sexes, ontogenetic shift on food requirement, and the different of food intake between seasons. Therefore, the composition of food in the pooled samples, as well as that of separated by sexes, body size and seasons were then investigated.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Stomach contents analysis

All the fish in the sub-samples collected in section 2.2.2 were opened their abdomens, preserved in 10% formalin solution and brought back to laboratory. In the laboratory, the characteristics and information on diet of each single fish were investigated. Each fish was measured body length (standard length: SL) in the nearest 0.1 millimeter. Using the information on body length, the specimens were grouped into 3 size class: SL≤30.0mm, 30.0mm<SL≤50.0mm and SL>50.0mm. They were also grouped by sampling seasons. There are 3 seasons in south Thailand demonstrated by monsoon: SE monsoon season (Jan.-Apr. 2007, Jan. 2008), SW monsoon season (May-Sep. 2007) and NE monsoon season (Oct.-Dec. 2007). Sex was examined by considering both secondary sexual characteristics and gonad (see section 2.2.3). Then a stomach was removed. The food in stomach was taken out and diluted in 2-$cm^3$ tap water in test tube. Because the stomachs of the fish were very small, therefore, it was assumed that the volume of diluted stomach contents of each fish were still equal to 2 $cm^3$.

Plant matter and microalgae in the diluted stomach contents were identified and quantified under a compound microscope using haemacytometer because there were plenty of them in the stomachs and their sizes were small. It was sampled 4 times per stomach. For zooplanktons and other animals which appeared in larger size, 5 fixed rows of Sedgewick-Rafter Cell (the $2^{nd}$, $5^{th}$, $9^{th}$, $13^{th}$ and $18^{th}$ rows) were used to qualify (diet identification) and quantify (volume estimation) the diet composition.

In order to assess the quantity of each food categories in a stomach, the volumetric method was used to find the volume of each food category. The dimensions of each food item were measured using micrometer, and then calculated volume of each food item using formula of three-dimensional shape the food item most closely resemble.

For a stomach, the volumes of each food category calculated from 4 measurements using haemacytometer were summed, and so on for the 5 rows of Sedgewick-Rafter Cell. The volume summation of each food category was then converted to be the total volume in the stomach (to multiply by 2000/3.6 and 4 for using haemacytometer and Sedgewick-Rafter cell respectively. Next, the volume of each food category found in every specimen were sum and the percent of summed volume (*%V*) of each food category was calculated (Equation 6.1).

Equation 6.1:

$$\%V_i = \frac{V_i}{V_{total}} \times 100$$

In the equation 6.1, $\%V_i$ is the percent volume of the food category *i*, $V_i$ is the volume summation of the food category *i*, and $V_{total}$ is the summation of the volume of all food types in pool specimen. Next, the percent of frequency occurrence (*%F*) of each food category was calculated (Equation 6.2).

Equation 6.2:

$$\%F_i = \frac{\text{the number of stomachs that the food category i presented}}{\text{the number of all stomachs}} \times 100$$

With the stomachs aim of comparing the importance between food categories, the Index of Relative Importance: *IRI* (Hyslop, 1980) was performed. IRI is the index incorporated by number (*N*), volume (*V*) and frequency of occurrence (*F*). However, some food categories found in the stomachs of *Poecilia velifera* such as plant matter and clump of microalgae was not possible to be counted, therefore, the number (*N*) of these food categories could not be evaluated. In order to solve this problem, the *IRI* was modified to be the index incorporated by only volume and frequency of occurrence, mentioned as $IRI_{mod}$ (Equation 6.3).

Equation 6.3: $$IRI_{\text{mod}} = \%V_i \times \%F_i$$

$IRI_{mod}$ could be used to compare the relative important between food categories within a group. Nevertheless, with the purpose of comparing the proportion of food composition between groups of *Poecilia velifera* that exhibit different sex, body size and sampling season, $\%\ IRI_{mod}$ were investigated (equation 6.4).

Equation 6.4: $$\%IRI_{\text{mod}} = \frac{\text{IRI}_{\text{mod}} \text{ of a food category in a group}}{\text{total IRI}_{\text{mod}} \text{ in the group}} \times 100$$

#### 6.2.2 Data analysis

In order to test whether there are the difference of food proportions between different sexes, body size classes and seasons, MANOVA was applied. Sex, size class and sampling season were set as independent variables. Every food category was set as dependent variable. MANOVA was analyzed using JMP IN software version 3.2.1 (SAS Institute Inc, 1997). Pie chart was used to display the proportion of $\%\ IRI_{mod}$ in overall specimens. Bar charts were used to show the proportion of *%* $IRI_{mod}$ comparing between different sex, size class and sampling season.

## 6.3 RESULTS

There are 776 *Poecilia velifera* were collected from the Songkhla Lake Basin during the study. Stomach contents analysis revealed the fish fed on various types of food including plants, microalgae, invertebrates and vertebrates. However, some taxa appeared in the stomach in a small proportion compared to the others. Therefore, the diets were regrouped into 5 main groups: “Plant”, “Algae”, “Insect”, “Crustacean” and “Other” (table 6.1).

The plant matter found in the stomachs was identified as vascular plant. These were supposed to be aquatic vascular plants and submerged terrestrial plants in flooding season.

Table 6.1 The composition of each food category found in the stomach of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, from Jan. 2007-Jan. 2008

| **Food category** | **Composition** |
|---|---|
| Plant | -plant materials |
| Algae | -green algae<br>-blue-green algae<br>-brown algae |
| Insect | -mosquito larvae<br>-other terrestrial insects |
| Crustacean | -shrimps<br>-copepods<br>-ostracods |
| Other | -hydrozoans<br>-flat worms<br>-nematodes<br>-annelids<br>-mollusks<br>-rotifers<br>-fish larvae (its offspring)<br>-eggs of aquatic organisms |

Although *Poecilia velifera* in the Songkhla Lake Basin fed on various types of food, the proportion of food categories shown by $\%IRI_{mod}$ revealed a trend of preference. Stomach contents analysis showed that plant matter and algae took great importance in the diet composition of the fish. The importance combination of plant matter and algae in the stomach reached 94 $\%IRI_{mod}$. Crustaceans, insects and other animals, in contrast, appeared in a small proportion of importance (Figure 6.1).

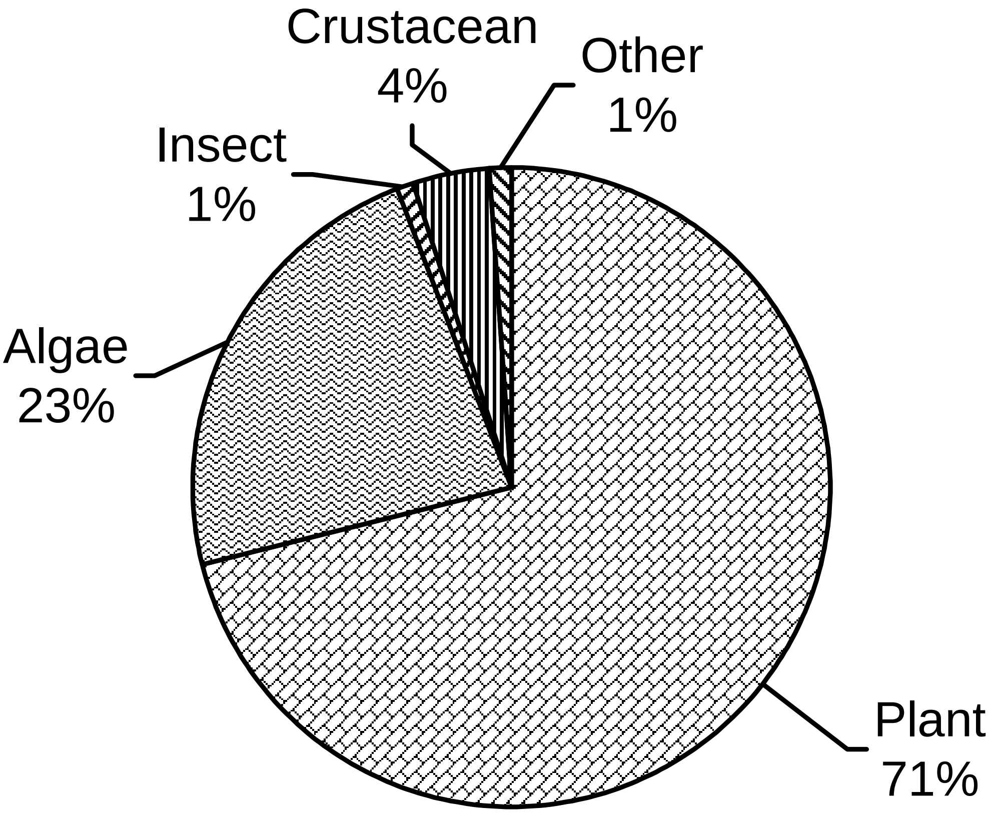


Figure 6.1 Proportion of food (%IRI) in stomachs of *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan 2008)

Comparing $\%IRI_{mod}$ between different groups, there were the differences of diet composition between sexes (F=3.8144; $d.f._1=4$; $d.f._2=755$; $p=0.0044$), body size classes (F=10.5762; $d.f._1=8$; $d.f._2=1512$; $p<0.0001$), and sampling seasons (F=5.0071; $d.f._1=8$; $d.f._2=1512$; $p<0.0001$). There was no interaction between Sex, Size class and Season detected (Table 6.2).

Table 6.2 MANOVA table testing the difference between diet compositions of *Poecilia velifera* (Regan, 1914) exhibited different sexes, size classes and seasons of collection (using Pillai's Trace). The specimens were collected from the Songkhla Lake Basin, from Jan. 2007-Jan. 2008

| **Effect** | **Value** | **F Value** | **DF Num** | **DF Den** | **Prob>F** |
|---|---|---|---|---|---|
| sex | 0.0198084 | 3.8144 | 4 | 755 | 0.0044** |
| sizeclass | 0.1059862 | 10.5762 | 8 | 1512 | <0.0001*** |
| season | 0.0516181 | 5.0071 | 8 | 1512 | <0.0001*** |
| sex*sizeclass | 0.0049772 | 0.4715 | 8 | 1512 | 0.8768 |
| sex*season | 0.0061752 | 0.5854 | 8 | 1512 | 0.7907 |
| sizeclass*season | 0.0228071 | 1.0867 | 16 | 3032 | 0.3616 |
| sex*sizeclass*season | 0.0113553 | 0.5395 | 16 | 3032 | 0.9275 |

Comparing sexual difference in the stomach contents of *Poecilia velifera* obtained from the Songkhla Lake, plant matter was found as the main category in the stomach of both sexes, followed by microalgae. It is interesting that the small aquatic and terrestrial animals (the combination of “Insect”, “Crustacean” and “Other”) took relatively greater proportion of food in the stomach of males rather than females (Figure 6.2; see also Appendix 5).

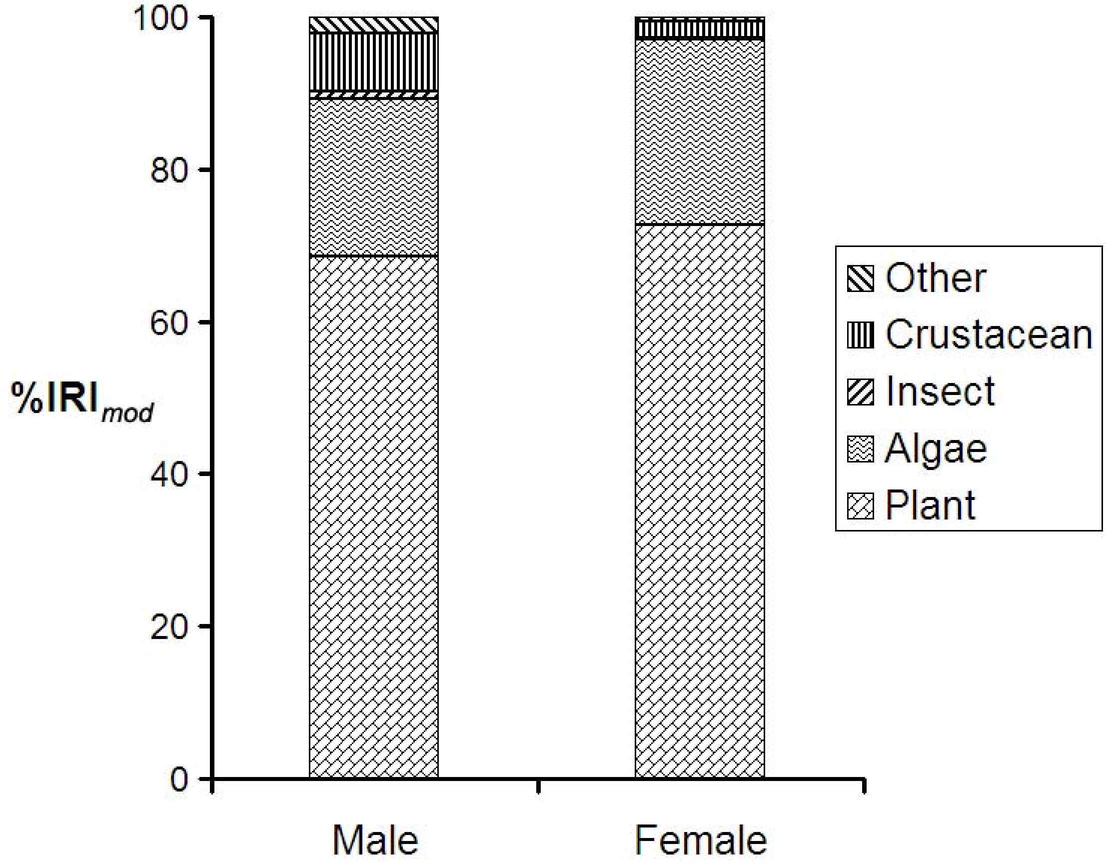


Figure 6.2 Proportion of food composition (*%IRI$_{mod}$*) in stomachs of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan. 2008) comparing between sexes

Dividing the fishes by size class (Figure 6.3; see also Appendix 5), it was found that the proportions of plant matter and algae in the smallest size class were comparable to each other. The two bigger size classes showed a different proportion. Both of them showed the bigger proportion of plant matter than microalgae. In addition, food in the stomach of the smallest size class exhibited greater proportion of aquatic and terrestrial animals than the other size class.

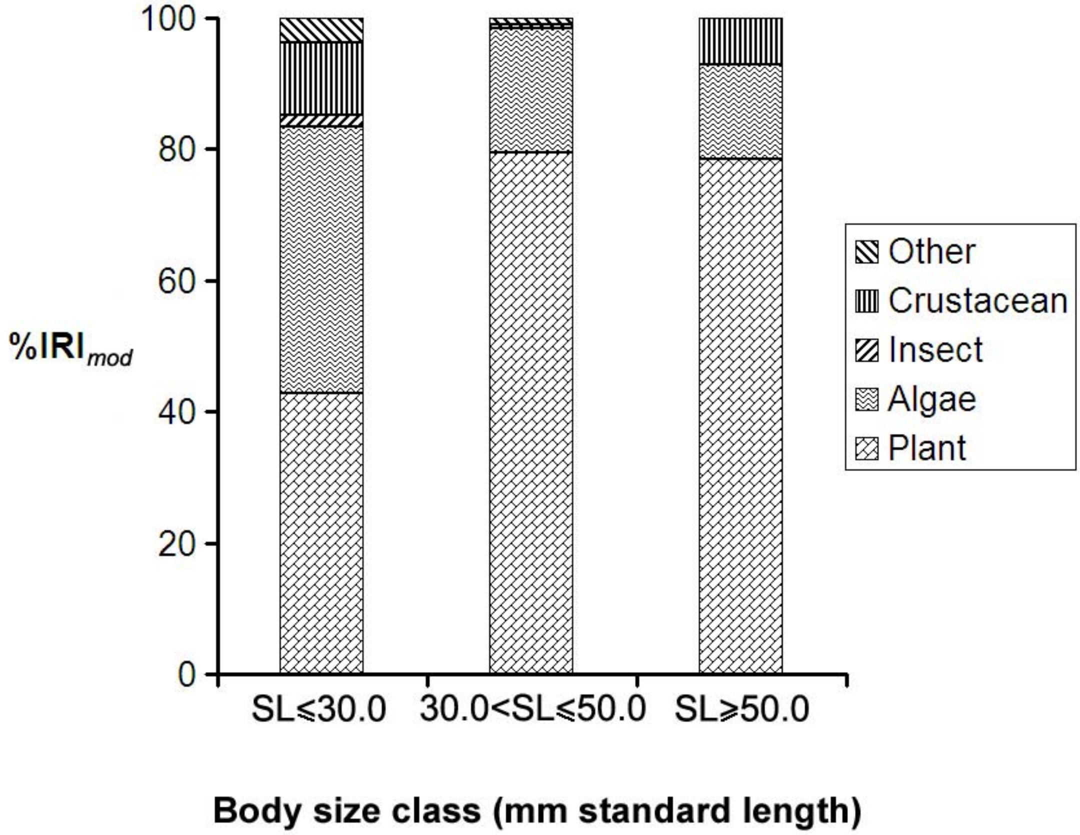


Figure 6.3 Proportion of food composition (*%IRI*$_{mod}$) in stomachs of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan. 2008) comparing between body size classes (mm standard length)

Focusing on the season the specimens were collected (Figure 6.4; see also Appendix 5), the diets composed in the stomach of *Poecilia velifera* collected in SE and SW monsoon seasons were only plant matter and microalgae, about 85-90% were plant matter. Stomach contents in the NE monsoon season showed a different composition. Plant matter and microalgae exhibited in a comparable proportion whereas “Insect”, “Crustacean” and “Other” showed a smaller but remarkable proportion.

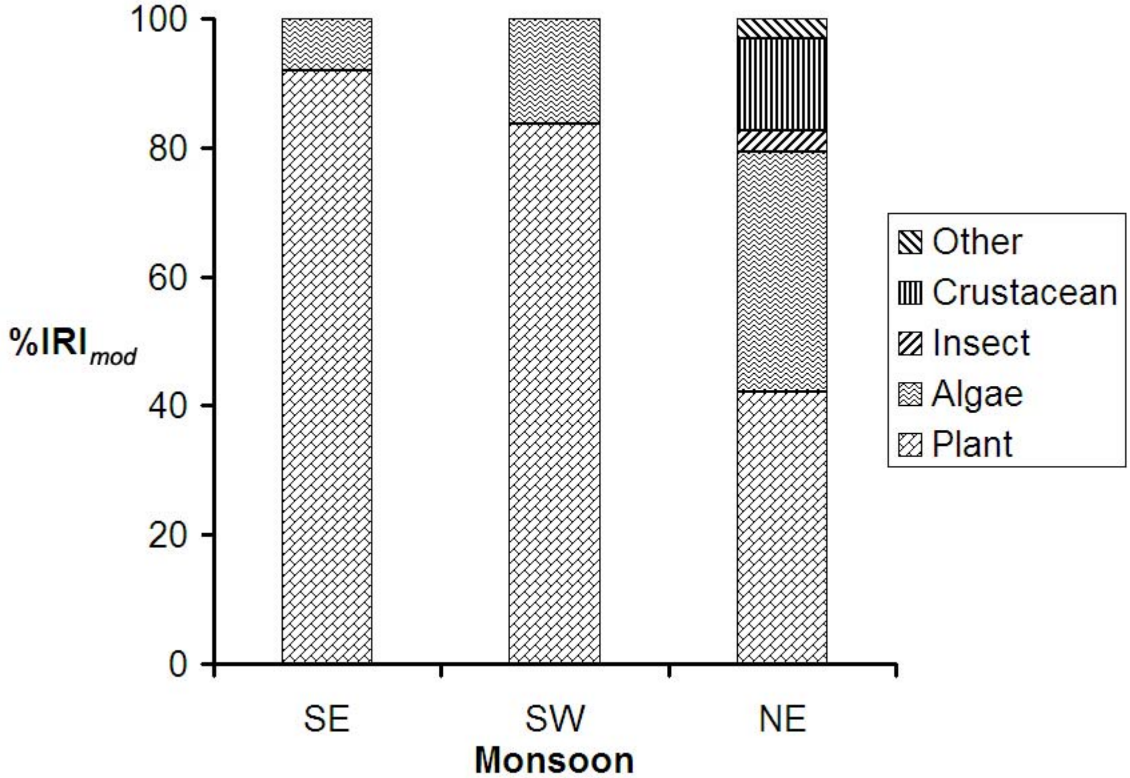


Figure 6.4 Proportion of food composition (*%IRI$_{mod}$*) in stomachs of Yucatan molly *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin (Jan. 2007-Jan. 2008) comparing between sampling seasons

**6.4 DISCUSSION**

This study indicates that Yucatan molly *Poecilia velifera* invading the Songkhla Lake Basin feed on diverse food types (Table 6.1) like that was claimed by Axelrod *et al.* (1981) and Mills and Vevers (1989). Their foods include 4 kingdoms: Plantae, Animalia, Protista and Monera. Interestingly, for Animalia, almost all phyla of the kingdom found in the stomach, including phylum Cnidaria, Platyhelminthes, Nematoda, Annelida, Arthropoda, Rotifera, Mollusca and Vertebrata.

Although *Poecilia velifera* feeds on various types of food, some food especially that were included in the category "Other" (but fish larvae) might be eaten accidentally because these food items appeared in very small proportion. In addition, *Poecilia velifera* usually picks up food from water and underwater surface (personal observation). Therefore, the mentioned animals which generally present in the surface might be eaten unintentionally while Yucatan molly was feeding on plant matter or microalgae as the main diet.

It is interesting that *Poecila velifera* collected from the Songkhla Lake Basin showed cannibalism behavior (Table 6.1). However, it was found in only 2 specimens and each specimen contained only one fish larva. Meffe and Snelson (1989) claimed that many poeciliids cannibalize in natural habitats whereas some species cannibalize only in laboratory. Cannibalism may provide particularly good nutritive source which allows the enhancement of growth and reproduction (Meffe and Crump, 1987). Moreover, cannibalism may be a natural mechanism taking action to limit population size of some poeciliids (Moore and McKay, 1971).

Performing stomach contents analysis of *Poecilia velifera* between sub-groups, remarkable information appeared. It was found there are the differences of stomach contents between different sexes, body size classes, and sampling seasons (Figure 6.2, 6.3 and 6.4).

Sexual variation on the diet of *Poecilia velifera* between sexes (Figure 6.2) was a noticeable difference of animal composition (the summation of "Insect", "Crustacean" and "Other") in the stomach. Male *P. velifera* feeds on small terrestrial and aquatic animals rather than female does. On the other hand, female *P. velifera* feeds on greater amount of plant and microalgae than male. This difference of food proportion may be the result of the different nutritional requirement between sexes. Small terrestrial and aquatic animals may provide specific essential nutrition male needed to maintain its regulating system and/or growth and/or reproduction. On the other hand, higher protein gain from animals may not profit the growth and reproduction of female *P. velifera* as the study of Dahlgren (1980) on its relative, *Poecilia reticulata*. If this is the case, it is reasonable that female *P. velifera* feed on insects, crustaceans and other animals less than males.

Ontogenetic shift in the diet of *Poecilia velifera* also detected, defined by the different of food proportion between different body sizes (Figure 6.3). Although the fish in every size feed mainly on plant and microalgae, the proportion of plant fed by *P. velifera* in the smallest size class is more or less the same amount of microalgae, whereas the fish in the middle and biggest size class feed on plant about 4-5 times greater than of microalgae. It is speculated that the teeth of the fish in the smallest size class are not well-developed, therefore, they feed on microalgae rather than plant. However, there is not any report mentioned about this. In addition, the fish in the smallest size class feed on relatively large proportion of small terrestrial and aquatic animals than other size classes. It suggests that the fish in the smallest size class which is sexually immature or maturing needs more proteins or lipids (Lovell, 1998; Izquierdo *et.al.*, 2001) gain from these food categories. Moreover, despite the proportion of animals in the diet of *P. velifera* decreases in the middle size class, it increases again in the biggest size class. It is not completely understood why the fish in biggest size class needs to feed more animals again. One assumption is that the fish in this size needs more lipid and fatty acid to support its higher fecundity as shown in Figure 4.2 (Izquierdo *et.al.*, 2001).

Sampling season also influences stomach contents of *Poecilia velifera* in the Songkhla Lake Basin. It is obviously appeared that insects, crustaceans and other aquatic animals were found in the diet of only the fish collected during NE monsoon season. It is the flooding season, thus, it is more opportunity for insects to fall into water or else they are flooded. Then they were fed by the fish. The NE monsoon season is also the zooplankton-predominated period during a year (Angsupanich, 1997). Some of these zooplanktons are expected to be the planktonic larvae of many aquatic animals. This might cause the rise of aquatic animals in the stomach of *P. velifera* in the NE monsoon season.

Additionally, the proportion of microalgae fed by the fish varies seasonally. The relative importance of microalgae is greatest in the fish collected during NE monsoon season as well, where it is a bloom of phytoplankton (Anguspanich and Rakkheaw, 1997). It indicates that the diet composition of *P. velifera* in the Songkhla Lake Basin possibly varies as the result of variation of food availability in the area.

Balcombe *et al.* (2005) has studied the fish assemblages of an arid zone floodplain river in Queensland, Australia and proposed that fish caught during flooding season possibly reveals the real preference of the fish under favorable conditions. In the flooding season, fishes can move into floodplain and gain more feeding opportunities. Thus, the fishes can choose what they want. For *Poecilia velifera*, diet composition of the samples collected during NE monsoon season should be the information showing the food requirement of *P. velifera*. Therefore, it suggests that *P. velifera* in the Songkhla Lake Basin needs to feed on plant, algae and small proportion of small terrestrial and aquatic animals (omnivore), not herbivore as revealed in the stomach contents of the pool sample and as their close relative, *Poecilia latipinna* (Lesueur, 1821) (Alkahem *et al.*, 2007). Or else, *P. velifera* may be claimed as opportunistic feeder, eating what is available because the proportion of stomach contents of the fish related to the availability of food in an area in a specific of time.

Although feeding preference of *Poecilia velifera* in the Songkhla Lake Basin is revealed, more information on the following topics are required. The study of stomach contents of the fish together with temporal availability of its diet in the study area is required to ensure whether their diet is affected by availability of food in the study site. In addition, the study of stomach contents of *P. velifera* in the Songkhla Lake Basin comparing with another native or alien population could be useful since this fish feed on various types of food and the proportion of its stomach contents varies.

# CHAPTER 7

# GENERAL DISSCUSSION

## 7.1 *POECILIA VELIFERA* (REGAN, 1914): A POTENTIAL INVASIVE SPECIES IN THE SONGKHLA LAKE BASIN

According to this study, a lot of information on distribution and biology of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin were provided. It was found that *P. velifera* currently distributed in the entire Thale Sap Songkhla and Haad-kaew Lagoon (Sa-nguansil and Lheknim, inpress). Reproductive biology of the populations of *P. velifera* in the area displays rapid maturity. They begin to mature about one month after hatched. The populations also exhibit female biased sex ratio, males to females ratio was 1.0:1.8 (Figure 4.1), high fecundity (fecundity of average mature female size is 29.5 offspring; see also Part 4.3 and Figure 4.2) and all year round reproduction (Figure 4.3). Moreover, growth curve (age-length and length-weight relationship), growth parameters (asymptotic length and growth coefficient), mortality (natural mortality, fishing mortality coefficient and total mortality coefficient) and recruitment pattern were obtained (see Chapter 5). This fish shows rapid growth and short life span suggested by relatively high K and M value (Sparre and Venema, 1992). Finally, diet of *P. velifera* in the Songkhla Lake Basin was assessed, indicating that this fish feed on various types of food and their food varied between different sexes, size classes and seasons. These results support that this fish could maintain their population and may be able to increase their population sizes and extend their distribution range to the uninvaded area in the Songkhla Lake Basin.

Although this fish has been an alien species in the Songkhla Lake Basin for at least eight years, its distribution range is restricted only in the Thale Sap Songkhla (Figure 3.1). It is assumed that the distribution of this fish had just extended slowly to Klong Pak Ror, the canal connecting Thale Sap Songkhla and the upper un-invaded part, in the time the survey was manipulated. Otherwise, there were some barriers acting against their further distribution. The effective barriers for *Poecilia velifera* may be the strong water current at Klong Pak Ror, a deep narrow canal connecting the Thale Sap Songkhla and the Thale Sap. Since this fish cannot swim against that strong water current (Hankison *et al.*, 2006), the fish cannot spread further to the Thale Sap. However, in the rainy season when it is severe flooding, this fish may go further by swimming through the flooded forest which could lowering the water current.

## 7.2 TREND OF POPULATION STATUS OF *POECILIA VELIFERA* TO THE SONGKHLA LAKE BASIN

It is expected that *Poecilia velifera* in the Songkhla Lake Basin has an ability to extend their population size. From the information mentioned in section 7.1, all of their reproductive status are supporting that they have a fantastic reproductive ability, reproduce continuously with relatively bigger batch size comparing to other poeciliids (see Part 4.3). Although this fish does not produce great amount of eggs as many other r-selected species, their reproduction has high probability to succeed because of internal fertilization and ovoviviparity. Furthermore, they occupy high vegetated shallow water protecting them from being eaten by large piscivorous fishes.

Another factor playing as a role against the appearance of *Poecilia velifera* is the causes of mortality. The information from interviewing local people shows that people in the invaded area do not exploit the fish for their food or any other benefits. Therefore, most of their mortality is expected to be positioned on natural mortality.

For *Poecilia velifera* invasion, not only the ability to enlarge their population size but also the ability to expand their distribution range should be concerned about. Although the limited distribution was observed (see Part 3.3.2), the uninvaded water is still vulnerable to the introduction of this invasive alien species. Since *P. velifera* prefers high vegetated area, tolerates to high range of salinity (Wannachote, 2008) and feed on various type of food (see Chapter 6), it could extend to the new uninvaded habitats which presents fresh water and a lot of preferred habitats.

In conclusion, from the results of this study, it is predicted that *Poecilia velifera* established initially in the Thale Sap Songkhla will continuously enlarge their population size and distribution range in the Songkhla Lagoon. If there is nothing to stop them, it is possible for them to occupy the entire water in the Songkhla Lake Basin.

## 7.3 POSSIBLE IMPACTS OF *POECILIA VELIFERA* TO THE SONGKHLA LAKE BASIN

Each specific ecosystem has evolved for so long time. This causes the ecosystem to be in a specific equilibrium. By putting a new element into the ecosystem, it becomes more or less unsteady and shifts to another equilibrium (Ricklefs, 1990; Begon *et. al.*, 2006). The point of issue is that whether the new equilibrium of the ecosystem is preferable.

Indicating the impacts of an alien species is subjective. However, it does not mean that the prediction of the impacts is valueless. The way used to assess the impacts is the best educated guess based on biological and ecological knowledge of the alien species and associated native species and ecosystem (Courtenay and Meffe, 1989). The result of consideration appears as negative or positive or both, depends on the judge. Followings are the considered impact of *Poecilia velifera* to the ecosystem in the Songkhla Lake Basin, separated as positive and negative.

### 7.3.1 Positive impacts

Some positive impacts have been reported for introduced poeciliids. One is pest control, especially mosquito control. There are three species that was introduced to many nations to serve this purpose, including *Gambusia affinis*, *Poecilia latipinna* (Lesueur, 1821) and *Poecilia reticulate* (Welcome, 1981). According to this purpose of introduction, many purposeful introductions indicated positive result (Welcome, 1988; Lever, 1996). Another positive impact is that the reintroduction of poeciliids into their native range for the purpose of conservation (Johnson and Hubbs, 1989).

For the occurrence of *Poecilia velifera* in the Songkhla Lake Basin, the positive impact as mosquito control cannot be defined. Although mosquito larvae were found in the stomach of some specimens (Table 6.1), it appeared in a very low frequency. In order to ascertain this aspect, more information on availability of mosquito larvae in the habitats *P. velifera* present is needed.

One another positive impact possibly provided by the occurrence of *Poecilia velifera* in the Songkhla Lake Basin is the increase of prey availability for native piscivorous species, like introduced invertebrates that provide food availability for a native generalist predator - red-backed salamanders *Plethodon cin*ereus in the North America forests (Maerz et.al., 2005). As the body size of *P. velifera* is not big comparing to many native predators such as snappers, sea perches, groupers, breams or even piscivorous birds for example, the fish may be the food supporting these predators. This is considered to be the positive impact to local fisheries because the prices of mentioned piscivorous fishes are high in the market.

### 7.3.2 Negative impacts

An obvious negative impact of the introduced poeciliids to local ecosystem is the decrease or extinction of larvae, juveniles or small adults of other native species which are their preys. It was claimed that this species has eradicated most or all of the smaller indigenous fishes as a result of predation and competition for food and space (Couternay and Robins, 1973; Mckay, 1984; Couternay and

Stauffer, 1990; Lever, 1994; Lever, 1996). However, the information on the stomach contents of *Poecilia velifera* invading the Songkhla Lake Basin (See Part 6.3) revealed that the fish feed on small amount of small terrestrial and aquatic animals. Therefore, it seems no need to concern about this impact.

Another impact caused by hybridization resulting as the genetic deterioration of allied native species (Courtenay and Meffe, 1989). The exotic poeciliids may interbreed with their native congener and causes unwanted hybrids. Furthermore, the introduction of associated parasites and diseases may also be included as the negative impacts of the introduction of poeciliids, but the report on these impacts have not yet been found.

For the occurrence of *Poecilia velifera* in the Songkhla Lake Basin, its impact to the ecosystem and native species is still not clearly indicated. The major impact may be the competition for space as suggested in Schoenherr (1981) because *P. velifera* usually occupies shallow water with high vegetated which is supposed to be a nursery area of many indigenous species. In addition, the present of *P. velifera* in the area may reduce reproductive ability of coexisted native species as the study of Howe *et. al.* (1997). He found that in the captive situation, *Gambusia holbrooki* (Girard, 1859) could reduce breeding performance and hence possible survival of *Pseudomugil signifer* (Kner, 1865) in Australia.

Considering possible trophic alteration caused by the fish, it seems not to be a serious problem for the ecosystem because *P. velifera* feeds mainly on vascular plants and microalgae which are dominant in tropical environment. However, although this fish also feeds on larvae, juveniles or small adults of other native species, it consumes in a small proportion which is expected not to harmful for the area.

Finally which is supposed to be the negative impact of *Poecilia velifera* in the Songkhla Lake Basin is being a nuisance species. This is only the problem of local fishermen fishing around the infected area. A lot of *P. velifera*, an unwanted species, trapped in their fishing gears may annoy their work.

## 7.4 SUGGESTED IMPLICATION TO MANAGE *POECILIA VELIFERA* IN THE SONGKHLA LAKE BASIN

There are many management actions taken world wide to deal with invasive alien species. These can be categorized as 6 types of action including prevention, mechanical methods, biological methods, chemical methods, indirect methods and integrated methods (Mosquin, 1997). Prevention is the action used to prevent the introduction and the dispersal of an alien species. This is the only action taken before the introduction of alien species happened. Mechanical methods is the use of machines, hand picking, soil tillage, deliberate fire, shooting, trapping, *etc.* to get rid of an alien species. Biological methods is the use of organisms, may be the predators or parasites or diseases of the alien species to eliminate them. Chemical methods are the use of chemical in the form of herbicides, pesticides, pheromones for example to carry out the alien. Indirect methods are the restoration of the ecosystem invaded by the alien. These methods include the replacement of the alien by native species. The last one is integrated methods: the combination of the methods mentioned above.

Normally, since an alien species is introduced and established in an area, there are two ways of action to deal with it. One is to prevent their further distribution expansion. Another is the management of alien population – to control its population not to be as big as to cause severe adverse impacts. These two actions should be operated strictly and continuously to manage the impacts of an alien species. For the case of *Poecilia velifera* in the Songkhla Lake Basin, the prevention of further distribution expansion seems difficult to operate. Though it is a narrow canal connected the Thale Sap Songkhla with the Thale Sap (Klong Pak Ror: Pak Ror canal), there is no appropriate barrier could construct to prevent their dispersion effectively especially in the flooding season. However, there are some ways to manage the population of *Poecilia velifera*. These are the use of biological methods and indirect methods.

The biological method could approach to this situation is the re-introduction of native piscivorous fishes such as snappers, sea perches, groupers, breams. It seems to be an interesting approach to deal with this alien species. Piscivorous fishes may be able to control the population size of *Poecilia velifera* in the area. However, the population sizes of these predators have been decreased because of overfishing in the area. Performing this method profits not only the management plan for the alien fish, but also the restocking of the high-commercial-value fish in the Songkhla Lake Basin.

Another approach could be taken to manage the population of *Poecilia velifera* is to do indirect methods such as the habitat restoration, for example recovering the lake side forest, polluted water management and stop overfishing. This method based on the idea that the native species living in an undisturbed or more natural habitat may have more ability to tolerate to the invasion of an alien species because these kind of habitat contributed to evolution of those native species (Southwood, 1988; Ricklefs 1991). Scoppettone *et al.* (2005) also proved that habitat restoration can be used to reduce populations of non-native fishes.

## 7.5 FUTURE DIRECTIONS

Although a lot of information on the distribution and biology of *Poecilia velifera* in the Songkhla Lake Basin have been investigated in this study, further study on the biology of this alien species and more information at least in the population level and community level are required. The repeat survey on the distribution range of this fish should be investigated to provide the most updated information. In addition, the abundance of this fish and risk assessment in each infected area should be defined to list the hot spot of its invasion.

More study to find out the potential native predators of *Poecilia velifera* in the Songkhla Lake Basin should also be investigated. These information together with the information on predators available in the area can be used to support clarify the cause of mortality of the alien species. This information can also be approached to plan an effective action to manage the fish.

Finally, in order to encourage local people and local authorities, the intensive study to clarify the potential negative impacts of *Poecilia velifera* in the Songkhla Lake Basin. If this fish really affect the ecosystem or the folkways of local people, especially in the negative ways, the co-operation between local people and local authorities to manage this alien fish in the Songkhla Lake Basin may be raised more easily and sustainable.

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# APPENDIX

**Appendix 1** **Published record of *Poecilia velifera* (Regan, 1914)**

| **Country** | **Location** | **Status** | **Establishment** | **Reference** |
|---|---|---|---|---|
| Mexico | Yucatan Peninsula | native | yes | Hankison et al. 2006 |
| America | coastal Florida | alien | yes | Ferriter et al. 2006 |
| Colombia | no data | alien | yes | Welcomme 1988 |
| Israel | no data | alien | no data | Golani 2000 |
| Peru | no data | alien | yes | Ortega et al. 2007 |
| Taiwan | no data | alien | no data | Shen 1993 |
| Vietnam | Maekong River Delta | alien | no data | Welcomme and Vidthayanon 2003 |
| Singapore | no data | alien | no data | Ng et al. 1993 |
| Thailand | Chao Phraya River Delta | alien | yes | Vidthayanon and Premcharoen 2002; Nico et al. 2007 |
| Thailand | Songkhla Lake Basin | alien | yes | present study |

**<u>Appendix 2</u> The distribution survey of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand**

| Record No. | Sampling Station | Record coordinates | | Record date | The occurrence of the fish |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| 1 | Ban Tha Sa Arn | 7º13'04"N | 100º34'28"E | 25 Nov 2007 | Yes |
| 2 | Ban Kok Rai | 7º09'57"N | 100º35'02"E | 25 Nov 2007 | Yes |
| 3 | Klong Khwang | 7º09'04"N | 100º34'03"E | 25 Nov 2007 | Yes |
| 4 | Klong Pa-wong | 7º08'31"N | 100º33'26"E | 25 Nov 2007 | Yes |
| 5 | Ban Tha Nanghom 1 | 7º08'13"N | 100º32'04"E | 25 Nov 2007 | Yes |
| 6 | Ban Tha Nanghom 2 | 7º08'17"N | 100º30'41"E | 25 Nov 2007 | Yes |
| 7 | Seasonally-closed Haadkaew Lagoon | 7º15'12"N | 100º33'00"E | 26 Nov 2007 | Yes |
| 8 | Open Haadkaew Lagoon | 7º14'23"N | 100º33'36"E | 26 Nov 2007 | Yes |
| 9 | Ban Hua Khao Daeng | 7º13'04"N | 100º34'28"E | 26 Nov 2007 | Yes |
| 10 | Ban Khao Nui | 7º11'49"N | 100º33'34"E | 26 Nov 2007 | Yes |
| 11 | Ban Bor Ang | 7º13'01"N | 100º32'03"E | 26 Nov 2007 | Yes |
| 12 | Koh Yor | 7º09'20"N | 100º31'56"E | 13 Sep 2008 | Yes |
| 13 | Lhaem Poh | 7º09'16"N | 100º28'04"E | 25 Nov 2007 | No |
| 14 | Shrimp farm 1 | 7º14'16"N | 100º30'15"E | 26 Nov 2007 | No |
| 15 | Sa Thing Mor | 7º14'53"N | 100º29'26"E | 26 Nov 2007 | No |
| 16 | Pak Ror 1 | 7º15'35"N | 100º25'39"E | 26 Nov 2007 | No |
| 17 | Pak Ror 2 | 7º15'03"N | 100º26'03"E | 18 Nov 2007 | Yes |
| 18 | Pak Ror 3 | 7º16'2"N | 100º24'45"E | 18 Nov 2007 | No |
| 19 | Ban Koh Nang Kam | 7º16'18"N | 100º25'20"E | 18 Nov 2007 | No |

**<u>Appendix 2</u> The distribution survey of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand** (continue)

| Record No. | Sampling Station | Record coordinates | | Record date | The occurrence of the fish |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| 20 | Ban Tha Nam | 7º15'59"N | 100º24'15"E | 18 Nov 2007 | No |
| 21 | Ban Tha Nam 2 | 7º16'33"N | 100º24'22"E | 18 Nov 2007 | No |
| 22 | Ban Tha Hin | 7º23'32"N | 100º25'5"E | 18 Nov 2007 | No |
| 23 | Khu Khut | 7º27'45"N | 100º24'41"E | 18 Nov 2007 | No |
| 24 | Klong Ree | 7º31'45"N | 100º24'10"E | 18 Nov 2007 | No |
| 25 | Ban Lhaem Had | 7º34'5"N | 100º16'37"E | 18 Nov 2007 | No |
| 26 | Ban Rong | 7º41'29"N | 100º19'11"E | 18 Nov 2007 | No |
| 27 | Pak Ja | 7º15'47"N | 100º23'54"E | 5 Dec 2007 | No |
| 28 | Ban Tha Prapa | 7º16'25"N | 100º23'5"E | 5 Dec 2007 | No |
| 29 | Ban Tha Prapa 2 | 7º17'14"N | 100º22'53"E | 5 Dec 2007 | No |
| 30 | Ban Tarn | 7º19'50"N | 100º23'3"E | 5 Dec 2007 | No |
| 31 | Ban Tarn 2 | 7º19'26"N | 100º22'53"E | 5 Dec 2007 | No |
| 32 | Tambon Koh Nang Kam 1 | 7º18'31"N | 100º22'54"E | 5 Dec 2007 | No |
| 33 | Tambon Koh Nang Kam 2 | 7º16'10"N | 100º23'48"E | 5 Dec 2007 | No |
| 34 | Shrimp farm 2 | 7º19'34"N | 100º24'16"E | 5 Dec 2007 | No |
| 35 | Koh Mak | 7º22'29"N | 100º20'53"E | 5 Dec 2007 | No |
| 36 | Pakbang Nakkarat | 7º23'21"N | 100º20'44"E | 5 Dec 2007 | No |
| 37 | Ban Chong Fuen | 7º23'6"N | 100º19'26"E | 5 Dec 2007 | No |
| 38 | Ban Lhaem | 7º22'59"N | 100º16'13"E | 5 Dec 2007 | No |
| 39 | Haad Khai Tao | 7º26'27"N | 100º14'3"E | 5 Dec 2007 | No |
| 40 | Lhaem Jong Thanon | 7º29'32"N | 100º13'33"E | 5 Dec 2007 | No |

**<u>Appendix 2</u> The distribution survey of *Poecilia velifera* (Regan, 1914) in the Songkhla Lake Basin, Thailand** (continue)

| Record No. | Sampling Station | Record coordinates | | Record date | The occurrence of the fish |
|---|---|---|---|---|---|
| | | Latitude | Longitude | | |
| 41 | Haad Jong Kae | 7º29'23''N | 100º12'30''E | 5 Dec 2007 | No |
| 42 | Ranode | 7º47'37''N | 100º15'30''E | 5 Dec 2007 | No |
| 43 | Klong Nang Riam | 7º45'37''N | 100º10'75''E | 5 Dec 2007 | No |
| 44 | Thale Noi | 7º48'22''N | 100º6'57''E | 5 Dec 2007 | No |

**<u>Appendix 3</u> Morphological counts and measurement of 31 *Poecilia velifera* (Regan, 1914) collected from the Songkhla Lake Basin, Thailand**

| Specimen number | Standard length (mm) | Total length (mm) | body depth (mm) | predorsal distance (mm) | dorsal fin base length (mm) | Number of dorsal fin rays | Number of scale occiput-dorsal | Number of scale around caudal peduncle | Number of lateral scale |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 52.7 | 72.7 | 20.6 | 18.1 | 27.8 | 18 | 7 | 20 | 27 |
| 2 | 56.7 | 74.8 | 22.2 | 17.9 | 28.7 | 18 | 7 | 20 | 27 |
| 3 | 51 | 62.6 | 19.9 | 15.7 | 25.1 | 17 | 7 | 20 | 27 |
| 4 | 48.3 | 63.9 | 18.3 | 17.6 | 22.6 | 17 | 7 | 20 | 27 |
| 5 | 50.0 | 65.7 | 19.4 | 17.8 | 21.9 | 17 | 7 | 20 | 27 |
| 6 | 45.9 | 60.5 | 16.7 | 14.9 | 21.1 | 16 | 7 | 20 | 27 |
| 7 | 47.2 | 62.2 | 18.7 | 14.8 | 20.4 | 18 | 7 | 20 | 28 |
| 8 | 50.0 | 64.8 | 19.8 | 18.9 | 26.6 | 18 | 7 | 20 | 28 |
| 9 | 47.8 | 62.4 | 17.3 | 15.3 | 23.3 | 17 | 7 | 20 | 28 |
| 10 | 46.1 | 61.5 | 17.5 | 15.6 | 19.9 | 17 | 7 | 20 | 27 |
| 11 | 45.9 | 62.1 | 18.7 | 15.1 | 23.0 | 17 | 7 | 20 | 28 |
| 12 | 44.4 | 58.3 | 17.7 | 17.0 | 20.0 | 18 | 7 | 20 | 27 |
| 13 | 45.0 | 60.5 | 17.6 | 14.3 | 20.5 | 17 | 7 | 20 | 28 |
| 14 | 44.5 | 59.3 | 17.4 | 15.3 | 21.0 | 16 | 7 | 20 | 28 |
| 15 | 43.1 | 52.2 | 17.4 | 18.7 | 17.7 | 16 | 7 | 20 | 27 |
| 16 | 43.1 | 57.7 | 16.1 | 13.6 | 19.0 | 17 | 7 | 20 | 29 |
| 17 | 43.6 | 57.2 | 15.9 | 16.3 | 18.8 | 17 | 7 | 20 | 29 |
| 18 | 39.4 | 53.0 | 16.0 | 15.0 | 15.9 | 17 | 7 | 20 | 29 |
| 19 | 40.2 | 53.3 | 15.7 | 14.9 | 18.7 | 19 | 7 | 20 | 29 |
| 20 | 41.8 | 53.8 | 15.9 | 13.7 | 17.5 | 17 | 7 | 20 | 29 |
| 21 | 38.2 | 50.4 | 14.1 | 16.1 | 14.0 | 16 | 7 | 20 | 28 |
| 22 | 35.1 | 47.3 | 14.3 | 11.3 | 15.6 | 18 | 7 | 20 | 28 |
| 23 | 35.4 | 46.7 | 13.7 | 11.3 | 13.3 | 17 | 7 | 20 | 29 |
| 24 | 31.5 | 48.1 | 14.1 | 15.3 | 14.3 | 19 | 7 | 20 | 28 |
| 25 | 38.4 | 51.2 | 14.3 | 13.8 | 16.5 | 18 | 7 | 20 | 28 |
| 26 | 36.8 | 52.5 | 14.6 | 12.8 | 16.8 | 17 | 7 | 20 | 27 |
| 27 | 38.0 | 50.1 | 14.8 | 13.8 | 14.6 | 17 | 7 | 20 | 27 |
| 28 | 39.2 | 50.9 | 15.8 | 18.2 | 15.0 | 17 | 7 | 20 | 27 |
| 29 | 39.4 | 50.6 | 15.0 | 14.3 | 15.1 | 17 | 7 | 20 | 27 |
| 30 | 38.9 | 52.4 | 15.6 | 14.2 | 17.3 | 18 | 7 | 20 | 27 |
| 31 | 33.8 | 44.6 | 13.7 | 12.8 | 14.0 | 17 | 7 | 20 | 27 |

**Appendix 4 Length frequency distribution of *Poecilia velifera* (Regan, 1914) collected from Songkhla Lake Basin, from January 2007 to January 2008**

| Standard length (mm) | Month | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
| **>5.0-7.5** | | | | | | | | 1 | | | | | |
| **>7.5-10.0** | 1 | | | | | 3 | 8 | 31 | 1 | | 1 | | |
| **>10.0-12.5** | 1 | 1 | 1 | 3 | 1 | 38 | 31 | 205 | 22 | 7 | 1 | 6 | |
| **>12.5-15.0** | 2 | 4 | 1 | 19 | 18 | 110 | 39 | 411 | 76 | 21 | 16 | 35 | |
| **>15.0-17.5** | 8 | 11 | 1 | 37 | 75 | 163 | 85 | 302 | 119 | 37 | 120 | 64 | |
| **>17.5-20.0** | 7 | 18 | 3 | 31 | 135 | 100 | 134 | 182 | 231 | 56 | 347 | 153 | 3 |
| **>20.0-22.5** | 22 | 23 | 8 | 28 | 129 | 86 | 92 | 176 | 493 | 135 | 271 | 294 | 11 |
| **>22.5-25.0** | 11 | 27 | 8 | 27 | 84 | 116 | 91 | 108 | 631 | 186 | 151 | 503 | 11 |
| **>25.0-27.5** | 11 | 37 | 32 | 34 | 52 | 107 | 116 | 94 | 538 | 218 | 108 | 675 | 13 |
| **>27.5-30.0** | 13 | 37 | 35 | 41 | 31 | 103 | 128 | 63 | 331 | 192 | 58 | 450 | 42 |
| **>30.0-32.5** | 10 | 35 | 22 | 28 | 27 | 66 | 97 | 44 | 230 | 114 | 38 | 203 | 91 |
| **>32.5-35.0** | 12 | 37 | 24 | 36 | 17 | 47 | 88 | 25 | 157 | 92 | 22 | 91 | 128 |
| **>35.0-37.5** | 8 | 35 | 24 | 26 | 12 | 30 | 91 | 28 | 121 | 69 | 32 | 51 | 116 |
| **>37.5-40.0** | 17 | 26 | 11 | 30 | 23 | 12 | 99 | 17 | 78 | 44 | 19 | 20 | 81 |
| **>40.0-42.5** | 18 | 11 | 16 | 28 | 17 | 12 | 96 | 23 | 72 | 29 | 15 | 18 | 68 |
| **>42.5-45.0** | 20 | 15 | 12 | 28 | 18 | 6 | 58 | 20 | 30 | 22 | 15 | 9 | 56 |
| **>45.0-47.5** | 14 | 11 | 5 | 24 | 16 | 6 | 27 | 22 | 33 | 25 | 6 | 2 | 40 |
| **>47.5-50.0** | 15 | 8 | 5 | 19 | 8 | 3 | 22 | 6 | 24 | 15 | 7 | 2 | 17 |
| **>50.0-52.5** | 9 | 8 | 4 | 7 | 17 | 7 | 11 | 17 | 18 | 6 | 4 | 2 | 10 |
| **>52.5-55.0** | 6 | 9 | 4 | 9 | 7 | 5 | 6 | 4 | 6 | 10 | 8 | 1 | 4 |
| **>55.0-57.5** | 6 | 9 | 5 | 5 | 9 | 5 | 3 | 6 | 5 | 4 | 4 | | 3 |
| **>57.5-60.0** | 4 | 8 | 1 | | 1 | 1 | 1 | 1 | 2 | | 1 | | |
| **>60.0-62.5** | 2 | 2 | | 1 | 1 | 3 | 2 | 1 | 2 | 3 | | 1 | |
| **>62.5-65.0** | | | | | | 1 | | | | 2 | | | |
| **>65.0-67.5** | 1 | | | | 1 | 1 | | | | | | | |
| **>67.5-70.0** | | | | | | | | | | 2 | | | |

**<u>Appendix 5</u> Stomach content of *Poecilia velifera* (Regan, 1914) collected from Songkhla Lake Basin, from January 2007 to January 2008 defined by %IRI$_{mod}$ for pool specimens and that grouped by sexes (M= male; F= female), size classes (1: SL ≤ 30.0mm; 2: 30.0mm < SL ≤ 50.0mm; 3: SL > 50.0mm) and seasons (SE= Southeast monsoon season; SW= Southwest monsoon season; NE= Northeast monsoon season)**

| **Food categories** | ***%IRI$_{mod}$*** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **pool** | **sex** | | **size class** | | | **season** | | |
| | | **M** | **F** | **1** | **2** | **3** | **SE** | **SW** | **NE** |
| Plant matter | 71 | 69 | 73 | 43 | 79 | 79 | 92 | 84 | 42 |
| Microalgae | 23 | 21 | 24 | 41 | 19 | 14 | 8 | 16 | 37 |
| Insects | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 3 |
| Crustaceans | 4 | 8 | 2 | 11 | 0 | 7 | 0 | 0 | 14 |
| Other | 1 | 2 | 1 | 4 | 1 | 0 | 0 | 0 | 3 |
| **total** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| **n** | 776 | 332 | 444 | 324 | 363 | 89 | 144 | 379 | 253 |

# VITAE

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- TRF/BRT Special program for Biodiversity Research and training grant BRT T_251003
- The Development and Promotion of Science and Technology Talent Project (DPST)
- Research assistantship from Faculty of Science Prince of Songkla University
- Faculty of Science and Department of Biology, Faculty of Science, Prince of Songkla University supported for participating in the International Conference on Managing Wetlands for Sustainable Development: Innovative Research and Lessons Learned, Effective Partnerships, and the Need for Co-Management. Thumrin Thana Hotel, Trang Province, Thailand on 9-11 January 2008
- Association for Tropical Biology & Conservation, Asia-Pacific Chapter (ATBC) supported for participating in the conference of the Association for Tropical Biology & Conservation, Asia-Pacific Chapter hosted by King Mongkut's University of Technology Thonburi in the title "Assessing and Restoring Biodiversity in a Human Dominated Landscape" at Imperial Mae Ping Hotel, Chiang Mai, Thailand on 12-15 February 2009

**List of Publication and Proceeding**


- Sa-nguansil, S. and Lheknim, V. 2010. The occurrence and reproductive status of Yucatan molly *Poecilia velifera* (Regan 1914) (Poeciliidae; Cyprinodontiformes): an alien fish invading the Songkhla Lake Basin, Thailand. Aquatic Invasions. (in press).
- Sa-nguansil, S. and Lheknim, V. Diet of sailfin molly *Poecilia velifera* (Regan, 1914) (Poeciliidae; Cyprinodontiformes): an alien fish invading Songkhla Lake Basin, Thailand (in preparation)
- Sa-nguansil, S. and Lheknim, V. Otolith validation and growth of sailfin molly *Poecilia velifera* (Regan, 1914) (Poeciliidae; Cyprinodontiformes): an alien fish invading Songkhla Lake Basin, Thailand (in preparation)