นิเวศวิทยาการกินอาหารของปลาไหลงู *Pisodonophis boro* (Hamilton, 1822) บริเวณปากแม่น้ำปราณบุรี จังหวัดประจวบคีรีขันธ์

นายภากร ณ ลำปาง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์ทางทะเล ภาควิชาวิทยาศาสตร์ทางทะเล
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2559

FEEDING ECOLOGY OF THE SNAKE EEL *Pisodonophis boro* (Hamilton, 1822) IN PRANBURI RIVER ESTUARY, PRACHUAP KHIRI KHAN PROVINCE

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Marine Science
Department of Marine Science
Faculty of Science
Chulalongkorn University
Academic Year 2016

Thesis Title	FEEDING ECOLOGY OF THE SNAKE EEL *Pisodonophis boro* (Hamilton, 1822) IN PRANBURI RIVER ESTUARY, PRACHUAP KHIRI KHAN PROVINCE
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ภากร ณ ลำปาง : นิเวศวิทยาการกินอาหารของปลาไหลงู *Pisodonophis boro* (Hamilton, 1822) บริเวณปากแม่น้ำปราณบุรี จังหวัดประจวบคีรีขันธ์ (FEEDING ECOLOGY OF THE SNAKE EEL *Pisodonophis boro* (Hamilton, 1822) IN PRANBURI RIVER ESTUARY, PRACHUAP KHIRI KHAN PROVINCE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ดร.เจษฎ์ เกษตระทัต, หน้า.

ปลาไหลงู *Pisodonophis boro* เป็นปลาที่มีแนวโน้มในการถูกพัฒนาเป็นปลาเศรษฐกิจได้ในปากแม่น้ำปราณบุรี อย่างไรก็ตามยังไม่มีการรายงานการวิจัยที่แสดงถึงบทบาท และการใช้ประโยชน์จากพื้นที่ของปลาไหลงูภายในระบบนิเวศบริเวณปากแม่น้ำปราณบุรี จังหวัดประจวบคีรีขันธ์ ดังนั้นการศึกษานิเวศวิทยาการกินในรูปแบบขององค์ประกอบของอาหารในกระเพาะ สัณฐานวิทยาและมิญชวิทยาของทางเดินอาหาร ตลอดจนการศึกษาโครงสร้างมิญชวิทยาของอวัยวะสร้างเซลล์สืบพันธุ์ของปลาไหลงูจึงมีความสำคัญและจำเป็น การศึกษานี้ทำการเก็บตัวอย่างปลาไหลงู 77 ตัวอย่าง จากปากแม่น้ำปราณบุรี จังหวัดประจวบคีรีขันธ์ ตั้งแต่เดือนมีนาคม พ.ศ. 2558 ถึงเดือนมีนาคม พ.ศ. 2559 เพื่อศึกษาองค์ประกอบอาหารในกระเพาะอาหารของปลาไหลงูและสัณฐานวิทยาและมิญชวิทยาของทางเดินอาหาร และเก็บตัวอย่างเนื้อเยื่ออวัยวะสร้างเซลล์สืบพันธุ์ของปลาไหลงูจาก 105 ตัวอย่าง (77 ตัวอย่างเดิมรวมกับตัวอย่างเพิ่มเติม 28 ตัวอย่าง) เพื่อศึกษามิญชวิทยาของอวัยวะสร้างเซลล์สืบพันธุ์ พบว่าปลาไหลงูชนิดนี้มีลักษณะการกินอาหารแบบปลากินเนื้อ และมีการเลือกกินอาหารเพียงอย่างเดียวมากกว่ากินอาหารตามโอกาส โดยองค์ประกอบหลักของอาหารที่พบคือปู 4 ชนิดได้แก่ *Metaplax elegans, Perisesarma bidens, Sarmatium germaini* และ *Uca perplexa* และจากผลการศึกษาดัชนีความสำคัญของอาหาร (IRI) ปูชนิด *S. germaini* มีความสำคัญมากที่สุดเป็นอันดับแรก รองลงมาคือ *M. elegans, U. perplexa* และ *P. bidens* ตามลำดับ สัณฐานวิทยาของทางเดินอาหารมีลักษณะเป็นเส้นตรงขนานกับลำตัว และมีกระเพาะอาหารเป็นรูปตัววาย ลักษณะมิญชวิทยาของทางเดินอาหารประกอบด้วย 4 ชั้น คือ mucosa, submucosa, muscularis และ serosa หลอดอาหารมีรอยพับยกตัวสูงตลอดแนวและบริเวณเนื้อเยื่อบุผิวชนิดแบนบางถูกแทรกด้วยเซลล์สร้างเมือก ขณะที่โครงสร้างทางมิญชวิทยาของกระเพาะอาหารพบว่ามีต่อมกระเพาะอาหารแทรกตัวอยู่ในชั้น mucosa ทั้งกระเพาะอาหารส่วนต้นและส่วนปลาย ลำไส้ของปลาไหลงูแบ่งออกเป็น 3 ส่วน คือ ลำไส้ส่วนต้น ลำไส้ส่วนกลาง และลำไส้ส่วนท้าย พบเซลล์กอบเลทแทรกอยู่ในชั้นเนื้อเยื่อบุผิวเป็นจำนวนมาก นอกจากนี้ยังพบอีกว่าเซลล์กอบเลทยังมีปฏิกิริยากับกับสีย้อมเพอริโอดิกเอสิดชิฟฟ์ (PAS) และอัลเซียนบลู (AB) ตลอดทั้งลำไส้แสดงให้เห็นว่าเซลล์กอบเลทมีการสร้างสารจำพวกมิวโคโพลีแซ็กคาไรด์ และไกลโคโปรตีน อวัยวะสร้างเซลล์สืบพันธุ์ในปลาไหลงูปรากฏเพียงแค่โครงสร้างรังไข่เท่านั้นที่จัดเป็นการพัฒนาเซลล์สืบพันธุ์เป็นแบบพร้อมกัน (synchronous development oocyte) เนื่องจากประกอบด้วยเซลล์ไข่ระยะเพรินิวคลีโอลาร์ (perinucleolar stage) เพียงแค่ระยะเดียว (จำนวนตัวอย่าง 105 ตัวอย่าง) จากการศึกษาครั้งนี้แสดงให้เห็น ผลจากการศึกษาสัณฐานวิทยาและมิญชวิทยาของระบบทางเดินอาหารแสดงให้ถึงความสอดคล้องกับองค์ประกอบอาหารภายในกระเพาะอาหารของปลาไหลงูที่สนับสนุนว่าระบบทางเดินอาหารของปลาไหลงูชนิดนี้มีการวิวัฒนาการให้สามารถย่อย และดูดซึมอาหารที่มีโครงสร้างที่แข็งเช่นปู และขับถ่ายชิ้นส่วนที่ไม่สามารถย่อยได้เช่นกระดอง และก้ามปูออกไปได้อย่างมีประสิทธิภาพสูงสุด การศึกษานี้ยืนยันว่าปลาไหลงูชนิดนี้เป็นปลาที่กินเนื้อ โดยเป็นปลาที่มีการเลือกกินอาหารแบบเฉพาะเจาะจงมากกว่าเป็นปลาที่มีการกินอาหารตามโอกาส จากภาพรวมของการศึกษานี้แสดงให้เห็นถึงความสำคัญและการใช้ประโยชน์จากพื้นที่ปากแม่น้ำปราณบุรีของปลาไหลงูเพื่อเป็นแหล่งอาศัยและแหล่งอาหารเท่านั้น ไม่ได้ใช้ประโยชน์เพื่อเป็นพื้นที่สืบพันธุ์วางไข่

ภาควิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อนิสิต .....

สาขาวิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2559

5672049623 : MAJOR MARINE SCIENCE
KEYWORDS: FISH / FEEDING ECOLOGY / HABITAT UTILIZATION / MICROANATOMY / OVARIAN HISTOLOGY

PHAKORN NA LAMPANG: FEEDING ECOLOGY OF THE SNAKE EEL *Pisodonophis boro* (Hamilton, 1822) IN PRANBURI RIVER ESTUARY, PRACHUAP KHIRI KHAN PROVINCE. ADVISOR: JES KETTRATAD, Ph.D., pp.

The snake eel, *Pisodonophis boro* (Hamilton, 1822), one of the economically important species, is a potential aquaculture fish candidate at Pranburi River estuary. However, the roles in the food web of the ecosystem and the habitat utilization of this fish are still lacking. Hence, feeding ecology, morpho-histological structures of the digestive tract and gonadal histological study are required. Seventy-seven of *P. boro* were collected from the Pranburi River estuary, during March 2015 to March 2016 for the study of the gut content and morpho-histological structures of the digestive tract. Gonadal tissues of 105 specimens (77 former specimens and 28 additional specimens) were collected for the gonadal histological study. The resulted of gut content and morpho-histological structures of the digestive tract revealed that *P. boro* is a carnivore and it is considered as a specialist feeder rather than an opportunistic feeder. The results of the gut content demonstrated that the important prey item was crabs: *Metaplax elegans*, *Perisesarma bidens*, *Sarmatium germaini*, and *Uca perplexa*. The index of relative importance revealed that the most important prey was *S. germaini* followed by *M. elegans*, *U. perplexa* and *P. bidens* respectively. Morpho-histological study of the digestive tract of *P. boro* demonstrated the overall anatomical morphology of the digestive tract was elongated, and the stomach appeared as Y-shaped. The digestive tract consisted of four layers: mucosa, submucosa, muscularis and serosa. The histology of the esophagus demonstrated the longitudinal folds and the mucosal epithelium, which was lined with simple squamous epithelium inserting with numerous mucous cells. The mucosal layers in both anterior and posterior regions of stomachs were similarly formed as gastric rugae. Histology of the intestine regions divided into three regions: anterior intestine, middle intestine, and posterior intestine. Numerous intestinal folds were exclusively presented in the anterior region, which was lined by simple columnar cells. Goblet cells were also seen among the epitheliums and positively reacted to Periodic Acid Schiff (PAS) and alcian blue (AB) staining throughout the intestine tract. It indicated that the intestine tract contained mucopolysaccharides and glycoprotein. All specimens (n=105) had the similar gonadal morphology of ovarian structure which contained perinucleolar stage oocyte. The snake eel was considered as a synchronous developmental oocyte based on the histological observation. The morpho-histological study of *P. boro* digestive tract supported the index of relative importance of the diets of *P. boro* which implied that the digestive tract of *P. boro* has undergone evolutionary modifications to optimize digestion and absorption of crab-derived metabolites while also being able to breakdown and then eliminates hard body parts (e.g., carapace, chelipeds) that are not digestible. This study suggested that the role of estuary ecosystem in Pranburi River is important in providing food sources and habitat for snake eel, *P. boro*. The snake eel did not use the estuary of Pranburi River for spawning purpose but rather as a spawning migration route to the spawning ground.

Department: Marine Science
Field of Study: Marine Science
Academic Year: 2016

Student's Signature .....
Advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Lect. Dr. Jes Kettratad, for his support, guidance, invaluable advice in this thesis and his great contribution to every phase in my field/ laboratory studies.

I would like to express my sincere thank my thesis committee members, Assoc. Prof. Dr. Voranop Viyakarn, Assoc. Prof. Dr. Charoen Nitithamyong and Dr. Apichat Termvidchakorn, for their valuable suggestions and comments throughout my thesis.

I would like to thank Assoc. Prof. Dr. Wannee Jiraungkoorskul for her advice and support on the histological study, Prof. Dr. F. Gerald Plumley, for his helpful advice and valuable suggestion on the grammatical structures in my manuscripts and Dr. Sinlapachai Senarat, for his advice on the useful information, advice and revise in my manuscripts.

I also would like to thank the staffs of Sirinart Rajini Mangrove Ecosystem Learning Center at Pranburi River, Prachuap Khiri Khan Province and also thanks to Mr. Sema Phonha and Mr. Somrak Roopchom, the local fisherman, for their kind help in field specimen collection.

This work was financially supported by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

Last but definitely not least, I wish to express my beloved mother and my family for their support, encouragement, and understanding throughout my academic work.

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Figure 4.3 Schematic diagram showing the undeveloped stage of *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016. **Abbreviations:** AD, adipocyte; BN, Balbiani body; BM, basement membrane; GC, a germinal compartment; FC, follicular cells; N, nucleolus; OP, ooplasm. 49

CHAPTER 1

GENERAL INTRODUCTION

The snake eel, *Pisodonophis boro* (Hamilton, 1822) belongs to family Ophichthidae, Order Anguilliformes. It is widely distributed in the Indo-West Pacific, southern India, Sri Lanka, Indonesia, and Thailand. The snake eel is an anadromous fish and usually found along the estuaries and the tidal zone around the upstream areas of coastal rivers. Subramanian (1984) observed that this species was selective feeder which feeds only on a single species of crab (*Uca annulipes*) in India (n=87). The study from Cambodia reported that this fish forage on small fishes in the water column (Rainboth, 1996). Hence, *P. boro* may show selective feeding habit based on the diversity of organisms present in each location.

In Thailand, there has not been any study on the feeding ecology and the overall basic biology of the *P. boro*. Most studies focused on the distribution of the species (Chaudhry, 2010; Moravec *et al.*, 2007; Paphavasit *et al.*, 2014). Paphavasit *et. al*. (2014) reported that *P. boro* is found in the estuarine of the Pranburi River. This species has a high economic value and has a potential for the commercial fish market. Unfortunately, the population of *P. boro* in this area is on the decline due to several reasons such as the community development and the environmental problems (pers. comm.). If the population is still decreasing, it is likely that they will be extinct from the estuary of the Pranburi River.

In order to properly manage this species, it is vital to understand their ecological role and reproductive biology. Feeding ecology primarily leads to the understanding of the ecological roles of fish species, population dynamics, resources partitioning, and trophic ecology (Guedes & Araújo, 2008; Ross, 1986; Svanbäck & Bolnick, 2007). Moreover, the feeding information is a great value for developing the conservation strategies (Braga *et al.*, 2012). Not only is the feeding information required for the task but also the aspects of histological knowledge as well. The study of histological of fishes has been well studied in teleost. The morphology and histology of the alimentary tract have been suggested to correlate with the feeding habits (Al-Hussaini, 1949) and shows variation in morphology, histology, and

function relates to its feeding guild (Abdulhadi, 2005). The histological of the digestive system is well-known as a good indicator of fish nutrition requirement (Rašković *et al.*, 2011). The second aspect of this study focuses on the reproductive biology of *P. boro*. The reproductive information is important for both management and aquaculture (Tongnunui *et al.*, 2016). These knowledges also would be providing the information to explain how *P. boro* utilizing the Pranburi River estuary. Therefore, this study aims to investigate the gut content and the aspect of histological knowledge of digestive tract and gonadal histology of *P. boro*.

Objectives of this study

1. To study gut contents of *Pisodonophis boro* (Hamilton, 1822)
2. To study morpho-histology of digestive tract of *Pisodonophis boro* (Hamilton, 1822)
3. To study gonadal histology of *Pisodonophis boro* (Hamilton, 1822)

CHAPTER 2
REVIEW OF LITERATURES

2.1 The snake eel *Pisodonophis boro* (Hamilton, 1822)

2.1.1 Taxonomy and Morphology

The snake eel, *Pisodonophis boro* (Hamilton, 1822) is an anadromous fish with taxonomic classification belongs to Order Anguilliformes: family Ophichthidae. The general morphology of *P. boro* is shown in Figure 2.1. The body is elongated snake-like with common length about 70 cm and maximum length up to 100 cm (Rainboth, 1996). They have no dorsal spine and anal spine. The dorsal fin begins at least 1 pectoral-fin length behind the tip of pectoral fin (Fig. 2.1). Talwar and Jhingran (1991) reported that the vertebrae of *P. boro* ranged from 171 to 173. The color of the body is olive brown on the dorsal side, pale silver on the ventral side in both male and female. The tail is finless tail tip form. The head is large. The anterior nostril is tubular. Teeth are granular to molariform, multiserial bands on jaws and vomer, but sharper on jaws and pointed. The lateral line is inconspicuous.

Figure 2.1 External morphology of female snake eel (SL = 67 cm), *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016. Arrowhead indicates the origin of pectoral fin. Scale bar: 2.5 cm.

2.1.2 Natural history

Pisodonophis boro is a tropical anadromous fish. It can survive in a wide range of habitats such as lagoons, estuaries, freshwater, marine and paddy fields. It is common in tidal reaches areas and nearby upstream areas of coastal river (Rainboth, 1996; Sommer *et al.*, 1996). The *P. boro* has a very wide distribution in the Indo-West Pacific region. Its distribution covers India, Bangladesh, Viet Nam, China, Indonesia, Taiwan, Philippines, Australia, South Africa, Madagascar, Kenya, Yemen, and Saudi Arabia. It is considered as a carnivorous fish. They live in holes in the river bottom and bank and actively forages at night (Rainboth, 1996). Little is known about maturity, reproductive biology, and breeding season but there are some reported suggested that *P. boro* spawn in rice-paddy field during the rainy season (Rainboth, 1996).

2.2 General life history of Anguillids

2.2.1 Life history of the catadromous eel

Generally, eels have five life cycle stages: leptocephali, metamorphosis, the glass Eel (elver), the yellow eel, and the silver eel respectively (Fig. 2.2). However, the life history of the family Ophichthidae (snake eel) has not been well studied. The life cycle of the family Anguillidae has been well studied for a century. Aida *et al.* (2003) described the life cycle stage of the eel as follows:

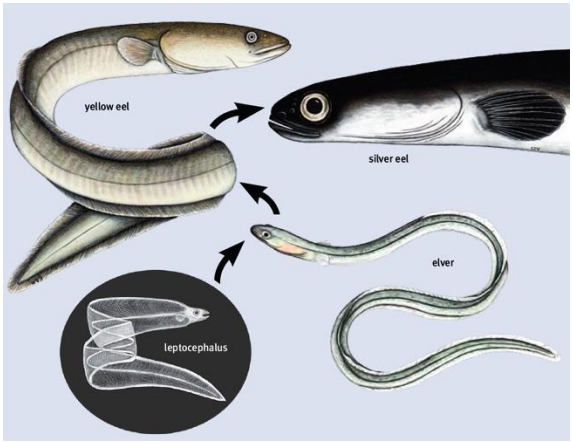

Figure 2.2 Ontogenetic stages of *Anguilla rostrate* (Illustrated by Ethan Nedeau, retrieved from http://www.gulfofmaine.org/times/summer2005/eel.html; date: 27/09/2016).

1. Leptocephali stage (Mochioka, 2003)

The Anguillid leptocephalus is one of the most typical larvae of Anguilliform fishes. They have simple and primitive morphological features. The body form is an olive leaf-like shape (Fig. 2.3). It has no melanophores except on the tip of the tail in early-stage larvae. It has moderately few myomeres and moderate tail. The maximum size is approximately 80 mm. The maximum size varies among species. The gut is simple without swellings, loops, or switchbacks, and the anus is about three-quarters of the total length. The kidney ends slightly behind the midpoint of the gut. The dorsal fin is moderately short and begins slightly before the level of the anus. The caudal fin is obviously separated from the tips of the dorsal and anal fins. The head and snout are moderately short and the eyes are medium size. The food sources of leptocephalus larvae are believed to be small particles, which can uptake by pinocytotic digestion or feed on low trophic level materials such as dissolved and particulate organic matter existing in seawater. It is possible that the foods of leptocephalus larvae are detrital materials such as larvacean houses and zooplankton fecal pellets.

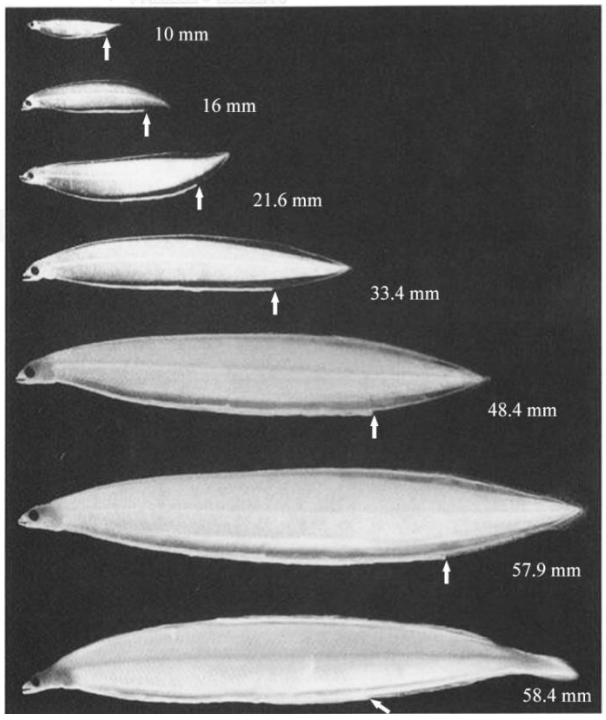

Figure 2.3 Ontogenetic changes of *Anguilla japonica* leptocephali: 10, 16, 21.6, 33.4, 48.4, 57.9, and 58.4 mm total length (TL), respectively, from the top. At bottom shows a metamorphosing stage. Arrowheads indicate the anal position (figure from Mochioka (2003)).

2. Metamorphosis stage (Otake, 2003)

Anguillids have an unusual type of larva and a prolonged larval period. The unusual and successful developmental strategy of leptocephali is that they can grow to a large size with minimal metabolic consequence. The morphological change during the metamorphosis includes the placement of dorsal fin, anal fin, and anus. The anus moves anteriorly with the initiation of metamorphosis, as do the origins of the dorsal and anal fins. The relative position of the anus (i.e., the ratio of preanal myomeres to total myomeres, PAM/TM ratio, or the ratio of preanal length to total length, PAL/TL ratio) is used as a criterion of the external metamorphic stage in anguilliform leptocephali. For example, the Japanese eel, *Anguilla japonica* has the PAM/TM ratio of leptocephali averages 0.66 and decreases to 0.36 in glass eel (juvenile). The metamorphosis stage from leptocephali to glass eel can be divided into three stages base on the relationship between TL and PAL/TL ratio: 1) premetamorphosis (PAL/TL ratio more than 0.85), 2) early metamorphosis (0.8-0.43), and 3) late metamorphosis (less than 0.43) as shown in Figure 2.4 (Yamano *et al.*, 1991). Modeling of physiological changes takes place during the metamorphosis from leptocephalus to glass eel (juvenile) is shown in Figure 2.5.

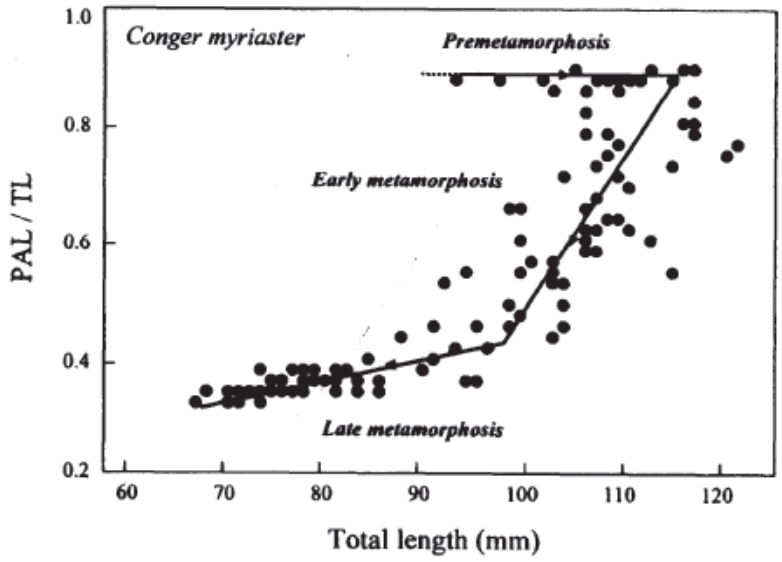

Figure 2.4 Relationship between total length (TL) and the ratio of preanal length to total length (PAL/TL ratio) of *Conger myriaster*. Straight line indicates the metamorphic process. The metamorphic process is divided into three stages: premetamorphosis, PAL/TL ratio more than 0.85; early metamorphosis, 0.85-0.43; late metamorphosis, less than 0.43 (figure from Yamano et al. (1991)).

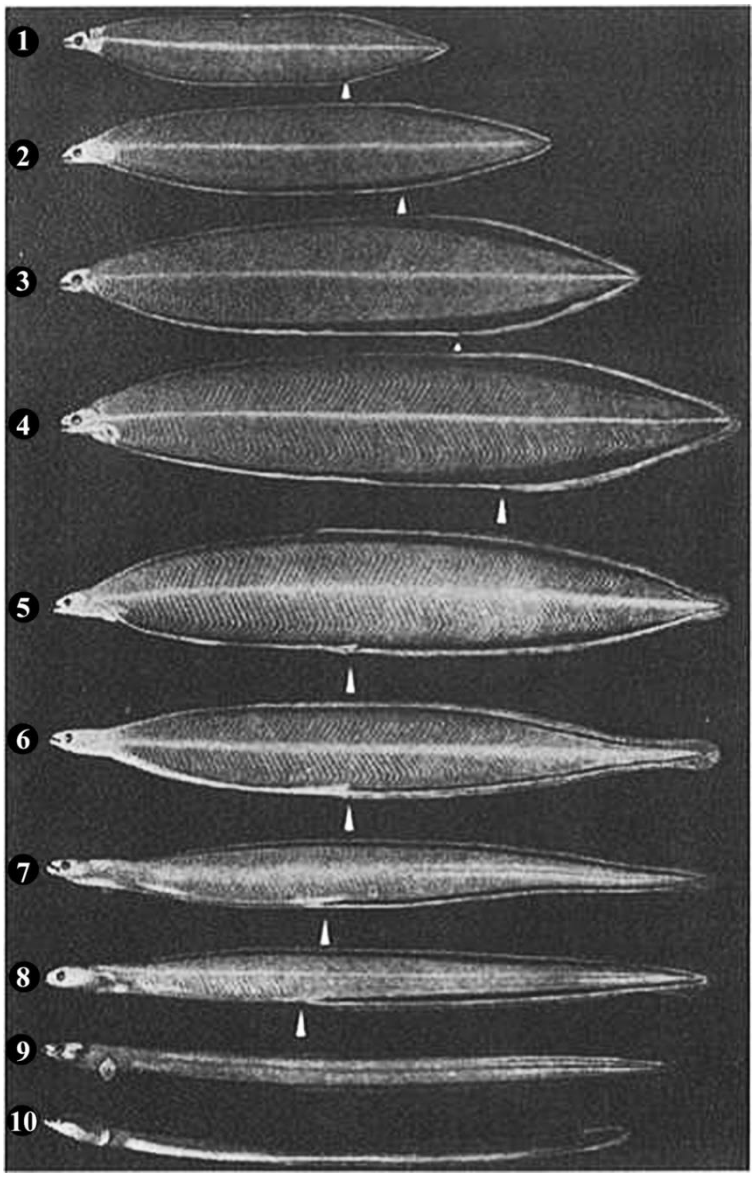

Figure 2.5 Morphological change in metamorphosing from leptocephalus to glass eel (juvenile). Leptocephali (1-4), metamorphosing leptocephali (5-8), glass eel (9), and elver (10) of *Anguilla anguilla*. Arrowheads indicate the anal position (figure from Sterba (1967)).

During the metamorphosis, the body shape of leptocephali larva rapidly changes from the leaf-like laterally shape to the cylindrical shape of the juveniles-like with the PAM/TM ratio decreasing to less than 0.35 (late metamorphic period). The internal tissues and organs such as muscle, kidney, and intestinal organs are also rapidly transformed into juvenile-like forms while the vertebral column is fixed. The duration of the late metamorphic period is likely to be 14 days from 70 days of the total metamorphic period in *Conger myriaster* (Otake *et al.*, 1997).

3. The glass eel stage (Tabeta & Mochioka, 2003)

The metamorphosis from the leptocephalus larva to glass eel occurs prior to entering the freshwater. The "Glass eel" is defined here as a developmental stage from the end of the metamorphosis of the leptocephalus to the beginning of pigmentation. The development of pigmentation begins at the base of the dorsal fin (Fig. 2.6). These eels stages are also known as elvers. During metamorphosis from leptocephalus larvae to glass eel, the external brain shape of leptocephali slowly changes from being laterally compressed to being dorsoventrally compressed and elongated (Fig. 2.7). The brain transformed with a well-developed cerebellum and important granular is much more flattened in appearance. The brain function will develop a visual system in leptocephali then the chemosensory and mechanosensory systems being developed in juveniles. Furthermore, gonads were found only in the body cavity of glass eels, and these gonads had one or two primordial germ cells (PMG) per cross section, indicating that thyroid hormone (TH) production started before thyroid stimulating hormone (TSH) production and that TSH and TH are both secreted during metamorphosis from leptocephalus to glass eel.

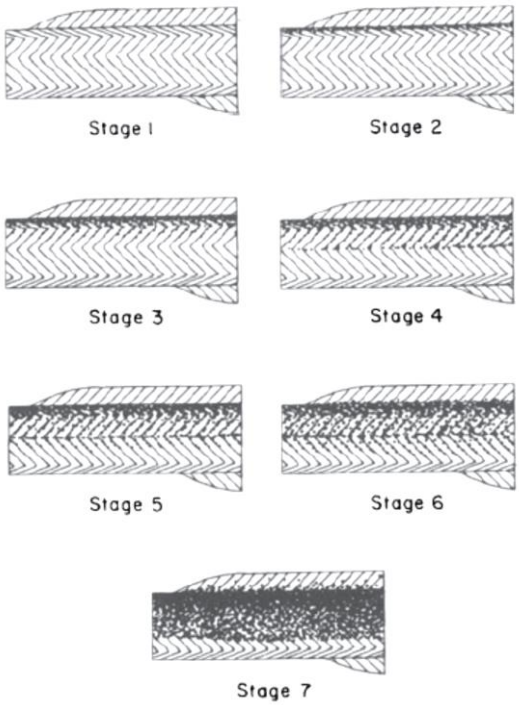

Figure 2.6 The pigmentation stages of glass eels *Anguilla rostrata* (figure from Haro and Krueger (1988)).

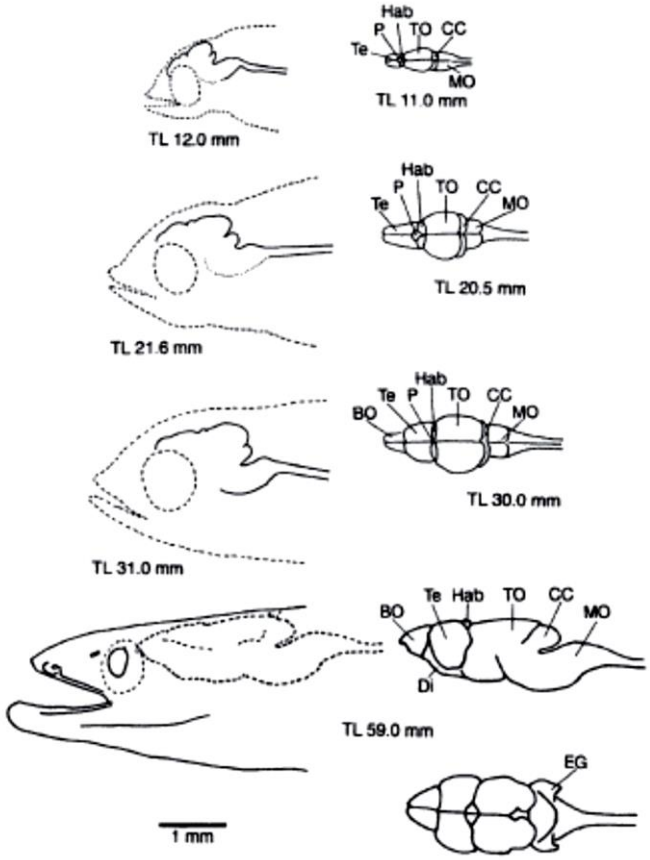

Figure 2.7 External brain morphology of *Anguilla japonica* changes in leptocephali and glass eels. TL, total length; Te, telencephalon; P, pineal organ; Hab, habenula; TO, optic tectum; CC, corpus cerebelli; MO, medulla oblongata; BO, olfactory bulb; EG, eminentia granularis (figure from Tomoda and Uematsu (1996)).

4. The yellow eel stage (Moriarty, 2003)

After entering fresh water, the glass eels transform into the yellow eel. The name of "yellow eel" is due to the brownish-yellow color of lateral sides and belly (Fig. 2.8). Most species of *Anguilla* are found far upstream in continental river systems. Some species are present in fully marine waters, including lagoons and coastal waters than in freshwater. The studies on strontium levels in the otoliths of both *A. anguilla* and *A. japonica* indicated that yellow eels reach the silver stage in brackish or saltwater without migrating into freshwater (Tsukamoto & Nakai, 1998; Tzeng *et al.*, 1997). The yellow eels are known to forage at night. Many studies on the stomach contents of yellow eels showed the composition of a wide range of invertebrates and fishes. In freshwater, a majority of studies show a diet of small

invertebrates for eels (Anguillidae) less than 30 to 40 cm with increasing number of fish when eel grows larger (Moriarty, 2003). Estuarine and marine *Anguilla* in general sense feeds on larger invertebrates, including various species of crab and oligochaete worms, and on a variety of fishes (Kaifu *et al.*, 2012). The average growth rate of yellow eel, *A. anguilla* range from 3 cm to cm per year and most did not exceed 10 cm per year (Berg, 1990). Female eels tend to grow faster than male eels. The yellow eel live upstream in freshwater for a long time. After 10 to 15 years, *Anguilla* eels have matured and reach a length of approximately 60 cm in males to 80 cm in a female. At this stage, the yellow eel changes their morphology and physiology which includes changes like their eyes become large and pigment pattern change to adapt living in the deep ocean. Then the transformed and matured eel are call “silver eel”. They generally swim downwards to the open seawater for spawning.

Figure 2.8 The yellow eel, *Conger conger*, showing the brownish-yellow color of their sides and belly (Online retrieved from http://www.eelregulations.co.uk/cont-007.html; date 28/09/2016).

5. *The silver eel stage* (Aoyama & Miller, 2003)

The average age at downstream migrants of silver eels has been reported to range widely from about 3 to 20 years such as Atlantic eels *A. Anguilla* was 3 to 12 years and *A. rostrata* was 4 to 20 years. At this stage, the morphological and physiological of yellows eels are changed, such as the color of the skin (Fig. 2.9), enlargement of the eyes and pectoral fins, degeneration of the alimentary tract, changes in fat content and musculature, swim bladder modifications, and increases of chloride cells in the gills. The ecological function of silvering is seemed to be to develop countershading to reduce their striking contrast during the oceanic spawning migration. These changes would facilitate the new demands of deep-water swimming, predator avoidance, and possibly visual mate location, their body changes to prepare to spawn and then die in the open ocean.

Figure 2.9 Coloration changes in *Anguilla rostrata*. Top, typical yellow eel; Middle, a downstream migrant silver eel; Bottom, an artificially matured silver eel (open ocean) (photograph courtesy of Alex Haro).

2.2.2 Life history of the anadromous eel

When compared to the eel in the family Anguillidae, little is known about the life history of the eel in the family Ophichthidae. This probably caused by the fact that the eel in the family Anguillidae had more commercial valuable in the fish markets, especially in the European eel (*Anguilla anguilla*), Japanese eel (*A. japonica*), and American eel (*A. rostrata*). However, as mentioned above, most literatures focus on the temperate eel species. Tropical species may have some difference in their development as well as their basic biology. The knowledge of tropical eel species is still unknown. Some studies have been done for the family Ophichthidae in the area listed below:

1. *Reproductive biology of the family Ophichthidae*

Richardson (1974) as cited by (Wenner, 1976) collected the eggs and larvae of *Pisodonophis cruentifer* from continental shelf around 74-148 km far from the Virginia coastal, USA. The eggs were taken by the surface plankton tows. The eggs diameter was greater than 2 mm and the number of eggs found ranged from 658 to

9013 eggs. Wenner (1976) reported the smallest matured *P. cruentifer* in Norfolk Canyon area with a total length of 19.7 cm in male and 22.7 cm in a female. Female eel was significantly larger than male. The eels entered the matured stage in June. They had peak spawned period in July. Histology of ripe gonad of both male and female *P. cruentifer* is shown in Figure 2.10.

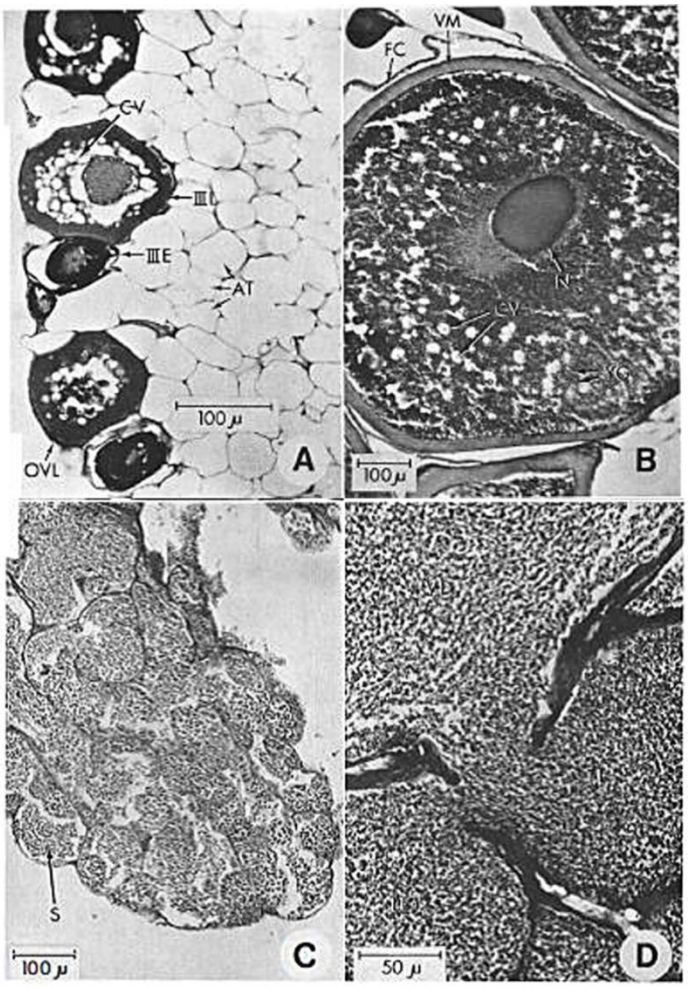

Figure 2.10 Gonadal histology of *Pisodonophis cruentifer*. A, cross section of immature ovary; B, cross section of mature ovary; C, cross section of testicular lobe from mature male showing the presence of spermatozoa in seminiferous tubules; D, cross section of gonadal tissue from mature male showing the emptying of the ripe spermatozoa from the testicular lobes into the vas deferens. CV, cytoplasm vesicle; IIIE, early stage III oocyte; IIIL, late stage III oocyte; AT, adipose tissue; OVL, ovigirous lamellae; N, nucleus; FC, follicle cells; VM, vitelline membrane; YG, yolk granules; S, spermatozoa (figure from Wenner (1976)).

Casadevall *et al.* (2001) collected the Mediterranean snake eels *Ophichthus rufus* samples from Blanes (Costa-Brava, Catalan sea, northwest Mediterranean). Based on a total of 679 specimens, collected monthly between 1985 and 1987, *O. rufus* is an oviparous species (fish that undergo external fertilization) with a group synchronous ovary and buoyant eggs. The annual fecundity estimates ranged from 1,426 to 23,605 oocytes. Mean gonadosomatic index (GSI) indicated the development of maturation started in May and achieved maximum values in August in males. In females, the development started in January and achieved maximum values in August, with a sudden decrease in September. The smallest mature individual in a female and male was 23 cm TL. This reproductive period was similar to what was found in *P. cruentifer* (Wenner, 1976). In the relative of sex ratio (SR), *O. rufus* was the sex-ratio generally 0.15; 88 of 679 specimens were male (Fig.2.11).

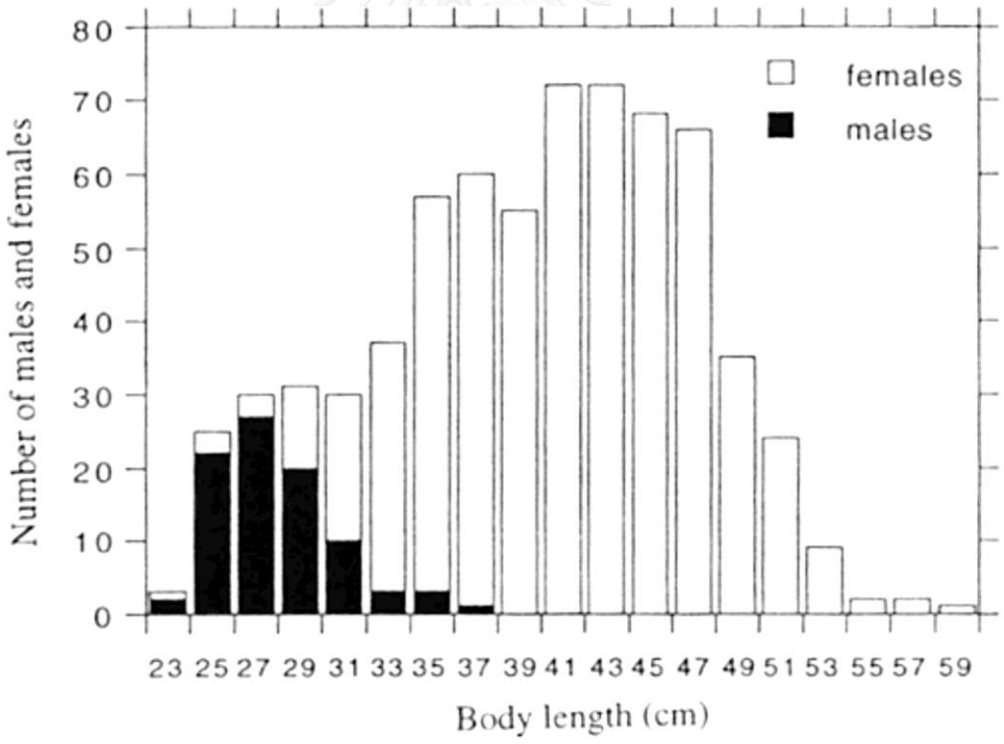

Figure 2.11 Size distribution of males and females of *Ophichthus rufus* (figure from Casadevall et al. (2001)).

According to distribution of males and females in the sample, Kartas and Quignard (1984) as cited by Casadevall *et al*. (2001) suggested that this phenomenon may explain by 1) male maturing earlier; 2) a tendency for slow growth in males; 3) a high

mortality rate in males; and another possible reason is a spatial displacement of sexes, which was already observed for *Conger conger* (Cau & Manconi, 1983). Furthermore, however, the parthenogenetic events have been reported over in a few the vertebrate taxa, about 70 taxa vertebrates had a form of parthenogenesis which only female genomes transferred to the next generation (Neaves & Baumann, 2011; Wootton & Smith, 2014). This mode of reproductive also occurs a few in the teleosts, which are about 8 taxa or represent only about 0.03% of all teleost species (Pandian, 2011; Wootton & Smith, 2014). The parthenogenetic mode mostly found in Poeciliids, and rarely in Atheriniformes and Cypriniformes (Pandian, 2011). Recently, the parthenogenesis has occurred in the captive female white spotted bambooshark for the first time (Straube *et al.*, 2016). Straube *et al.* (2016) claimed that this event was the first genetically confirmed evidence for second-generation facultative parthenogenesis in vertebrates and significantly support the evolutionary of parthenogenetic reproductive mode as an alternative to sexual reproduction. It is possible that parthenogenic event may occur in the Anguilliforms, but more details are needed to conclude this situation.

2. Morphology of egg and larva of the snake eel (*Ophichthidae*)

Several studies have described the morphology of an egg and larva of the snake eel (the family Ophichthidae) (Ji *et al.*, 2012; Naplin & Obenchain, 1980; Richardson, 1974). Egg development, *P. cruentifer* have large pelagic eggs with a smooth chorion, a wide perivitelline space, and a segmented yolk. Eggs diameter ranged from 1.62 to 2.98 mm. Generally, the small eggs within this range did not complete the expansion of the chorion following the fertilization. The diameter of 436 oil globules ranged from 0.26 to 0.65 mm. Late stage eggs with well-developed embryos had the small oil globule measurements because the oil globules are attenuated; eggs in the earlier developmental stages usually possess larger oil globules (Richardson, 1974). The egg development of *P. cruentifer* was exhaustively described (see developing in Fig. 2.12) and suggested that the duration of embryonic life (spawning to hatching) was approximately 96 hrs (Naplin & Obenchain, 1980; Richardson, 1974).

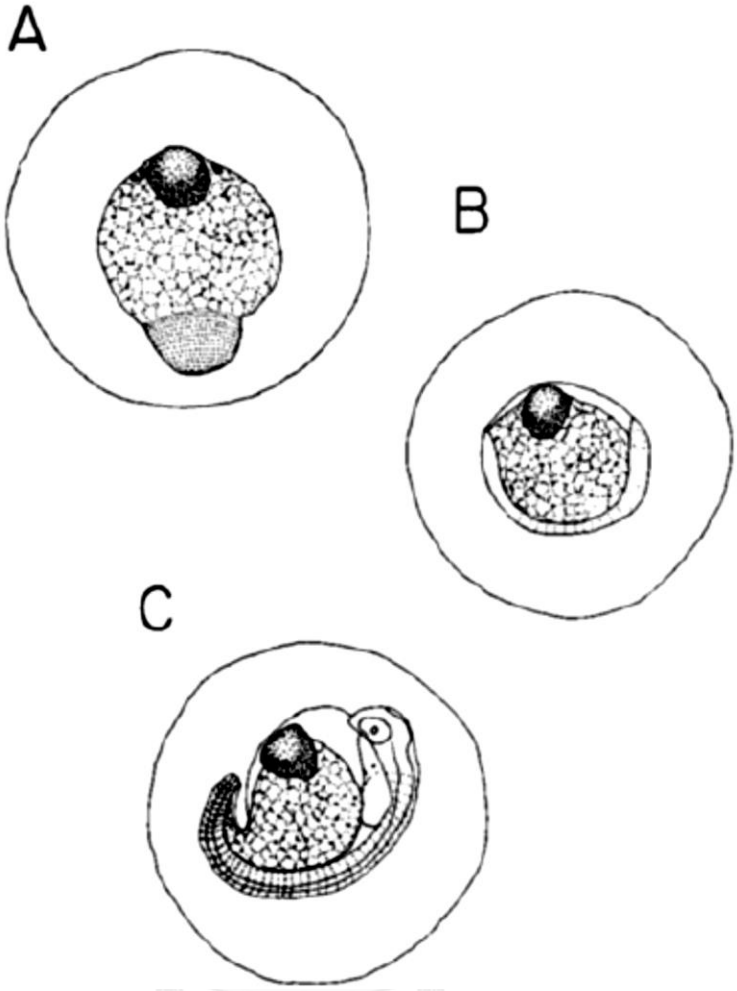

Figure 2.12 Developmental stages of *Pisodonophis cruentifer* eggs: A) Early, B) middle, and C) early late stage eggs (Richardson, 1974).

Pisodonophis cruentifer hatched at 5.5 to 6.5 mm SL (Fig. 2.13). After hatching, the jaws are not well developed; jaw formation was barely beginning to develop at about 6.4 to 7.0 mm larva (Fig. 2.13f). Liver development appeared in specimen about 6.4 mm (Fig. 2.13d), when the second loops of the intestine became more marked. The ratio of gut length to SL decreased during development, dorsal and anal fins were not presented in the young larvae but were evidenced on larvae over 19 mm. The metamorphosis began at about 80 mm (Naplin & Obenchain, 1980; Richardson, 1974).

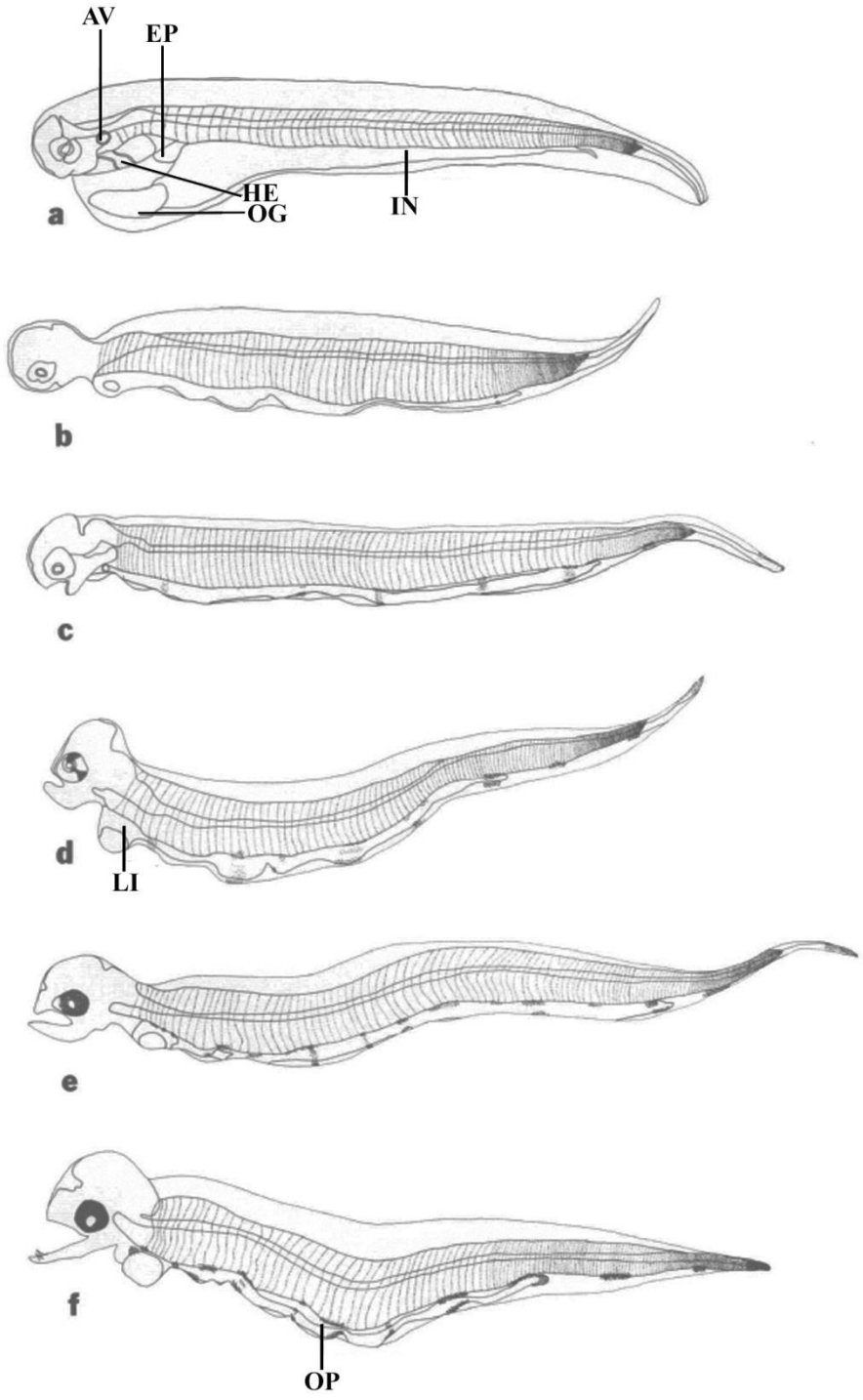

Figure 2.13 Developmental stages of *Pisodonophis cruentifer* larvae: a, 6.1 mm; b, 6.4 mm; c, 7.1 mm; d, 6.4 mm; e, 6.7 mm; f, 6.4 mm. AV, auditory vesicle; EP, esophagus pouch; HE, heart; IN, intestine; LI, liver; OG, oil globule; OP, opisthonephros (figure from Naplin and Obenchain (1980)).

Pigmentation of *P. cruentifer* can be classified into six stages. The first stage, the early larvae with the yolk sac has no pigment at the size about 6.1 mm mean length. Then, the second stage, the larvae size 6.4 mm had a small pair of pigment patched near the anus, and small pigment at a head. The third stage, the eyes become pigmented by size 6.7 to 7 mm and the first post-anal spot, as well as a small clump of dorsal tail pigment spots, appeared. The fourth stage, the melanophores concentrated at the gut loops and a small second post-anal spot developed. The dorsal tail pigment was more widespread. Fifth and six stages, the larvae show increased pigmentation along the gut, the tail, and on the eyes. A few melanophores have developed on the dorsal surface of the esophagus in more than 6.7 mm larva. After that, in the six stages, intervals pigment developed at along the ventral side of the gut usually at the opposite dorsal pigment patched. The clumps of melanophores along the gut start to take stable positions with respect to myomere number (Naplin & Obenchain, 1980; Richardson, 1974).

2.3 Feeding ecology of fish

Feeding ecology of fishes is one of the major tools to understand fish roles within their ecosystems because they can suggest relationships among species based on feeding resources, which helps in understanding competition and predation effects on the system (Hajisamae, 2012; Hajisamae *et al.*, 2003; Krebs, 1989; Ramírez-Luna *et al.*, 2008), population dynamics, resources partitioning (Guedes & Araújo, 2008; Ross, 1986) and trophic ecology (Svanbäck & Bolnick, 2007). Moreover, observation of the feeding information is a great value for developing the conservation strategies (Braga *et al.*, 2012). In this sense, the theory about foraging, or optimum foraging theory (OFT), was needed to determine feeding behavior of fishes. The optimum foraging theory is based on the evolutionary evidence that individuals within a population that forage most benefits rate of energy income have the greater fitness for contributing more genes to next future generations (Townsend & Winfield, 1985). On the other hand, the total energy that fish consumed as food minus the energy costs of finding that food is the net energy gain (Gerking, 1994). However, a large number of species of fish are flexible enough to shift from feeding on one food type to another when the opportunity arises. Therefore, the scientist tried to groups together fishes

that exploit a common food source based on fish feeding relationships or functional groups, namely as a generalist, specialist, and opportunist. The terms generalist, specialist, and opportunist should be used in a temporal sense to describe some range in life (ontogeny) or a variation in food source rather than as descriptive terms applied to the feeding history of a specific species (Gerking, 1994). Gerking (2014) was explained the terms of feeding types as follows;

Generalist species feeds on a broad spectrum of food sources in terms of a number of species or the microhabitats where food organism lives. Another word, generalist species have no preference for a specific food source. A large number of fish species in many taxonomic groups have feeding type as a generalist.

Specialist species unlike generalist, specialists concentrate their energies on a restricted type of food and rarely broaden their diet. Nevertheless, a specialist today may be generalists tomorrow if a specific food source suffers a quick decline, labeling a species as a specialist without knowing precisely the full range of its feeding potential can be risky. For example, prior to 1983-1984 two coral-dwelling toadfish, *Amphichthys cryptocentrus* and *Sanopus barbatus* (Batrachoididae), fed almost exclusively 85 to 100% of stomach contents on the sea urchin *Diadema antillarum* (Robertson, 1987). These two species were recognized as diet specialist because of their unwavering concentration on the urchin. However, after the catastrophic mass mortality occurs in the years mentioned, only about 5% of the sea urchin population was intact. The population of the toadfish *S. barbatus* shifted to feed on fish whereas *Am. cryptocentrus* quickly became a generalist, feeding on all the available mobile benthic invertebrates available. On the other hand, a true specialist will show the decline when food source demolished. Some butterfly fish (Chaetodontidae) are coral polyp eating. When a starfish (*Acanthaster planci*) decimated the hard coral (greatly diminished), two species of butterflyfish, *Chaetodon ramfordi* and *Ch. aureofasciatus*, suffered large population losses (Williams, 1986). Hence, these butterflyfish were evidently specialist feeder.

Opportunist species is the fish that take advantage of opportunities. A fish is an opportunist if it takes advantage of, or feeds on food source outside of its usual diet. Alternatively, opportunist can have a temporal shift (Gerking, 1994). For examples, the omnivore that switches between animal and plant diet is an opportunist

or the piscivore that feeds on one fish species then change to feed on another species when the opportunity happened. On the other hand, it does not mean that this fish can feed across the whole spectrum of all possible foods, but rather can shift from one food source to another if the fish can capture and digest it (Gerking, 1994).

2.4 Feeding ecology of eel

Many researchers have been studied on feeding ecology of eel for a decade but many of them had focused on the species from the family Anguillidae such as European eel (*A. anguilla*), Japanese eel (*A. japonica*), and American eel (*A. rostrata*) (Aida *et al.*, 2003; Kaifu *et al.*, 2013; O'Sullivan *et al.*, 2004). However, the feeding from study of the family Ophichthidae has been little known.

Kaifu *et al*. (2013) observed the diet of Japanese eels *Anguilla japonica* using stomach content and stable isotope methods in the Kojima Bay-Asahi River system, in Japan. Stomach contents and stable isotopes revealed that the eel feed on a single type of prey species, with different prey type, depends on the habitats. The eel *A. japonica* in Kojima Bay primarily feeds on prey from the pelagic food web such as mud shrimp and primarily feeds on prey from the littoral food web such as cray fish in the Asahi River. Based on these results, it appears that the eel can adapt to various feeding environments as opportunists, but also utilize the food resources by targeting a single type of prey species during a single feeding session. The European conger eel, *Conger conger*, had diet shift in difference habitat in Irish waters. The relative important index (IRI) shown the inshore dominant prey was common whiting fish (*Merlangius merlangus*) and offshore prey were Blue whiting fish (*Micromesistius poutassou*) while other preys (e.g., decapod crustaceans and cephalopods) contributed little to the overall diet in the stomach. However, the preponderance of no more than three species of fish, and the relative inadequacy of invertebrates, this study strongly suggested that these conger eels is a specialist rather than an opportunistic predator (O'Sullivan *et al.*, 2004). The study of *Anguilla bicolor* from the Bolgoda Estuary revealed that it fed mainly on invertebrates then change to piscivorous as they grow (Rupasinghe & Attygalle, 2006).

The stomach content analysis of Rufus snake eel *Ophichthus rufus*, the family Ophichthidae, was observed by Casadevall *et al.* (1994), the preference of *Op. rufus*

was a benthic organism that was confirmed by the index of relative important (IRI). The eel showed diet shift, which young of both sexes and adult males had a euryphagic carnivorous feeding mode while adult females became stenophagic piscivorous mode. They suggested that *Op. rufus* diet varied with the breeding season of prey, maximum occurrences of a specific prey species often coincided with the breeding periods of that species. Considering our fish target, studies on the feeding ecology of *Pisodonophis boro* are very limited. The snake eel *P. boro* showed preferences to burrows into a good mixture of sand silt and clay (loam) (Subramanian, 1984). They were not observed from sites where the composition such as medium and fine sand varies from such site where the *P. boro* was observed (Subramanian, 1984). It burrows into the mud bank at night searching for their food which was similar to other snake eel behavior. Subramanian (1984) used both field observation and food challenge experiment in the laboratory to investigate the feeding habit of *P. boro* from India reported that *P. boro* is a specialist feeder feds only on one single fiddler crab species (*Uca annulipes*) in India. On the contrary to Subramanian (1984), some reports suggested that *P. boro* is a carnivore and feeds mainly on crabs and small fishes (Froese & Pauly, 2016a; Rainboth, 1996). Feeding habit of *P. boro* is still unclear. An interesting question such as "Do *P. boro* have foods preferences (prey items based on the diversity of organisms present in each location) differently in the different locality?" Apart from the study on feeding ecology, histological of the digestive system in this fish is also necessary. It will provide insight information to determine and confirm whether the feeding ecology of this snake eel follows the optimal foraging theory (Gerking, 1994).

2.5 Analysis of stomach contents

Several studied working on the feeding ecology of fishes has been using a stomach content analysis as usually methodology to determine feeding habits of fishes. Moreover, to clarifying the feeding information, numerous methods have been established to evaluate the feeding ecology, for examples, gut content analysis (Cortés, 1997; Hynes, 1950; Hyslop, 1980), stable isotope analysis (Fanelli *et al.*, 2009; Graham *et al.*, 2007), and fatty acid analysis (Young *et al.*, 2010). Especially, the gut content analysis has been attractively used. This method is simple, cheap, and

provided the good outcomes (Ducommun *et al.*, 2010; Kaifu *et al.*, 2013; Tsai *et al.*, 2015; Wootton, 2012). Hyslop (1980) classified the gut content analysis into five methods as follows;

1. Occurrence method

Occurrence method is the possibly simplest way to determine the content in stomach of fishes. It is to record the number of stomachs containing one or more individuals of each food type. This number may then be expressed as a percentage of all stomachs (frequency of occurrence) or all those containing. The advantages of the frequency of occurrence method are that, provided food items are readily identifiable, it is quick and requires the minimum of tool. However, it gives little indication of the relative amount or bulk of each food category present in the stomach (Hajisamae, 2012; Hyslop, 1980).

2. Numerical method

This method is quick and requires the minimum apparatus as well. The number of individuals in each food type is recorded for all stomachs and the total is expressed as a proportion, usually a percentage, of the total individuals in all food categories. In some situations it may be the most appropriate method, for example, where prey items of different species are in the same size range. However, there were some disadvantages of this method; numerical estimate overemphasize the importance of small prey items taken in large numbers. For many stomachs, it is difficult to estimate numbers in each category because of mastication of the food and this method is not suitable for dealing with food items such as macroalgae and detritus which do not occur in discrete units (Arawomo, 1976 as cited by Hyslop, 1980).

3. Volumetric method

Currently, volumetric method is an attractively method to determine the stomach content of fish (Hjisamae, 2012). The analyses fall into two ways: direct and indirect estimation. In the direct, the displacement of each food item or group of items sorted from the stomach contents is measured, usually in some type of graduated measuring device, this displacement volume being equal to that of the food items. Alternatively, the settled volume of the stomach contents may be measured by

allowing them to settle in a graduated measuring vessel. However, direct method may have a major problem; the direct volume is not easily measured when the food is too small. So, the indirect volumetric analysis may be employed. Indirect analysis can be done in many ways such as comparing food items with blocks of known volume. In the case of measuring small stomach, stomach contents are squashed on a plate to a uniform depth and the area of the squash is measured (i.e. Sedgwick-Rafter counting cell or graph paper). The difference between the former and the sum of the latter values gives an estimate of the volume of the remaining stomach contents. Volumetric techniques probably give the most representative measure of bulk and may be applied to all food items.

4. Gravimetric method

In a gravimetric analysis of stomach contents, the weight of food may be determined 'wet ' or 'dry '. In dietary studies where large amounts of material are collected, wet weight is probably the more convenient measure; dry weight estimation is more time consuming and is usually employed where accurate determinations of calorific intake are required. However, the dry weight gives a lower error margin in bulk determination of the food of planktivorous fishes (Berg (1979) as cited by Hyslop (1980). When wet weight determinations are made, surface water is most often removed from prey items, by blotting them on tissue paper, drip drying, pre-drying on a warming plate, and/or centrifugation. The total weight of a food category can be expressed as a percentage of the overall weight of stomach contents, where weight is either ‘ wet ’or ‘ dry ’. Gravimetric methods give a reasonable estimate of bulk and, in the case of larger prey items, are relatively easy to apply; they have the advantage of being applicable to almost all prey items though they are perhaps less so than volumetric techniques (Hyslop, 1980).

5. Subjective method

The subjective method has been applied widely. It was developed from processing large amounts of material using in numerical, volumetric or gravimetric methods. For example, the percentage contribution by volume of each food type to the total contents may be estimated by eye or each food category is awarded points

proportional to its estimated contribution to stomach volume (Hyslop, 1950 as cited by Hyslop, 1980; Hajisamae, 2012). The advantage of estimation by an eye and awarded points is being simple and rapid to apply. However is less accurate when the observer has less experiences identified the food categories.

Pinkas *et al.* (1971) developed a more accurate technique to determine the composition of foods as called "index of relative importance (IRI)" and it was later modified by as by Hyslop (1980).

$$IRI = (\%N + \%V) \times \%F$$

Where *IRI* represents index of relative importance of the main food items, categories or type groups; *%N* represents percentage numerical abundance as the total number of food items in all stomachs in a sample; *%V* represents percentage volumetric composition as the total volume of the taxa of prey and *%F* represents percentages frequency of occurrence based on the number of stomachs in which a food items were found.

2.6 The snake eel *Pisodonophis boro* in relation to Pranburi River Estuary

It is well known that mangrove forest or estuary system provides a greater abundance of food sources due to the high productivity of the systems. This normally results in high fish diversity (Laegdsgaard & Johnson, 2001).

Currently, Pranburi estuary system is possibly the richest and most diverse in term of fishery resources in Southern Thailand. Previously this estuary confronted with mangrove deforestation as a result of shrimp farming and unplanned urban expansion. After that, the reforestation programs by the joint collaboration among the governmental sector, non-governmental organization, and the Pranburi coastal community was launched. Conflicts of interest in land use and deforestation have led to assessment on mangrove productivity and social interaction for the development of the mangrove ecosystem learning centers project. Sirinath Rajini Mangrove Ecosystem Learning Center in the area of Pranburi river mouth was established in order to prevent the intrusion of urban and to retain/recovery mangrove forest. The capacity building in the Sirinath Rajini Mangrove Ecosystem Learning Center aimed to be the lead learning center to promote the knowledge-enriched society (Paphavasit *et al.*, 2014). Nowadays researchers have evaluated the diversity and productivity of

mangrove forest when compared with the previous recorded, the study revealed no progress in the mangrove productivity, being in the developing stage. It is because that the water circulation and exchange of water masses in the river occurred under the influence of the mixed tidal regimes. The freshwater inflow was low due to the controlled released of freshwater by the Pranburi Dam. This regulation controls the salinity regime in the river which is brackish 19-32 psu throughout the years. Low water exchange between the mangrove forests and the coastal area indicated the low contribution of the forests in supporting coastal productivity (Paphavasit *et al.*, 2014).

Many species of marine fish utilize mangrove forests ecosystem transitorily as foraging areas, spawning ground, or move in when the environmental conditions are suitable e.g. high tide. Furthermore, the deposition of the liters within the mangrove forest creates a detrital based food web, often considered as one of the explanations why so many species of organism utilize the mangrove ecosystem (Chaves & Bouchereau, 2000; Robertson & Blaber, 1993; Wongchinawit, 2007). Fish in mangrove forest ecosystem can be categorized into three major groups according to life history includes true residents, partial residents, and marine migrants (Paphavasit *et al.*, 2014; Wongchinawit, 2007). Several groups of fishes have been reported in the estuarine of Pranburi River. Paphavasit *et. al*. (2014) categorized fishes in the Pranburi River estuary into three groups:

1. True residents, the fish that spends their complete life cycle in the estuary e.g., spawning area and feeding area within the habitat. True resident fishes can tolerate to a wide range of salinity which is often small size and numerous or dominant in the estuary fish communities (Blaber, 1977). True resident fishes that are often found in the Pranburi River estuary are Gobiidae *Periopthalmus* species, *Ambassis nalua*, *Leiognathaus* species, *Neostethus* species. and *Dermogynys* species (Paphavasit *et al.*, 2014).

2. Partial residents, the fish that transients enter the ecosystem seeking for nursery ground or feeding site, usually spawning and spending much of their adult stage offshore, then often returning seasonally to estuaries. However, partial resident fishes may include fishes that seek refuge from a predator (Wongchinawit, 2007). Partial resident fishes which utilized the Pranburi River estuary as spawning ground

and nursery grounds were fish in the families such as Muguilidae, Antherinidae, Ambassidae, Mullidae, and Teraponidae (Paphavasit *et. al*., 2014)

3. Marine migrant are often fishes that use the estuaries as feeding site in short period of time or long period of time following the tidal cycle and/or utilize the estuaries as the route to the spawning ground e.g. anadromous and catadromous fish (Aida *et al.*, 2003; Nelson, 2006; Rainboth, 1996; Riede, 2004; Tsukamoto & Nakai, 1998). Marine migrant fishes were also some marine species that seasonally visit the estuaries, usually as an adult seeking for food (Wongchinawit, 2007). Some marine migrant fishes also found in the Pranburi River estuary, usually found in some species of the families Gerreidae, Mugilidae, Engraulidae, Lutjanidae, and Clupeidae (Paphavasit *et. al*., 2014).

However, although, the snake eel *P. boro* are usually found in the Pranburi River estuary, little known of roles and utilization in the estuary area. Moreover, many aspects of knowledge such feeding ecology has not been studied in Thai estuaries. The studies on the feeding ecology of *P. boro* are important to determining the roles of mangrove and/or estuary food web as feeding ground or spawning ground. This knowledge can provide the crucial information for developing the management plans, conservation strategies and/or else in *P. boro*.

CHAPTER 3

GUT CONTENT AND MORPHO-HISTOLOGY OF THE DIGESTIVE SYSTEM IN *Pisodonophis boro* (Hamilton, 1822) FROM PRANBURI RIVER ESTUARY, THAILAND

3.1 Introduction

The snake eel, *Pisodonophis boro* (Hamilton, 1822) (Anguilliform: Ophichthidae), is found in the Indo-West Pacific, India, Indonesia, Sri Lanka, and Thailand (Froese & Pauly, 2016a). It is generally found in estuaries and the tidal zone around the upstream areas of coastal rivers (Subramanian, 1984). Based on previous reports on feeding behavior and habitat, the snake eels fed selectively on only a single species of crab in India (Subramanian, 1984) while they shifted to feed on small fish in the water column in Cambodia (Rainboth, 1996). This contradiction may show selective feeding habits based on the diversity of prey organisms present in each location. However, in Thailand, there has not been any study on the feeding ecology of this eel. The optimum foraging theory (OFT) entered the field of feeding ecology for the century. This theory has been used to predict how fish success a maximum net energy gains (Gerking, 1994). Scientists attempt to explain the foraging behavior and the pattern of fish feeding that optimizes benefits (e.g., energy) for the lowest cost, which could result in greater fitness for contributing more genes to the next generations (Townsend & Winfield, 1985). However, many fish species are flexible enough to shift from feeding on one food type to another when given the opportunity. Therefore, I hypothesize that *P. boro* in Thailand will select the most beneficial food based on the food availability in their habitat. *P. boro* has claimed that high economic value as a food source locally at Pranburi River (Paphavasit *et al.*, 2014). Unfortunately, the populations of *P. boro* in this area are declining due to community development, environmental degradation and fishing pressure (pers. comm.). If the populations are still declining, it is likely that they will be extinct from the estuary of Pranburi River. Even though, the existence of this snake eel in Pranburi River is well known (Paphavasit *et al.*, 2014), the feeding characteristics, as well as histological features of its digestive systems, are still lacking.

The current knowledge concerning feeding ecology in a various fish link to their ecological roles, population dynamics, resource partitioning, and trophic ecology in natural habitats (Guedes & Araújo, 2008; Ross, 1986; Svanbäck & Bolnick, 2007). Explicit feeding information is worthwhile for developing conservation strategies (Braga *et al.*, 2012). Several methods of studying feeding ecology of fish have been documented (Cortés, 1997; Fanelli *et al.*, 2009; Graham *et al.*, 2007; Hynes, 1950; Hyslop, 1980; Young *et al.*, 2010). Among those methods, fish stomach content analysis has been well recommended and accepted because it requires relatively less time and is simple to perform; it also provides scientifically valid outcomes (Ducommun *et al.*, 2010; Kaifu *et al.*, 2013; Wootton, 2012). The digestive morphology and histology (i.e., morpho-histology) are normally performed in parallel with the stomach content analysis. The purpose of the gut morpho-histology analysis was not only to understand the structure and function of the digestive tract but also to support information of feeding habits and management of fish species (Melo Germano *et al.*, 2014; Rodrigues & Menin, 2005). Morpho-histology of the digestive tract in teleosts has been reported in many fishes such as *Anguilla anguilla* (Clarke & Witcomb, 1980) *A. japonica* (Sun & Chen, 2012), and *Cyprinus rutilus* (Abdulhadi, 2005).

This study provides a test of the hypothesis that *P. boro* in Thailand will select the most beneficial and the most abundant food based on the food availability in their habitat. I assume the most abundant food source would be the least costly to consume. To test this hypothesis, field observation was conducted to clarify the feeding preference(s) of *P. boro* and to examine the gut content and the morpho-histological characteristics of the digestive tract of *P. boro* from the Pranburi River estuary.

3.2 Materials and methods

1. Study site and fish collection

Seventy-seven specimens of *Pisodonophis boro* (the total lengths of *P. boro* ranged from 24 to 97 cm, with a mean length±SE of 63.63±1.45 cm) were collected from the Pranburi River estuary, Prachuap Khiri Khan Province, Thailand (N 12°24'08.5"/E 099°59'00.2") (Fig. 3.1), during March 2015 to March 2016 by local

fisherman using traditional methods. They were euthanized by a rapidly cooling shock technique (Wilson *et al.*, 2009). The measurement of the snout-tail tip was made to the nearest 1 cm with a measuring tape. After dissecting fat and connective tissue, the length of digestive tract (esophagus to posterior intestine) was measured; the complete digestive system (GI tract plus liver and pancreas) was fixed in Davidson's reagent at least 36 h at room temperature before preserved in 70% ethanol for further gross anatomy and gut content analysis. The experimental protocol was approved by the Animal Care and Use Committee of Faculty of Science, Chulalongkorn University (Protocol Review No. 1523005).

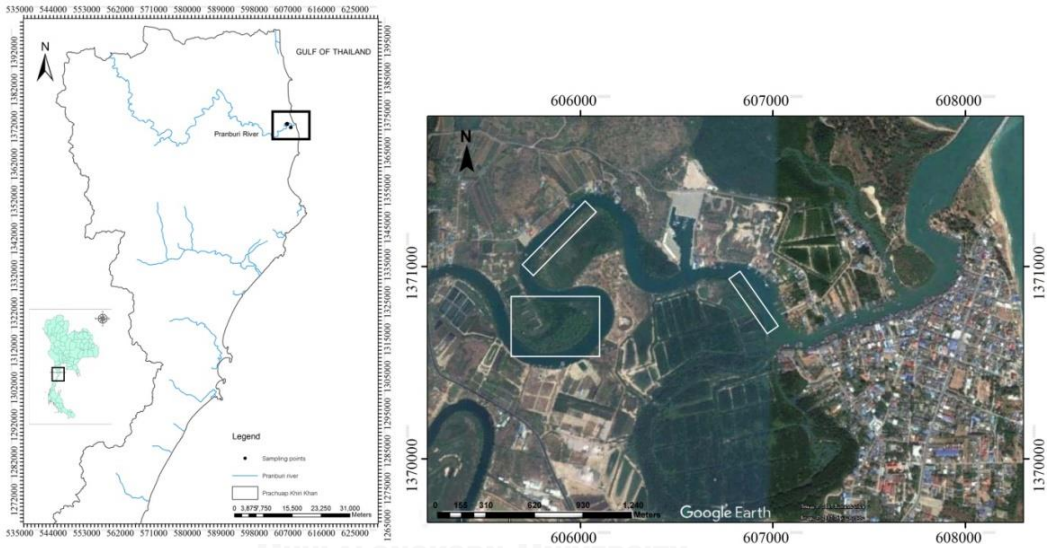

Figure 3.1 Sampling areas of *Pisodonophis boro* at Pranburi River estuary during March 2015 to March 2016, Thailand. Rectangles indicate sampling areas.

2. Gut content analysis

The gut was horizontally dissected (from the anterior stomach to posterior intestine) and the food was removed from the digestive tract under a stereoscopic light microscope. The food items were sorted, counted, and identified to the lowest possible taxonomic category according to the guidelines of Machjajib (1973) and Paphavasit *et al.* (2014). The importance of each consumed prey category was evaluated by calculating the index of relative importance (IRI, (Pinkas *et al.*, 1971), which incorporates percentage by number (N), volume (V) and frequency of occurrence (O) in the formula as: IRI = %O × (%N + %V). However, calculations of

percentage by volume (V) was converted by (Hajisamae, 2012) to the percentage by cover areas (C) for calculations of prey item in the stomach of fish. Hence, the present study combined these two indices into the formula as $IRI = \%O \times (\%N + \%C)$.

3. Histological and histochemical analysis

Ten randomly samples of the digestive tract with liver and pancreas were examined using standard histological techniques for histological analysis. The paraffin blocks were cut (6 µm thicknesses) and stained by Delafield's hematoxylin and eosin (H&E) (Humanson, 1979) as a routine stain, Masson's Trichrome (MT) for the detection of smooth muscle from connective tissue, Periodic Acid Schiff (PAS) for detection of glycoprotein, and alcian blue pH 2.5 (AB) for detection of mucopolysaccharide (Presnell & Schreibman, 1997; Suvarna *et al.*, 2013). The stained slides were photographed using an Olympus CX31 light microscope mounted with a Canon EOS 100d camera.

4. Semi-quantitative analysis

The goblet cells were counted from 12 randomized villi area (each with the area of 200 square µm) from each intestinal region and the mean number of counted goblet cells of each intestine region was tested for significant difference among the 3 regions by analysis of variance (ANOVA) and Multiple Range Tests with Bonferroni test.

3.3 Results

1. Composition of gut content

Seventy-seven specimens of *P. boro* were chosen for gut content analysis. Only 83.12% (sixty-four guts) contained food, while the remaining had empty guts. Normally, snake eels feed almost exclusively on crabs and a little on mantis-shrimp. The crabs that were found in the prey items include *Metaplax elegans*, *Perisesarma bidens*, *Sarmatium germaini* and *Uca perplexa*. The total list of prey species found is given in Table 3.1. Based on the IRI, the most important prey was *S. germaini*

(IRI=10913.6) followed by *M. elegans* (IRI=27.2). Other prey items showed low values of IRI (i.e., some values are 2, 3, 5 seen in Table 3.1).

Table 3.1 Summary of prey items found in the gut contents of *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016, n = 64 (empty gut = 13 not included in calculations).

Name	*O*	*N*	*C*	%*O*	%*N*	%*C*	*IRI*	%*IRI*
Sarmatium germaini	62	70	565	96.88	59.3	87.09	10913.609	99.65
Perisesarma bidens	1	1	7	1.563	0.85	1.079	2.2386282	0.041
Metaplax elegans	3	2	14	4.688	3.97	2.158	27.194104	0.495
Uca perplexa	2	1	4.7	3.125	1.33	0.725	5.1198695	0.093
Unidentified mantis-shrimp	1	1	8.5	1.563	1.33	1.310	3.8208412	0.069

Note: *IRI = the index of relative importance; O* = the frequency of occurrence of each prey category; *N* = the percentage of the number of items in all guts; *C* = the coverage areas of items in all guts.

2. Morphology of the digestive tract and accessory glands

The digestive tract composed of the anterior end of the esophagus, stomach, and intestines, respectively (Fig. 3.2a). The intestine coefficient was 0.32 (the intestine length/overall length). The esophagus was a slender tube, which extended from the pharynx to the stomach. The stomach was Y-shaped. It consisted of two main regions: anterior and posterior (Fig. 3.2b). No pyloric caeca were observed. The intestine consisted of three regions: anterior, middle, and posterior regions. The anterior intestine started from the pyloric sphincter. It initially had an almost straight shape in an anterior region while the middle region becoming more of a slightly coiled shape as it approached the straight shape in the posterior intestine. The liver was a dark brown-reddish color and structurally elongated at the dorsal regions. The anterior lobe of the liver was close to heart. The pancreas was located near the anterior intestine and stomach.

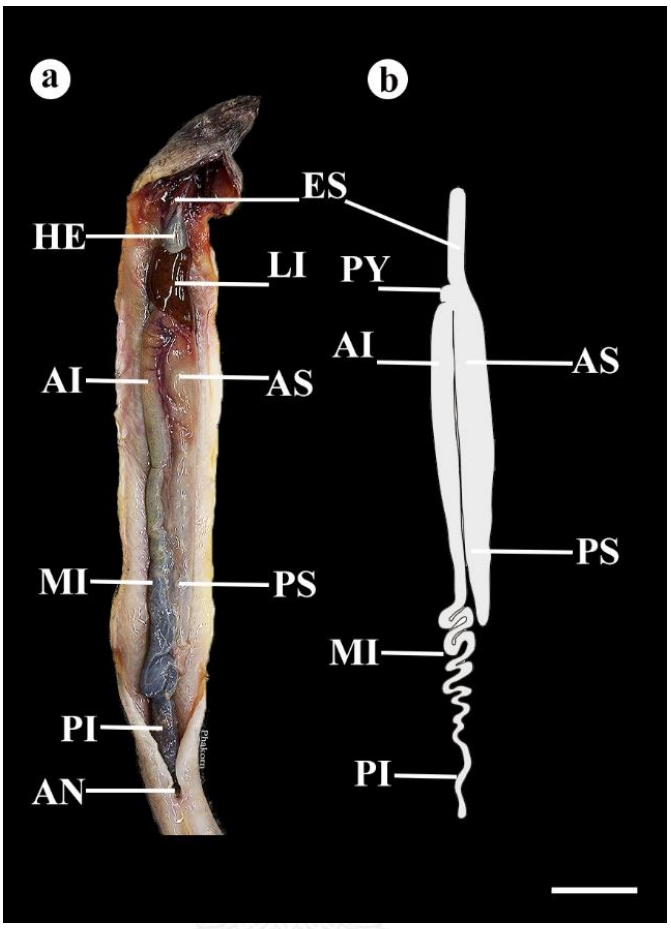

Figure 3.2 Anatomy of alimentary tract of *Pisodonophis boro* after a longitudinal incision collected from Pranburi River estuary during March 2015 to March 2016: **(a)** morphology of digestive tract; **(b)** Schematic diagram of digestive tract. **Abbreviations:** AI, anterior-intestine; AN, anus; AS, anterior-stomach; ES, esophagus; HE, heart; LI, liver; MI, middle-intestine; PI, posterior-intestine; PS, posterior-stomach; PY, pyloric sphincter. Scale bar = 2.5 cm.

3. Histological and histochemical observations of the esophagus

Histological sections of the esophagus revealed four distinct layers including mucosa, submucosa, muscularis and serosa. The mucosa contained longitudinal folds that protruded into the lumen (Fig. 3.3a). The deepest layer of the mucosal layer consisted of two layers, the epithelium and lamina propria which consisted of loose connective tissue (Fig. 3.3b, c). The muscularis mucosal layer was not observed in these sections. The epithelial layer was lined by a simple squamous epithelium which contained goblet cells (Fig. 3.3c). A strong positive reaction of both PAS and AB stains were detected in the mucous cells (Fig. 3.3d-i), indicating the presence of glycoprotein and mucopolysaccharides. Each mucous cell was oval shaped and was

filled with acidophilic cytoplasm. The submucosa layer consisted of loose connective tissue and blood vessels, whereas two sub-layers included inner longitudinal and outer circular muscles were found in the muscularis. The outermost layer of the serosa consisted of simple squamous epithelium (Fig. 3.3f).

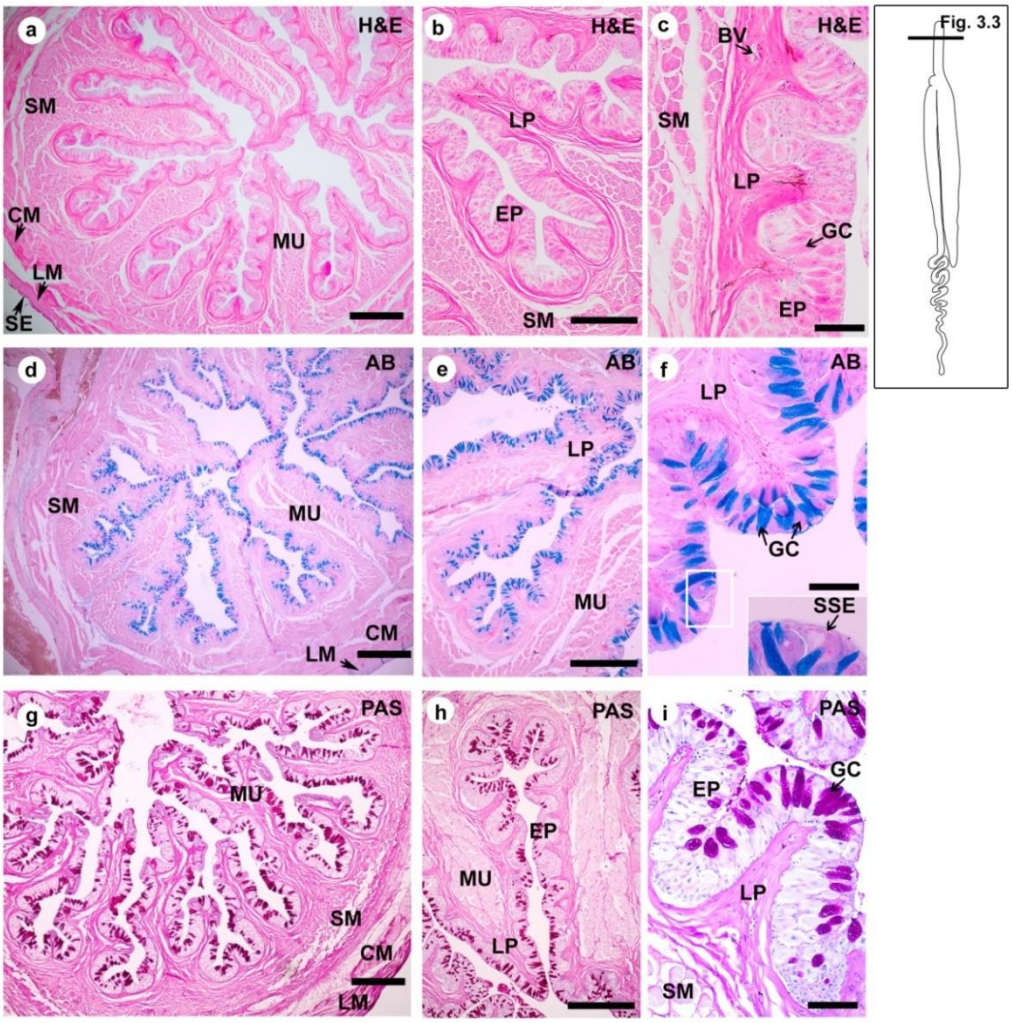

Figure 3.3 Transverse sections of *Pisodonophis boro* esophagus with different histochemical stains collected from Pranburi River estuary during March 2015 to March 2016. A schematic diagram of the location of the transverse section is shown to the far right. **Staining:** H&E, hematoxylin and eosin **(a, b, c)**; AB, alcian blue pH 2.5 **(d, e, f)**; PAS, Periodic Acid Schiff **(g, h, i)**. **Abbreviations:** BV, blood vessel, CM, circular layer of muscle; EP, epithelium; GC, goblet cells; LM, longitudinal layer of muscle; LP, lamina propria; MU, mucosa; SE, serosa; SM, submucosa; SSE, simple squamous epitheliums. Scale bars: a, d and g = 500 µm, b, e and h = 400 µm, c, f and i = 30 µm.

4. Histological and histochemical observations of the stomach

Histological sections of stomach samples were classified into two regions including anterior and posterior regions based on their localization and histological structures (Fig. 3.4a, b). PAS staining showed positive cells in throughout stomach regions (Fig. 3.4e, f). The histological structure of anterior regions was similar to the esophagus. However, the mucosal layer was formed into gastric rugae and was composed of simple columnar epithelium without mucous cells. The gastric rugae were supported by the lamina propria (Fig. 3.4c). The gastric gland was also exclusively seen in this layer; it was a simple tubule with uniform secretory cells. The muscularis layer was extensively observed in these sections and it was much thicker than in the other layers. It contained large layers of inner circular muscle as well as thinner outer longitudinal muscle (Fig. 3.4a). The histological structure of the posterior region was consistent with the anterior region. However, the posterior region contained less numerous gastric glands in the mucosal layer. In the muscularis layer, the thin layer of circular muscle was also observed (Fig. 3.4b).

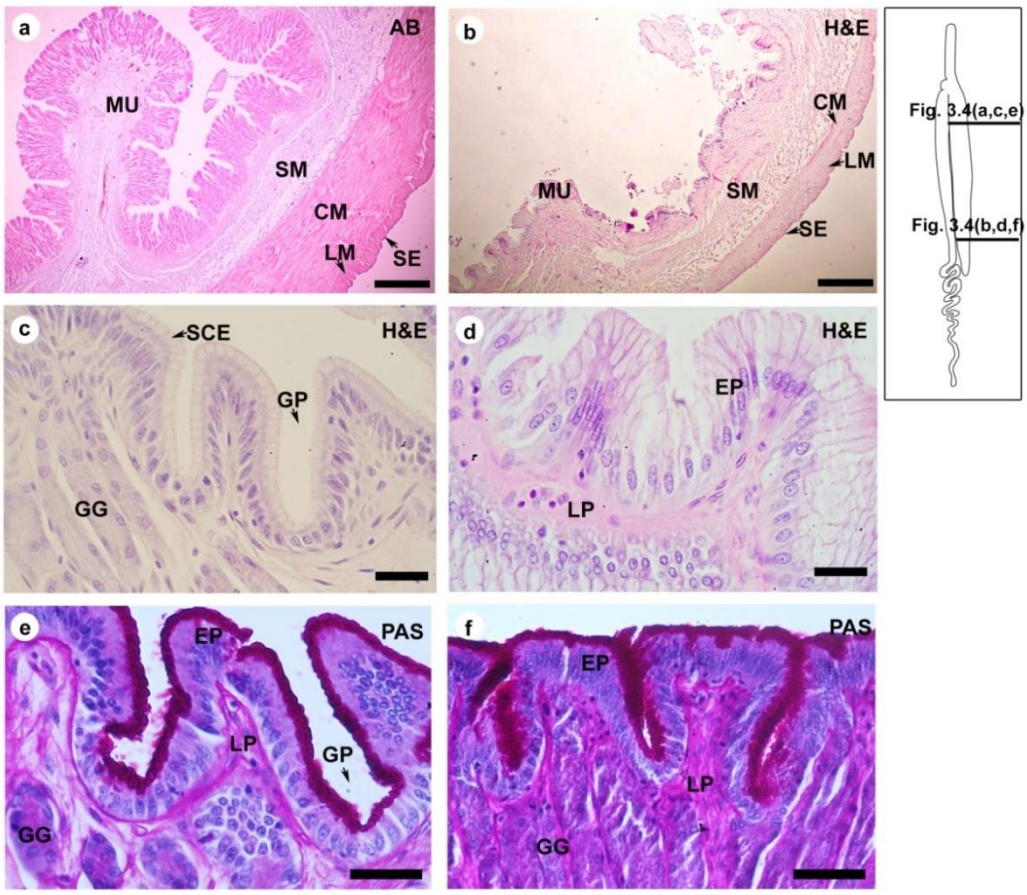

Figure 3.4 Transverse sections of stomach regions of *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016: anterior stomach **(a, c, e)** and posterior stomach **(b, d, f)**. A schematic diagram of the location of the transverse section is shown to the far right. **Staining:** AB, alcian blue stain; H&E, hematoxylin and eosin stain; PAS, Periodic Acid Schiff stain. **Abbreviations:** GG, gastric gland; GP, gastric pit; SCE, simple columnar epitheliums. Scale bars: a and b = 400 µm, c-f = 30 µm.

5. Histological and histochemical observations of the intestines

Histology of intestine was classified into three regions including anterior, middle and posterior intestines (Fig. 3.5a-c). AB and PAS staining showed scattered positive goblet cells with both acid and neutral mucopolysaccharides throughout all three regions (Fig. 3.5). The anterior intestine was clearly identifiable between the anterior stomach and the middle intestine. This region was composed of four histological layers similar to other organs of the digestive tract (Fig. 3.5d). However, the longitudinal fold was a more prominent feature in the mucosal layer of the anterior intestine (Fig. 3.5a). Several goblet cells were also observed and inserted in the

epithelial layer (Fig. 3.5g, j and m). The muscularis layer (inner circular muscle and outer longitudinal muscle) was very thin. The morphology of the middle and the posterior intestine was similar to other parts of the overall digestive tract. However, the longitudinal folds of the epithelial layer were thinner than in the anterior intestine (Fig. 3.5b, c).

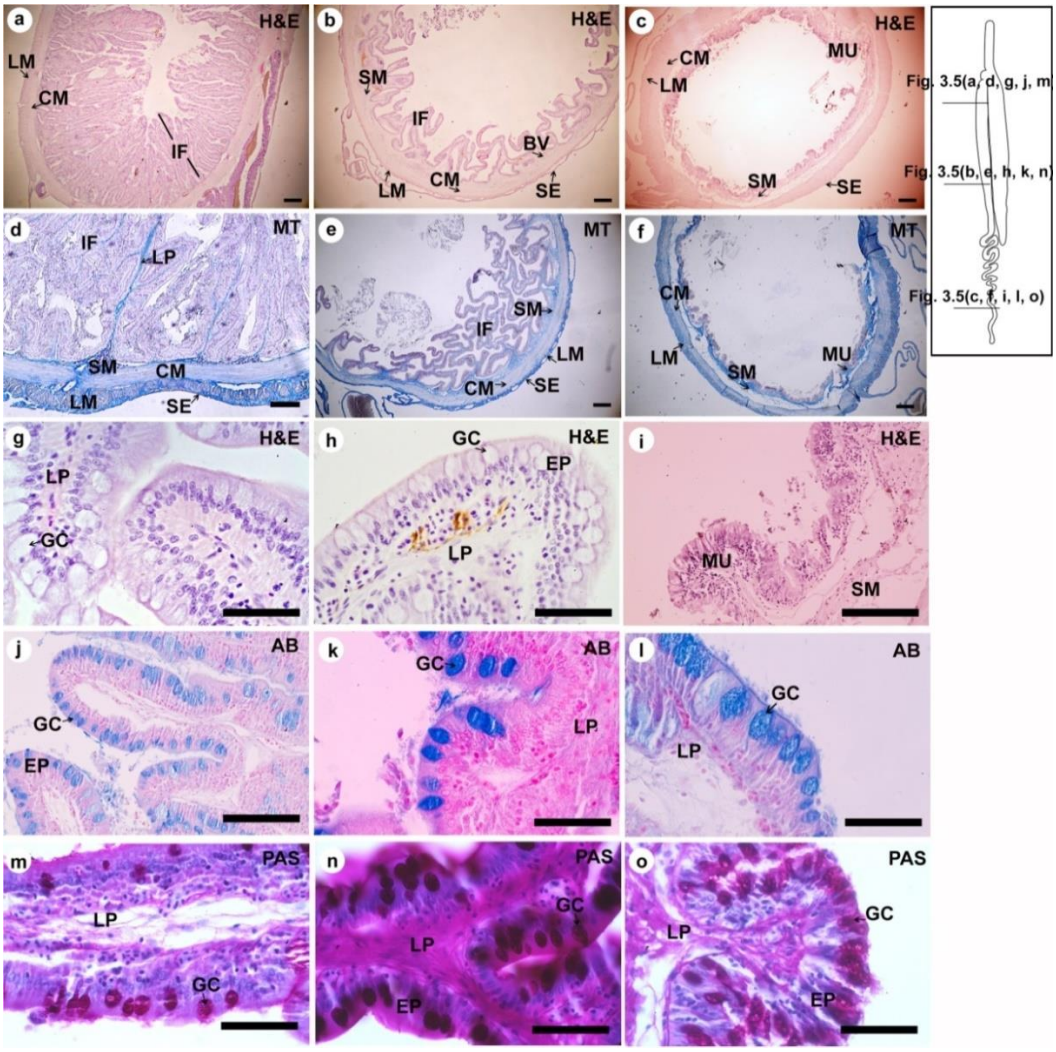

Figure 3.5 Transverse sections of *Pisodonophis boro* intestine regions with different histochemical stains collected from Pranburi River estuary during March 2015 to March 2016. A schematic diagram of the location of the transverse section is shown to the far right: anterior intestine **(a, d, g, j, m)**; middle intestine **(b, e, h, k, n)**; posterior intestine **(c, f, i, l, o)**. Masson's Trichrome staining showing loos connective tissue observed in the intestine **(d, e, f)**, Histochemistry of the intestine showing mucopolysaccharide in goblet cells **(j, k, l)**, and Periodic Acid Schiff stained showing glycoprotein in goblet cells observed in the intestine **(m, n, o)**. **Abbreviations:** IF, intestinal fold. Scale bars: a-f = 300 µm, g-o = 50 µm.

Moreover, the goblet cells in these two lower regions of the intestine were slightly larger when compared to the ones in the longitudinal fold of the anterior intestinal epithelial layers. The average number of goblet cells from the anterior intestine, the middle intestine, and the posterior intestine ranged from 18.66±5.74, 32.16±3.52 and 51.33±9.69 (mean±SD per 200 μm^2), respectively (Table 3.2). The number of goblet cells in the posterior region was significantly higher ($P<0.05$, $P=0.000$) than the anterior region and higher than the middle region.

Table 3.2 Major histologically variation among esophagus, stomach and intestines and goblet cell counts from different intestine regions of *Pisodonophis boro* (mean±SD per 200 μm^2) collected from Pranburi River estuary during March 2015 to March 2016; significant difference in mean is indicated by * ($p<0.05$).

Organs	Mucosal epithelium	Goblet cell	Gastric gland	Muscularis mucosa	Submucosa	Muscularis	Serosa
Esophagus	SSE	Present		Present	Present	Two layer	Present
Anterior stomach	SCE		Present	Present	Present	Two layer	Present
Posterior stomach	SCE		Present	Present	Present	Two layer	Present
Anterior intestine	SCE	Present (18.66±5.74)		Present	Present	Two layer	Present
Middle intestine	SCE	Present (32.16±3.35*)		Present	Present	Two layer	Present
Posterior intestine	SCE	Present (51.33±9.69*)		Present	Present	Two layer	Present

Note: SSE = simple squamous epithelium, SCE = simple columnar epithelium

6. Histological and histochemical observations of accessory organs

The liver was surrounded by a thin capsule, which contained fibro-connective tissue. The lobular structure of liver parenchyma consisting of polyhedral hepatocytes (i.e. the hepatic cord) was not detected in these samples (Fig. 3.6a). The oval nucleus in each liver cell was surrounded by eosinophilic cytoplasm. Sinusoidal portal blood (sinusoids) was present between cords. The central vein was located in the middle

area of the liver/of the sinusoids (Fig. 3.6b). Within the liver tissue, the bile duct was also identifiable and was surrounded by fibro muscular layers.

The pancreas was situated near the anterior of the intestine and near the stomach. Clusters of granular exocrine pancreatic cells were also observed (Fig. 3.6c, d). Pancreas cells had a strong basophilic cytoplasm with a basal nucleus. Moreover, eosinophilic zymogen granules were also observed.

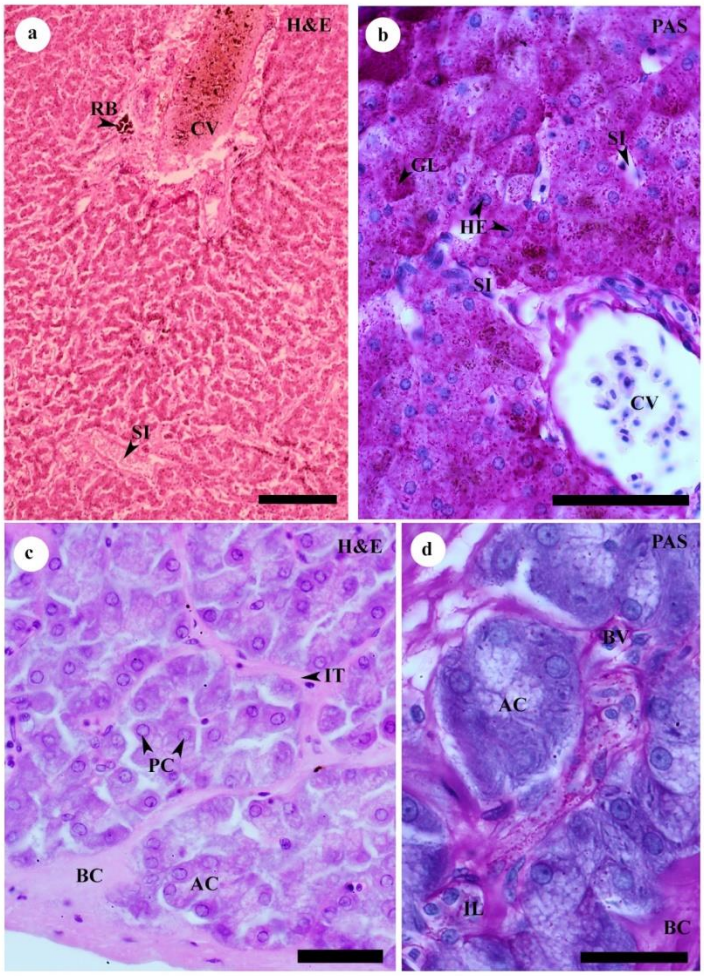

Figure 3.6 Light photomicrographs of histological structure of liver and pancreas of *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016. Organization of the liver showing hepatocytes, central vein, and glycogen accumulation **(a, b)**; pancreas features showing granular exocrine pancreatic cells with basophilic cytoplasm **(c, d)**. **Staining:** H&E, hematoxylin and eosin; PAS, Periodic Acid Schiff. **Abbreviations:** AC, pancreatic acini; BC, basophilic cytoplasm; CV, central vein; GL, glycogen; HE, hepatocyte; IL, islets of Langerhans; IT, interstitial tissue; PC, pancreatic cell; RB, red blood cell; SI, sinusoid. Scale bars: a and b = 50 µm, c and d = 40 µm.

The overall histology of the digestive tract of *P. boro* included interesting differences when comparing each of the three major regions (i.e., esophagus vs. stomach vs. intestine). The gastric gland was inserted throughout the stomach epithelium. Unlike the stomach, the esophagus and the intestine region was characterized by dominant mucous cells in the epithelial layer. The large muscular layer was observed in the anterior portion of the digestive tract while the smaller layer of muscularis was observed in the later portion. The overall histology is summarized in Figure 3.7.

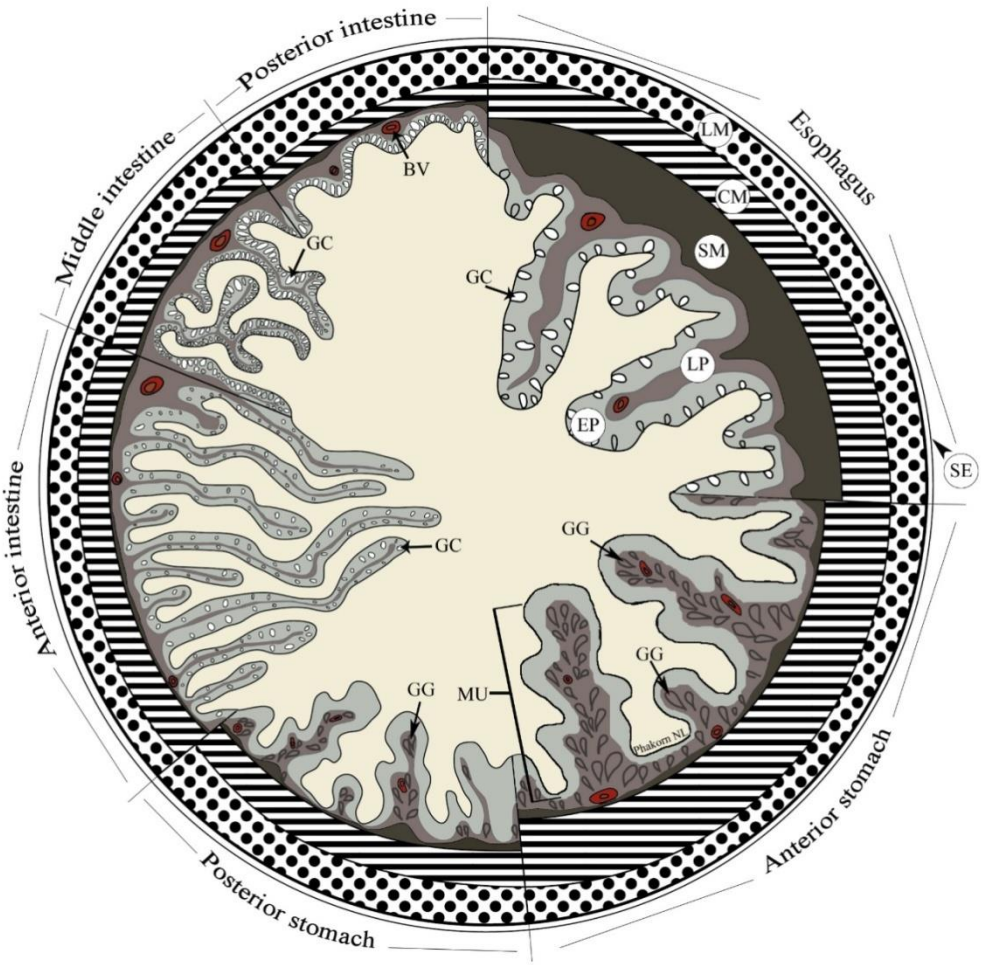

Figure 3.7 Schematic microscopic organization of the tubular digestive tract in *Pisodonophis boro*. All structures drawn to appropriate scale in terms of thickness and abundance.

3.4 Discussions

1. Gut content analysis and insights into optimal feeding strategies

Sarmatium germaini was the dominant prey item detected in the gut content of *P. boro*. The %IRI of *S. germaini* approached 100% whereas other prey had %IRI less than one percent. It is possible that *P. boro* can actually rank a variety of food items according to their energy income which is related to prey body size, influences energy value, and their escape ability as follows to the optimal foraging theory (Townsend & Winfield, 1985). However, where the *P. boro* occurred, *S. germaini* was not the major abundant crab species in Pranburi River estuary (Paphavasit. *et. al*., 2014). Other crabs i.e. *P. eumolpe M. elegans*, and *P. bidens* were more abundance than *S. germaini* (Paphavasit *et. al*., 2014). Therefore, *P. boro* seemed to only select on *S. germaini*. This sort of selective feeding was also observed in the *P. boro* found in India, which selected only a single species of crab *U. annulipes* (Subramanian, 1984). The specialist feeding type, which varies their prey among habitats, can also be found in other members of the order Anguilliformes (e.g., *Ophichthus rufus* (Casadevall *et al*., 1994); *Anguilla japonica* (Kaifu *et al*., 2013); *Conger conger* (O'Sullivan *et al*., 2004); *Anguilla bicolor* (Rupasinghe & Attygalle, 2006).

Hence, I suggest that *P. boro* might be considered as a specialist that feeds on *S. germaini* crab at Pranburi River estuary. Unfortunately, there have not been any studies on the feeding ecology of *P. boro* in Thailand. Based on the gut content analysis presented in this work and the prey availability in the ecosystem sampled, *P. boro* might be considered as a specialist feeder that feeds mainly on *S. germaini*. *Pisodonophis boro* seemed to select its prey depends on prey availability in the natural habitat.

2. Morpho-histology of Pisodonophis boro digestive tract

The overall gross morphology of the digestive tract of *P. boro* is similar to that of *P. cruentifer* (Wenner, 1976). The intestine coefficient (IC) of *P. boro* in the present study was 0.32, which compares with approximately 0.18 for *P. cruentifer* (Wenner, 1976). The IC value of *P. boro* in this study was similar to what was previously reported for carnivorous fish (ranging between 0.5-0.8) such as *Sparus*

aurata IC as 0.5-0.6 (Cataldi *et al.*, 1987) and *Glyptosternum maculatum* has IC as 0.8 (Xiong *et al.*, 2011). In contrast, herbivorous fish have IC ranging from 2 to 3 (Nie & Hong, 1963; Senarat *et al.*, 2013). Therefore, we suggested that *P. boro* can be classified as the carnivore, as based on the value of IC.

Our overall histology studies of the digestive tract of *P. boro* include similar to that of as *A. anguilla* (Clarke & Witcomb, 1980) and *A. bicolor* (Nasruddin *et al.*, 2014). However, there are still some slight differences. For instance, in the esophagus, the mucosal layer of *P. boro* did not contain taste buds; the reasons for this are unclear. In contrast, the mucosal histology of *A. anguilla* (Clarke & Witcomb, 1980) and *G. maculatum* (Xiong *et al.*, 2011) indicated the presence of taste buds. Also, the tip of the epithelial lining of the esophagus of *P. boro* was not covered with cilia; in contrast, the esophagus of the *Dentex dentex* (Carrassón *et al.*, 2006) and *A. bicolor* (Nasruddin *et al.*, 2014) was covered with cilia. The cilia lining is believed to involve with protecting the esophagus from injuries and lubricated the surface layer for food passage (Carrassón *et al.*, 2006; Nasruddin *et al.*, 2014). A simple squamous epithelium with numerous mucous cells in the esophagus of *P. boro* was observed following staining with H&E. These mucous cells were also positively stained with PAS and AB stains, similar to what was found in other eels such as *A. anguilla* (Clarke & Witcomb, 1980), *C. myriaster* (Takiue & Akiyoshi, 2013), *A. bicolor* (Nasruddin *et al.*, 2014) and other fish such as *D. dentex* (Carrassón *et al.*, 2006) and *G. maculatum* (Xiong *et al.*, 2011). Therefore, this strongly indicated that these cells produced glycoprotein and mucopolysaccharide in the mucosal layer of *P. boro* esophagus. The mucous produced from mucous cells act as lubrication for food when swallowed and it also involved in immunological responses against bacterial infections and osmoregulatory functions (Albrecht *et al.*, 2001; Nasruddin *et al.*, 2014; Senarat *et al.*, 2015). Another important observation is that the esophagus of *P. boro* consisted of two layers of muscularis; an inner longitudinal layer and an outer circular layer. These two features are directly involved with the movement of materials to the stomach and rejecting ingested materials, as generally found in Anguilliforms and Siluriforms (Abaurrea-Equisoain & Ostos-Garrido, 1996; Cao & Wang, 2009; Sis *et al.*, 1979; Takiue & Akiyoshi, 2013). The ability to reject ingested material would seem to be advantageous in *P. boro* that lives exclusively on crabs, as

these preys include substantial amounts of non-digestible body parts, such as the chitin-rich carapace and chelipeds.

The gastric gland was positively detected with PAS staining clearly demonstrated the existence of a gastric gland in *P. boro* stomach. Hence, I would expect that it produces gastric fluid with digestive enzymes and plays a major role in the secretion of pepsinogen for protein digestion, which is similar to the stomach of *A. bicolor* (Nasruddin *et al.*, 2014; Xiong *et al.*, 2011). Carrassón *et. al.* (2006) also suggested that protein secretion from the gastric gland may indicate that nutrient absorption occurs in the stomach, similar to several teleosts (Grau *et al.*, 1992; Ortiz-Delgado *et al.*, 2003; Senarat *et al.*, 2015; Xiong *et al.*, 2011). Other observations, the structural analyses of stomach between the two regions of *P. boro* stomach were different. The thickness of the mucosal projections, inner circular muscle, and the outer longitudinal muscle were observed from the anterior to the posterior regions. This is similar to *A. anguilla* and *A. bicolor* (Clarke & Witcomb, 1980; Nasruddin *et al.*, 2014). The stomach structural differences were probably related to the fact that the anterior stomach was the first section that received food from the esophagus and needed more muscle to breakdown and digests the foods during ingestion. Strong muscles are likely required in *P. boro*, for the physical breakdown of the hard body parts of crabs; in other words, this is an evolutionary adaptation that allows this species to be a specialist. In contrast, the posterior region of the stomach may act as storage suggesting it plays a less active role in the digestive breakdown (Xiong *et al.*, 2011). The pyloric caecum in *P. boro* was absent which is similar to other fishes, including *A. bicolor* (Nasruddin *et al.*, 2014) and *A. anguilla* (Clarke & Witcomb, 1980). Generally, the absence of pyloric caeca was observed in carnivore fish in contrast to the presence of pyloric caeca in herbivore. The presence of pyloric caecum was reported in *Rastrelliger brachysoma* (Senarat *et al.*, 2015), *Serrasalmus nattereri* (Raji & Norouzi, 2010), and *D. dentex* (Carrassón *et al.*, 2006). The presence of pyloric caeca was related to the natural habitat and food habits (Kapoor *et al.*, 1976; Raji & Norouzi, 2010). The function of the pyloric caeca was believed to increase the effectiveness of the absorption of carbohydrates and fats (Evans & Claiborne, 2006; Kapoor *et al.*, 1976). Hence, it is not surprising that *P. boro*, a carnivorous specialist, would not have pyloric caeca.

The mucosal epithelium of the *P. boro* intestine was covered by a simple columnar structure which contains several goblet cells. Moreover, these goblet cells were mostly found throughout the intestine mucosal. This is similar to other eels such as *A. anguilla* (Clarke & Witcomb, 1980), *C. myriaster* (Takiue & Akiyoshi, 2013), and *A. bicolor* (Nasruddin *et al.*, 2014). Many studies suggested that a high density of goblet cells in the intestine may serve as a lubrication aid to defecation in fish (Carrassón *et al.*, 2006; Clarke & Witcomb, 1980; Grau *et al.*, 1992; Senarat *et al.*, 2015; Xiong *et al.*, 2011). Moreover, these cells had been reported to play an important role in absorption, transportation, enzymatic cofactors, and also an additional role of defense from bacterial injects, which has been reported in *Senegal sole* (Arellano *et al.*, 2002) and *Tilapia* spp. (Scocco *et al.*, 1997). In addition, the goblet cells secrete many groups of mucopolysaccharides (i.e. sulphate groups, chondroitin, heparin, and chondroitinsulphates) which can provide protection for intestinal mucosae when fish consumed a larger quantity of hard solid foods (i.e. crab) (Scocco *et al.*, 1997). However, the present study found that the number of goblet cells in the middle and posterior intestine were significantly higher (P=0.000) than the anterior portion. This is similar to that of *A. bicolor* (Nasruddin *et al.*, 2014) and others carnivorous fishes (Kapoor *et al.*, 1976; Senarat *et al.*, 2015) such as *G. maculatum* (Xiong *et al.*, 2011), *Pseudoplatystoma fasciatum* (Rodrigues *et al.*, 2009), and *Monopterus albus* (Dai *et al.*, 2007). It is probably useful for increased mucous production to lubricate food particles, aid fecal expulsion, and also for protecting the epithelium (Murray *et al.*, 1994; Oliveira Ribeiro & Fanta, 2000; Purushothaman *et al.*, 2016). The mucosal fold was abundant in the anterior portion of the intestine. These increasing surface areas of the anterior portion enhances the absorptive ability (Oliveira Ribeiro & Fanta, 2000). The abundance of mucosal folds in the anterior intestine suggest this region is evolutionarily adapted to nutrient absorption in *P. boro*, and support the general idea that the middle and posterior intestine are adapted to an elimination of prey items (e.g., carapace, chelipeds) which are poorly brokendown and/or digested. The liver and pancreas were generally unremarkable in *P. boro* relative to other fish.

3.5 Conclusion

In conclusion, this study provided ecological and histological evaluations of that the gut content and morpho-histological characteristics of the digestive system in the *P. boro* from Pranburi River estuary. Based on IRI analysis, morphology, and histochemistry of digestive system, *P. boro* is a carnivore and is a specialist feeder on one crab species, *S. germaini.* The morpho-histology of the gut confirmed that *P. boro* is well adapted to a carnivorous lifestyle, including digestion and defection of the hard body parts of its crab prey. This information is useful in the feeding ecology required for aquaculture and management of this species. It would be interesting to conduct transplant experiments to test the feeding adaptations of this species in different river systems with different densities of its primary prey (*S. germaini*) and/or with different species of prey (especially habitats that lack *S. germaini*).

CHAPTER 4

SIZE DISTRIBUTION AND GONADAL HISTOLOGY OF *Pisodonophis boro* (Hamilton, 1822) (ANGUILLIFOMES: OPHICHTHIDAE) FROM PRANBURI RIVER ESTUARY, THAILAND

4.1 Introduction

The histological aspect of the gonad in fish is the key information in assessing the reproductive cycle, sex ratio and the cell structure of the gamete. Recently, there has been an incorporation of these data with the fishing gear regulation for the management of fish species(Dietrich & Krieger, 2009). There have been numerous studies on the histological aspect of the gonad in Anguilliforms. Several classifications of developing oocytes have been observed in several eel species including *Anguilla anguilla* (Colombo & Grandidr, 1996), *A. rostrate* (Sorensen & Pankhurst, 1988), *A. dieffenbachia,* and *A. australis* (Lokman *et al.*, 1998). On the other hand, family Ophichthidae which contains less commercial values has been ignored for examination.

Anguilliformes is a distinctive group of teleosts comprising more than 700 species (Nelson, 2006). Unique life cycles such the catadromous eels i.e. the Family Anguillidae (freshwater eel) and the anadromous eels i.e. the Family Ophichthidae (snake eel) (Froese & Pauly, 2016b; Nelson, 2006) were also observed in Anguilliforms. Previous observation also showed that mature Anguillids, freshwater eel can have hermaphrodite reproductive mode which the males develop through a transitional intersexual stage where the gonads contains both spermatogonia and oogonia (called a Syrski organ) (Colombo & Grandidr, 1996; Kearney *et al.*, 2011), whereas females develop ovaries directly from the undifferentiated primordial gonad such as *Anguilla australis* and *A. dieffenbachii* (Kearney *et al.*, 2011). However, Syrski organ in gonad have not been observed in snake eel, (Ophichthids).

Even though the ovarian structure have been described in some species of Ophichthidae such as *Pisodonophis cruentifer* and *Ophichthus rufus* (Casadevall *et al.*, 2001; Wenner, 1976) but this information is still lacking in *P. boro*. This fish is one of the most important commercial teleost fish at the estuary of Pranburi River

(approximately 200 – 250 baths per kilograms, pers. comm.). It's distribution ranges from the Indo-West Pacific to Sri Lanka, (Froese & Pauly, 2016a), and Thailand especially at the Pranburi River estuary (Paphavasit *et al.*, 2014). Up to date, this species may be listed as a threatened fish in this area. The wild fish population is dramatically declines from the natural habitat (pers. comm.). *Pisodonophis boro* population may be suffered from strong fishing pressure and the deterioration of their natural habitat (Moravec *et al.*, 2007; Paphavasit *et al.*, 2014). Similarly, Cheevaporn and Menasveta (2003) reported that the Pranburi River estuary has been well detected with environmental stressor and high pollutants of toxic compound especially lead and other heavy metals. If the decreasing still continues at this rate, *P. boro* may be extinct from the Pranburi River estuary. To resolve this problem, the previous studied suggested that development of the aquaculture is a primarily established with various manipulative strategies (i.e. hormone priming and artificial fertilization) (Zohar & Mylonas, 2001). However, these strategies require the basic knowledge about the size distribution and reproductive features before entering the complicated phase. However, this information still remains a mystery in *P. boro*.

Therefore, I primarily described the size distribution of *P. boro* in Pranburi River estuary. The ovarian structure and development relating to the reproductive mode/sex of *P. boro* during its annual reproductive season were secondarily observed by using histological and histochemical techniques. This work would be provided new insights the provision of scientific advice to aquaculture development and fisheries management strategies of this species.

4.2 Materials and Methods

1. Fish sampling

During March 2015 to March 2016, one hundred and five healthy specimens of *Pisodonophis boro* were captured by local fisherman from the Pranburi River Estuary, Prachuap Khiri Khan Province, Thailand (N 12°24'08.5" / E 099°59'00.2"). All fish was identified based on the taxonomic key of FAO (Rainboth, 1996). Then they were euthanized follows as a rapid cooling shock (Wilson *et al.*, 2009). The experimental protocol was approved by the Animal Care and Use Committee of

Faculty of Science in accordance with the guide for the care and use of laboratory animal prepared by Chulalongkorn University (Protocol Review No. 1423003).

2. Size distribution

All *Pisodonophis boro* specimens were measured for total length (the snout to the caudal fin to the nearest 1 cm) and total weight. In order to assess the size distribution, I carried out the criterion for ranges as well as size classes based on previous reported (Kearney *et al.*, 2011).

3. Histological and histochemical examinations

The ovarian tissue from all fish were dissected out into three regions (anterior, middle and posterior regions) and fixed in Davidson's fixative at 4 ºC (about 48 hrs). They were then processed by standard histological techniques (Presnell & Schreibman, 1997; Suvarna *et al.*, 2013). The paraffin blocks were serially sectioned at 4 µm thickness by the rotary microtome. Their paraffin sections were deparaffinized with xylene, rehydrated through a series of ethyl alcohol and stained with Delafield's hematoxylin and eosin (H&E) to study the basic structure of gonadal tissue. Some histological sections were specially stained with Periodic Acid Schiff (PAS) and alcian blue pH 2.5 (AB) to observe the chemical components (Presnell & Schreibman, 1997; Suvarna *et al.*, 2013). To determine in both sexes and gonadal development, the gonadal structures and gametogenesis was performed as following the criteria from Wenner (1976) and Dietrich and Krieger (2009) and photographed under light microscopic levels using Leica TE2000-U (10x and 40x).

4. Gonadal development

Each section of the gonadal tissue was examined under a light microscope in order to determine the stages of the ovarian development as described by Wenner (1976) and Dietrich and Krieger (2009).

4.3 Results and Discussion

1. Size distribution

The body size of *Pisodonophis boro* ranged from 24 cm to 97 cm with the mean total length of 66.28±1.3 cm (mean±SE; n = 105 individuals) (Fig. 4.1). The majority of the specimens were in the range from 50.5 cm to 80 cm in total length (n = 91 individuals) (Fig. 4.1). The largest size from this study was larger than that previously reported in *P. boro* in India, which ranged from 12.2 to 53.6 cm (Subramanian, 1984). This implies the difference size may due to the difference of natural habitat and collecting method; however, this observation needs more future study.

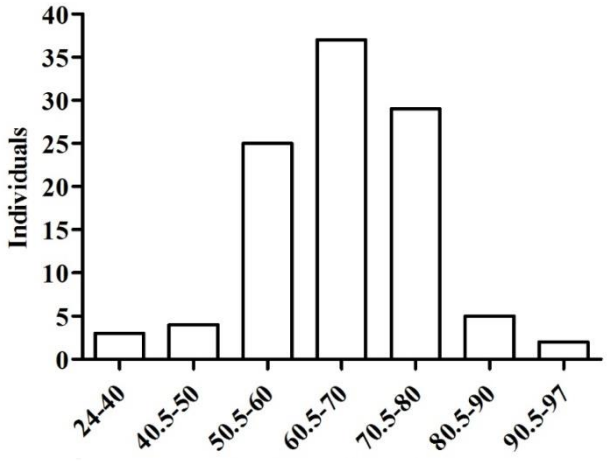

Figure 4.1 Size-classed distribution (cm) of *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016.

2. Morphology, histology, and development of the gonadal tissue with relating to sex

All sampling showed that the gonadal morphology of *P. boro* was elongated and paired. They are ribbon-liked in parallel to digestive tract (Fig. 4.2a). Each gonadal tissue was observed as a symmetrical elongation with milky white color throughout the study. The reproductive duct was absent. This suggests that it is a gymnovarian type fish. This is similar to previous observation in *P. cruentifer* (Wenner, 1976) and *Ophichthus rufus* (Casadevall *et al.*, 2001). Both species are without the reproductive duct. The gymnovarian type fish directly releases to the body cavity rather than through oviducts prior to spawning (Simon & Tomelleri, 2011).

According to histological observation, an only ovarian structure was detected throughout the ovaries. The ovary was structurally surrounded by a thin layer of tunica albuginea. The ovigerous fold protruded into a central lumen of the ovarian tissue, where could be distinguished into two compartments. (i) A germinal compartment (Figs. 4.2c and e) — a part that was covered by the ovigerous folds. This compartment composed of epithelial cells. (ii) A stromal compartment — a part contained one oogenic stage (Fig. 4.2b). It is also considered as "a synchronous developing type (Figs. 4.2b-e and 4.3).

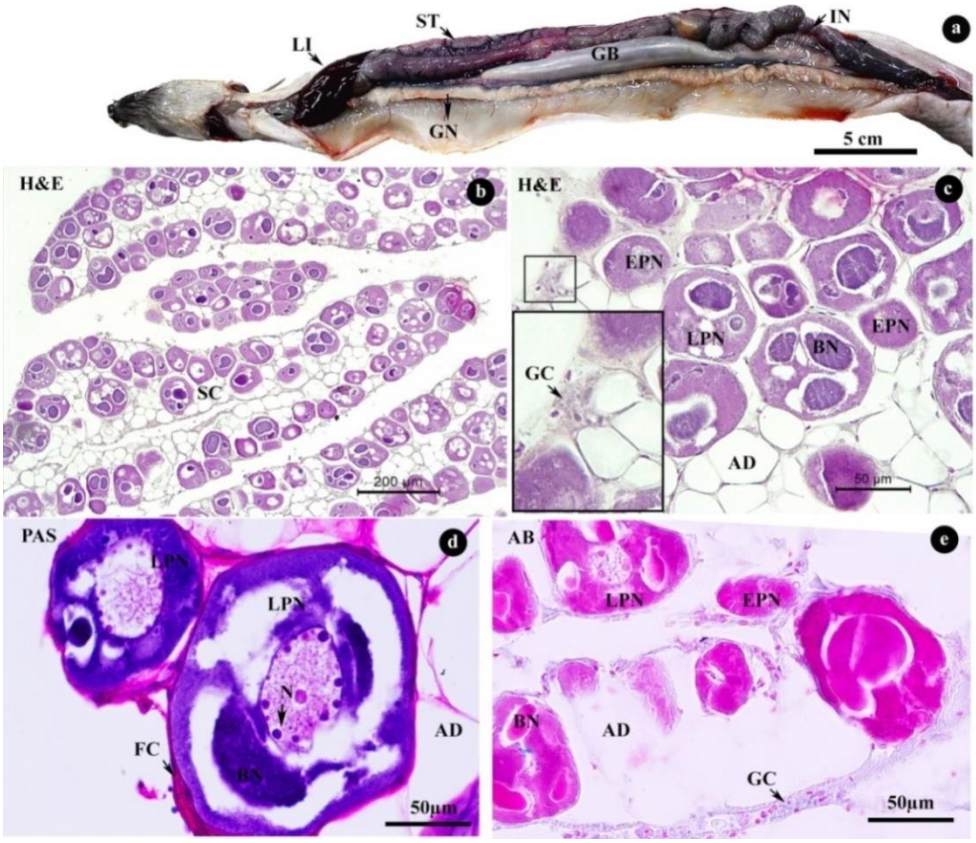

Figure 4.2 Micrograph of gross anatomy and histology of ovaries in *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016: **(a)** photograph of internal organs and ovary morphology of *P. boro*, **(b and c)** photomicrographs of ovarian histology of *P. boro* stained with hematoxylin and eosin staining, **(d and e)** high magnification photomicrographs of perinucleolar stages. **Abbreviations:** AD, adipocyte; BN, Balbiani body; FC, follicle cells; GB, gall bladder; GC, a germinal compartment; GN, gonad; IN, intestine; LI, liver; N, nucleolus; EPG, the early perinucleolar stage oocyte; LPN, the late perinucleolar stage oocyte; ST, stomach; SC, stromal compartment. **Staining:** AB, alcian blue stain; H&E, hematoxylin and eosin stain; PAS, Periodic Acid Schiff stain.

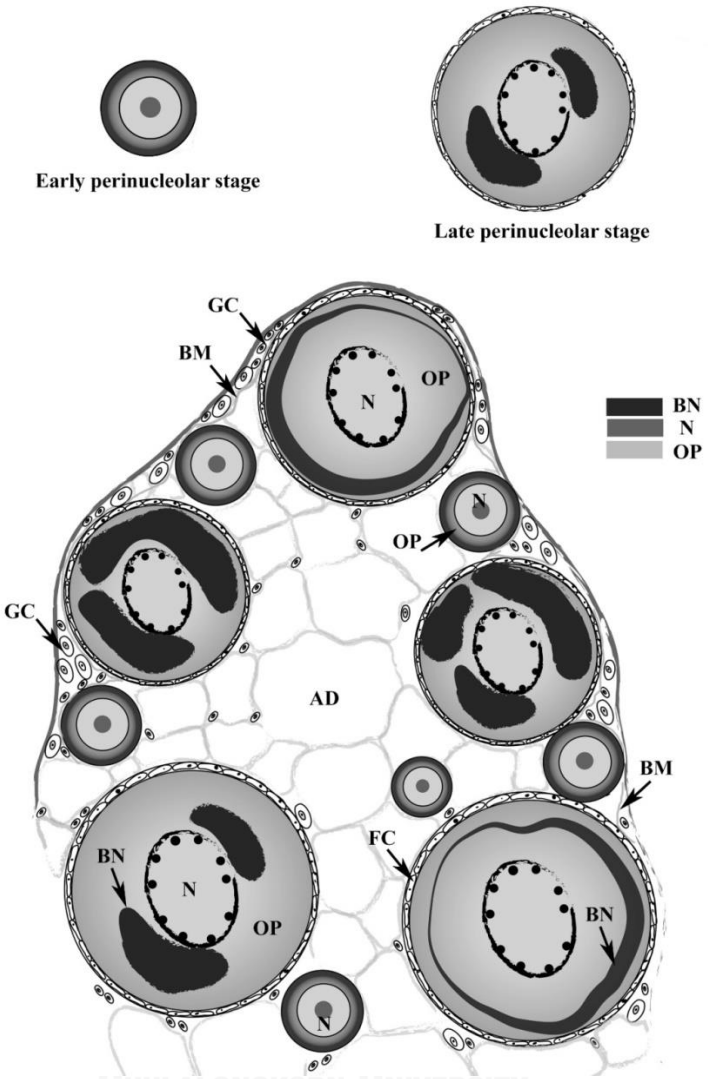

Figure 4.3 Schematic diagram showing the undeveloped stage of *Pisodonophis boro* collected from Pranburi River estuary during March 2015 to March 2016. **Abbreviations:** AD, adipocyte; BN, Balbiani body; BM, basement membrane; GC, a germinal compartment; FC, follicular cells; N, nucleolus; OP, ooplasm.

This type indicated that the ovary consists of oocytes develop in a unison. The example of fish that has this type of development is anadromous salmon (*Oncorhynchus* spp.) and catadromous eels (*Anguilla* spp.) (Casadevall *et al.*, 2001; McMillan, 2007). By contrast, a group synchronous developing type was found in *Op. rufus* in the northwest Mediterranean (Casadevall *et al*., 2001). It is possible that *Op. rufus* has a single and short breeding season (Dietrich & Krieger, 2009). Although a prior observation differs, this study clearly confirmed under histological observation

that *P. boro* had a synchronous ovary which found in fish that may spawn once in their lifetime or has a single breeding season. As a result, only undeveloped stage of *P. boro* was detected in all size-classes. The undeveloped stage oocyte composed of the early perinucleolar stage and the late perinucleolar stage with the presence of Balbiani bodies oocytes throughout the ovarian structure (Figs. 4.2 and 4.3). This is a novel finding; three possible explanations could explain this finding: a) a male maturing earlier than females (Kartas & Quignard, 1985); b) a hermaphrodite and parthenogenesis (Colombo & Grandidr, 1996) and c) a spatial displacement of sexes (Cau & Manconi, 1983). In the case of *Op. rufus*, the male was clearly disappeared at the large size (>39 cm in the body length), where the only female occur (Casadevall *et al.*, 2001). Casadevall and colleagues (2001) claimed that the more probable cause of that observation is a male maturing earlier in *Op. rufus*. Even though, the reason of a males maturing earlier than females were found in *Op. rufus* and commonly found in teleosts, this phenomenon do not apply to *P. boro* due to the smallest fish (a 24 cm in the total length) was sexual determined as female by histological observation. However, as mentioned above, the last two explanations may apply to this study.

Firstly, the information of the sex change and parthenogenesis, some *Anguilla* species has been reported as a hermaphrodite reproduction, which the gonad contains both spermatogonia and oogonia termed as “Syrski organ”. This phenomenon observed in many Anguillids such as *A. australis, A. anguilla, A. rostrata* and *A. japonica* (Colombo & Grandidr, 1996; Geffroy *et al.*, 2013; Kearney *et al.*, 2011). However, in the present day, I did not found a Syrski organ under the histological observation in all samples. This is similar to what was reported in *Op. rufus* gonad (Casadevall *et al.*, 2001). Therefore, hermaphroditism is probably less likely the case for *P. boro*. The occurrence of the parthenogenetic events had been reported over in a few the vertebrate taxa, about 70 taxa vertebrates had a form of parthenogenesis which only female genomes transferred to the next generation (Neaves & Baumann, 2011; Wootton & Smith, 2014). This mode of reproductive also occurs a few in the teleosts, which are about 8 taxa or represent only about 0.03% of all teleost species (Pandian, 2011; Wootton & Smith, 2014). Nevertheless, the parthenogenetic mode mostly found in the specific family such Poeciliids, and rarely in Atheriniformes and Cypriniformes (Pandian, 2011). However, recently, the parthenogenesis have

occurred in the captive female hammerhead shark (Chapman *et al.*, 2007) and white spotted bambooshark for the first time (Straube *et al.*, 2016). Straube and colleagues (2016) had claimed that this event was the first genetically confirmed evidence for second-generation facultative parthenogenesis in vertebrates and significantly support the evolutionary of parthenogenetic reproductive mode as an alternative to sexual reproduction. Therefore, *P. boro* in this study may be displayed the parthenogenetic mode of reproduction as similar to the bambooshark and hammerhead shark. But the parthenogenesis has not been any documented in the Anguilliforms, further studies would be conducted to test this hypothesis.

Second, the more probable explanation is a spatial displacement of sexes, there was a report of spatial displacement of sexes in Anguillid such as *Conger conger* (Cau & Manconi, 1983). Cau and Manconi (1983) claimed that *C. conger* showed spatial displacement between male and female eels. Male eels were absented at the depth below 400 m. At depth between 400 m to 800 m, the sex ratio of *C. conger* was close to 0.5 where the reproductive area take paced. Considering this model, it may apply to this study. Only female eel *P. boro* was found in this study at Pranburi River estuary; it may result from this fish shows sexes displacement behavior.

Overall resulted from this study, an only ovarian structure was found comprising of only undevelopmental oocytes throughout the years sampling; it may indicate that this *P. boro* utilized the Pranburi River estuary system just as feeding ground, not for spawning ground.

4.4 Conclusion

In this study, the size of *Pisodonophis boro* ranged from 24 cm to 97 cm with a mean body size of 66.28±1.3 cm (mean±SD). The gonad of *P. boro* was paired and elongated. It was parallel to the digestive tract. An only ovarian structure was found composing of early and late perinucleolar stage oocyte throughout the ovarian structure. This snake eel was considered as synchronous developmental oocytes. Our results contribute to the understanding of the female reproductive structure of this fish which use Pranburi River estuary for feeding area.

CHAPTER 5

GENERAL DISCUSSIONS AND CONCLUSIONS

The snake eel, *Pisodonophis boro* is one of the economically important fish and one of the aquaculture candidates in the Pranburi River Estuary, Thailand. Therefore, an understanding of the feeding ecology and reproductive biology of this species is required for this particular locality; however, this observation has never been studied.

Previous reports showed a variety of available food sources for the *P. boro* in the Pranburi River Estuary ecosystem. However, based on the gut analysis of *P. boro,* only *S. germaini* was the important prey based on gut content and percent IRI index with 99%. Based on the optimal foraging theory (OFT), it was possible that *P. boro* decided to choose this particular crab due to its energy, the escape ability of prey (Townsend & Winfield, 1985) and the most availability food sources (Kaifu *et al.*, 2013). Base on the result from this study and previous studies, *P. boro* selects the prey in each locality which could vary from locality to locality.

The external morphology of *P. boro* is slender and elongated which is suitable for living and forages their food in the hole of the mudflat of an estuary. Moreover, previous literature showed that teeth of *P. boro* were granular with multi-serial bands on jaws. The vomer teeth supported the breakdown of the hard structure of the crab. The intestine coefficient of *P. boro* had low value and was classified into the carnivorous type.

The esophagus had numerous mucous cells indicating increasing efficiency for lubricating and protecting the mucosa layer during the swallow of hard structures of crab i.e. cheliped (Albrecht *et al.*, 2001; Nasruddin *et al.*, 2014; Senarat *et al.*, 2015). The stomach of *P. boro* was Y-shaped. It contained several gastric glands in the mucosal layer. These glands produce gastric fluid with digestive enzymes. They play a major role in the secretion of pepsinogen for protein digestion (Nasruddin *et al.*, 2014; Xiong *et al.*, 2011). Pyloric caeca were not observed in this species supporting the fact that this species is a carnivorous fish. The portion of the intestine was typically divided into anterior, middle and posterior regions. Based on these

anatomical details of the digestive tract, *P. boro* is a specialist feeder on crabs rather than an opportunistic feeder.

The body size distribution of *P. boro* ranged from 24 cm to 97 cm with a mean total length±SE of 63.63±1.45 cm. The histological investigation revealed that only ovary was detected in all samples. This could be explained by a spatial displacement behavior of sexes as found in *C. conger* (Cau & Manconi, 1983), implying that each sex had their own habitat before breeding season occur. As the breeding season proceeds, then both sexes move to the same breeding ground to spawn. Gonadal histology revealed only one stage of oocytes development in all samples. This could imply that they are synchronous development oocyte.

These observations provided crucial information about habitat utilization of *P. boro* in the Pranburi estuary system. *Pisodonophis boro* utilized the system as a feeding ground and used the Pranburi River estuary as the pathway for upward migration to the freshwater habitat where the spawning ground was hypothesized by an earlier study (Rainboth, 1996). Ultimately, this study provided the knowledge of the ecological and histological evaluations of the gut content and morpho-histological characteristics of the digestive system as well as gonadal histology of *P. boro* from Pranburi River estuary. This information is useful for the feeding ecology required for aquaculture and management of this species.

Recommendation

It would be interesting to conduct transplant experiments to test the feeding adaptations of this species in different river systems with different densities of its primary prey (*S. germaini*) and/or with different species of prey (especially habitats that lack *S. germaini*).The further study is needed to understand the life cycle and distribution of *P. boro* along the Pranburi River to clarify and predict the spawning area of the *P. boro*. Moreover, the possible habitat for adult *P. boro* such as coral reef, the ocean, and the freshwater system should be observed to complete knowledge gap if the habitat requirement for *P. boro* whole life cycle.

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APPENDIX

APPENDIX

Appendix I Histological Staining Protocol

1.1 Mayer's Hematoxylin and Eosin staining

Procedure

1. Xylen I for 5 minutes
2. Xylen II for 5 minutes
3. Absolute alcohol I for 3 minutes
4. Absolute alcohol II for 3 minutes
5. 95% ethanol I for 3 minutes
6. 95% ethanol II for 3 minutes
7. 70% ethanol for 3 minutes
8. Distilled Water for 3 minutes
9. Mayer's Hematoxylin for 3 minutes
10. Tab Water (until clear)
11. 1% acetic acid for 10 second
12. 70% ethanol for 3 minutes
13. Eosin for 30 second
14. 95% ethanol I for 3 minutes
15. 95% ethanol II for 3 minutes
16. Absolute alcohol I for 5 minutes
17. Absolute alcohol II for 5 minutes
18. Xylen I for 5 minutes
19. Xylen II for 5 minutes
20. Mounting

Results: Nuclei – Blue with some metachromasia; Cyplasm – various shades of pink, identifying different tissue component.

1.2 Periodic acid Schiff (PAS) staining

Protocol

1. Xylen I for 5 minutes
2. Xylen II for 5 minutes
3. Absolute alcohol I for 5 minutes
4. Absolute alcohol II for 5 minutes
5. 95% ethanol I for 5 minutes
6. 95% ethanol II for 5 minutes
7. Tap Water for 5 minutes
8. 0.5% Periodic acid for 5 minutes
9. Tap Water for 5 minutes
10. Schiff's reagent for 30 minutes
11. Tab water for 10 minutes
12. Mayer's Hematoxylin for 15 minutes
13. Tab Water for 5 minutes
14. 95% ethanol I for 3 minutes
15. 95% ethanol II for 3 minutes
16. Absolute alcohol I for 5 minutes
17. Absolute alcohol II for 5 minutes
18. Xylen I for 5 minutes
19. Xylen II for 5 minutes
20. Mounting

Results: Strongly acidic sulfated mucosubstances – Blue; Nuclei – pink to red; Cytoplasm – pale pink.

1.3 Alcian blue staining

Procedure

1. Xylen I for 5 minutes
2. Xylen II for 5 minutes
3. Absolute alcohol I for 3 minutes
4. Absolute alcohol II for 3 minutes
5. 95% ethanol I for 3 minutes
6. 95% ethanol II for 3 minutes
7. 70% ethanol for 3 minutes
8. Distilled Water for 3 minutes
9. Mayer's Hematoxylin for 3 minutes
10. Tab Water (until clear)
11. Alcian blue pH 2.5 for 30 second
12. Distilled Water (until clear)
13. Eosin for 30 second
14. 95% ethanol I for 3 minutes
15. 95% ethanol II for 3 minutes
16. Absolute alcohol I for 5 minutes
17. Absolute alcohol II for 5 minutes
18. Xylen I for 5 minutes
19. Xylen II for 5 minutes
20. Mounting

Results: Glycogen, mucin, and some basement membranes – red/purple; Background – Blue.

Appendix II Photographs

Appendix 2.1 Pranburi River estuary

Fig. 2.1.1 Sampling area at Pranburi River estuary during low tide

Fig. 2.1.2 Sampling area at Pranburi River estuary during high tide

Appendix 2.1 Pranburi River estuary (Continued)

Fig. 2.1.3 The fisherman searching for the snake eel on the mudflat

Fig. 2.1.4 The fisherman digging through the mudflat looking for the snake eel

Appendix 2.2 Specimens collecting

Fig. 2.2.1 The fisherman digging through the mudflat looking for the snake eel

Fig. 2.2.2 The fisherman digging through the mudflat looking for the snake eel (Continued)

Appendix 2.2 Specimens collecting (Continued)

Fig. 2.2.3 The fisherman pouring traditional herbs medicine, one of the local method to catch the snake eel

Fig. 2.2.4 The snake eel, after exposed with the traditional herbs medicine, moving out of its hole

Appendix 2.3 Preys found in gut content of *Pisodonophis boro*

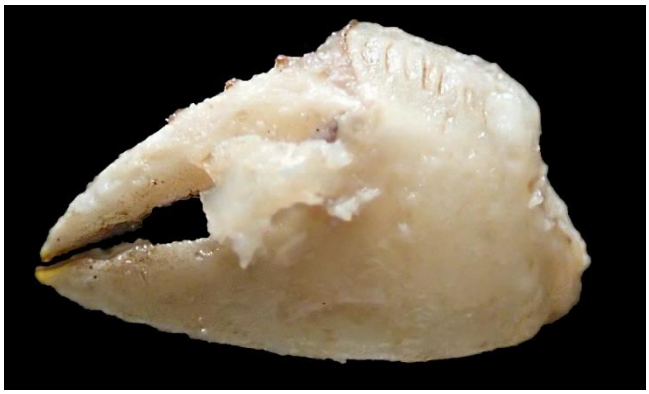

Fig. 2.3.1 Cheliped of *Sarmatium germaini*

Fig. 2.3.2 Cheliped of *Mataplax elegans*

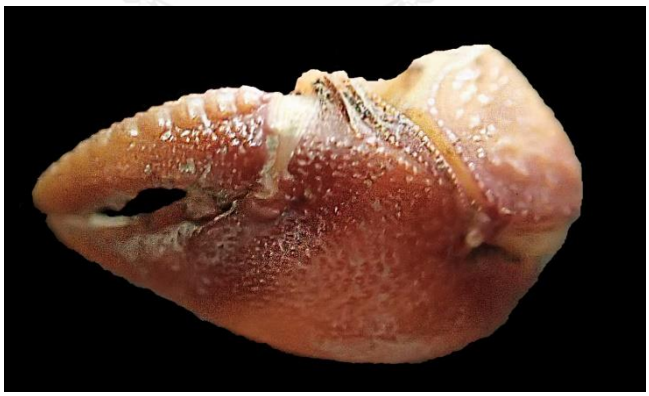

Fig. 2.3.3 Cheliped of *Perisesarma bidens*

Appendix 2.3 Gut contents (Continued)

Fig. 2.3.4 Cheliped of *Uca perplexa*

Fig. 2.3.5 Carcass of unidentified mantis shrimp

VITA

Mr. Phakorn Na Lampang was born on the 16 December 1990 in Chiang Rai Province. He graduated with his bachelor degree in Science from the Faculty of Science, Mahidol University (Kanchanaburi Campus) in 2012. After then, he enrolled as a graduate student in the program of Marine Science, Chulalongkorn University.

Poster Presentation

Phakorn Na Lampang, Amphornphan Palasai, Sinlapachai Senarat, Wannee Jiraungkoorskul, and Jes Kettratad. 2016. Renal histology and histopathology of the snake eel (Pisodonophis boro (Hamilton, 1822)) from Pranburi River, Thailand. Poster Presentation in Proceeding of the 5th Marine Science Conference, 1-3 June 2016, Bangkok, Thailand.

National proceeding

Phakorn Na Lampang, Amphornphan Palasai, and Jes Kettratad. 2016. Gut content analysis of the snake eel Pisodonophis boro (Hamilton, 1822) from the estuary of Pranburi River, Thailand. Proceeding of the 3rd National Meeting on Biodiversity Management in Thailand, 15-17 June 2016, Nan Province, Thailand.

International publications

1. Na Lampang, P., Palasai, A., Senarat, S., Jiraungkoorskul, W., and Kettratad, J. Observation of gut content and morpho-histology of the digestive system in Pisodonophis boro (Hamilton, 1822) from Pranburi River estuary, Thailand. (in preparation)

2. Na Lampang, P., Palasai1, A., Senarat, S., Jiraungkoorskul, W., Boonyoung, P., and Kettratad, J. Evidence for the existence of argyrophilic endocrine cells in the digestive system of the snake eel, in Pisodonophis boro (Hamilton, 1822). (in preparation)

3. Na Lampang, P., Palasai, A., Senarat, S., Jiraungkoorskul, W., Uribe, M. C., and Kettratad J. Body size distribution and gonadal histology of the Pisodonophis boro (Hamilton, 1822) (Anguilliformes: Ophichthidae) from Estuarine-Pranburi River, Thailand. (in preparation)