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Original Article

Observation of gut content and morpho-histology of the digestive system in *Pisodonophis boro* (Hamilton, 1822) from Pranburi River Estuary, Thailand

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Abstract

The snake eel, *Pisodonophis boro*, is an important ecological and subsistence component of the Pranburi River estuary. Here, we used gut content analysis and morpho-histology to examine optimum foraging theory in *P. boro* in the Pranburi River estuary. Gut content analysis revealed that four species of crabs, including *Metaplax elegans*, *Perisesarma bidens*, *Sarmatium germaini*, and *Uca perplexa*, but *S. germaini* were consistently present at high levels in the gut throughout a year-long study. The index of relative importance showed that the most important prey was *S. germaini*. The overall anatomical morphology of the digestive system of *P. boro* was elongated, and the stomach appeared as Y-shaped. The histology of the *P. boro* esophagus, stomach, and intestine were examined followed the standard histological techniques. Goblet cells through the digestive tract of *P. boro* were also seen among the epitheliums and reacted positively to periodic acid schiff and alcian blue staining methods. Overall, we suggest that *P. boro* is a specialist feeder on crabs rather than an opportunistic feeder.

Keywords: gut content, microanatomy, *Sarmatium germaini*, optimum foraging theory

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, 2016). It is generally found in estuaries and the tidal zone around the upstream areas of coastal rivers (Subramanian, 1984). Based on previous reports on their feeding behavior and habitat, the snake eel feeds selectively on only a single species of crab in India (Subramanian, 1984) while they feed on small fishes 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, but there has not been any study on the feeding ecology of this eel in Thailand. The optimum foraging theory (OFT) entered the field of feeding ecology at the end of the century. This theory has been described to predict how fish succeed in 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

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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, we hypothesize that in Thailand *P. boro* will select the most beneficial food based on the food availability in their natural habitat. *P. boro* has high economic value as a food source locally at Pranburi River (Paphavasit, Siriboon, Jaiperm, & Mookui, 2014). It is of interest to know to how the theoretical framework of OFT of this fish. Unfortunately, the populations of *P. boro* in this area tend to decline 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 various fishes are linked to their ecological roles, population dynamics, resource partitioning, and trophic ecology in natural habitats (Guedes & Araújo, 2008). Explicit feeding information is worthwhile for developing conservation strategies (Braga, Bornatowski, & Vitule, 2012). Several methods of studying feeding ecology of fish have been documented (Young, Guest, Lansdell, Phleger, & Nichols, 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 (Kaifu, Miyazaki, Aoyama, Kimura, & Tsukamoto, 2013). The digestive morphology and 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 *et al.*, 2014).

This study provides a test of the hypothesis that *P. boro* in Thailand will select the most beneficial and the most abundant food items in their habitat. We 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 preferences 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.

2. Materials and Methods

Seventy-seven specimens of *P. boro* (the total lengths of *P. boro* ranged from 24 to 97 cm, with a mean lengths of 63.63±1.45 cm) were collected from the Pranburi River estuary, Prachuap Khiri Khan Province, Thailand (12°24'08.5" N/ 099°59'00.2" E), during March 2015 to March 2016 by local fisherman using traditional methods. The local fisherman poured local liquid herbs extract mixed with tobaccos into the eel's hole. Due to the irritation to the respiration system of the eel from the extracts, the eel would exit the hole and was dipped net by the fisherman. Once the eel were caught, they were euthanized by rapid cooling shock technique (Wilson, Bunte, & Carty, 2009). The measurement of the snout-tail tip was made to the nearest 1 cm with a measuring tape. 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 hrs before preserved in 70% ethanol for further gross anatomy and gut content analysis.

The gut was horizontally dissected (from 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, Oliphant, & Iverson, 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 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: $IRI = \%O \times (\%N + \%C)$.

Ten 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) 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 (Suvarna, Layton, & Bancroft, 2013). The stained slides were photographed using an Olympus CX31 light microscope mounted with a Canon EOS 100d camera.

2.1 Semi-quantitative analysis

The intensity of staining from the various histochemical methods was assessed by visual observations using the following scores: − no reaction, + weak reaction, ++ moderate reaction and +++ strong reaction. 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. Results

3.1 Composition of gut content

Seventy-seven specimens of *P. boro* were obtained for gut content analysis. Only 83.12% (sixty-four guts) contained food, while the remaining had empty guts. 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 (Table 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.

Table 1. Summary of prey items found in the gut contents of *Pisodonophis boro*, 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

3.2 Morphology of the digestive tract and accessory glands

The digestive tract was composed of the anterior end of the esophagus, stomach and intestines (Figure 1a). 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 (Figure 1b). No pyloric caeca was observed. The intestine consisted of three regions: anterior, middle, and posterior regions. The anterior intestine started from the pyloric sphincter. It was initially relatively straight in the anterior region while the middle region had a slightly coiled shape as it approached the posterior intestine. The liver was dark brownish in color and structurally elongated at the dorsal regions. The anterior lobe of the liver was located close to the heart; however, the anatomical observation of pancreas was not morphologically distinguished.

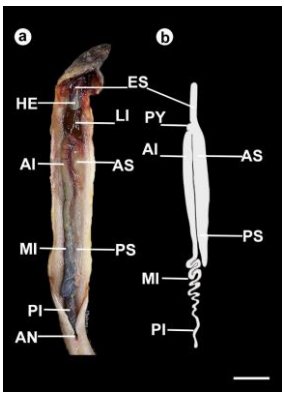

Figure 1. Anatomy of alimentary tract of *Pisodonophis boro* after a longitudinal incision: (a) morphology of digestive tract; (b) Schematic diagram of digestive tract. Abbreviations: AI, anterior-intestine; AN, anus; AS, anterior-stomach; ES, esophagus; HE, heart; LI, liver; MI, middle-intestine; PI, posterior-intestine; PS, posterior-stomach; PY, pyloric sphincter. Scale bar = 2.5 cm

3.3 Histolo-histochemical observations of the digestive tract

The esophagus histologically revealed four layers including mucosa, submucosa, muscularis and serosa. The mucosa contained longitudinal folds that protruded into the lumen (Figure 2a). The deepest layer of the mucosal layer consisted of two layers, the epithelium and lamina propria which consisted of loose connective tissue (Figure 2b, c). The muscularis mucosae layer was not observed in these sections. The epithelial layer was lined by a simple squamous epithelium, which contained goblet cells (Figure 2c). A strong positive reaction of both PAS and AB stains were detected in the mucous cells (Figure 2d-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 (Figure 2f).

The stomach was classified into two regions including anterior and posterior regions based on their localization and histological structures (Figure 3a, b). PAS staining showed positive cells in throughout stomach regions. 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 (Figures 3c). 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 (Figure 3a). 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 (Figure 3b).

Histology of intestine was classified into three regions including anterior, middle and posterior intestines (Figures 4a-c). AB and PAS staining showed scattered positive goblet cells with both acid and neutral mucopolysaccharides throughout all three regions of intestine (Figures 4a-4o). The anterior intestine was clearly identifiable between the anterior stomach and the middle intestine. This region was composed of four histological layers similar with other organs of the digestive tract (Figure 4d). However, the longitudinal fold was a more prominent feature in the mucosal layer of the anterior intestine (Figure 4a). Several goblet cells were also observed and inserted in the epithelial layer (Figures 4g, j and m). The muscularis layer (inner circular muscle and outer longitudinal muscle) was very thin. The morphology of the middle and the posterior intestines were similar to other parts of the overall digestive tract. However, the longitudinal folds of the epithelial layer were thinner than in the anterior intestine (Figures 4b, c). Moreover, the goblet cells in these

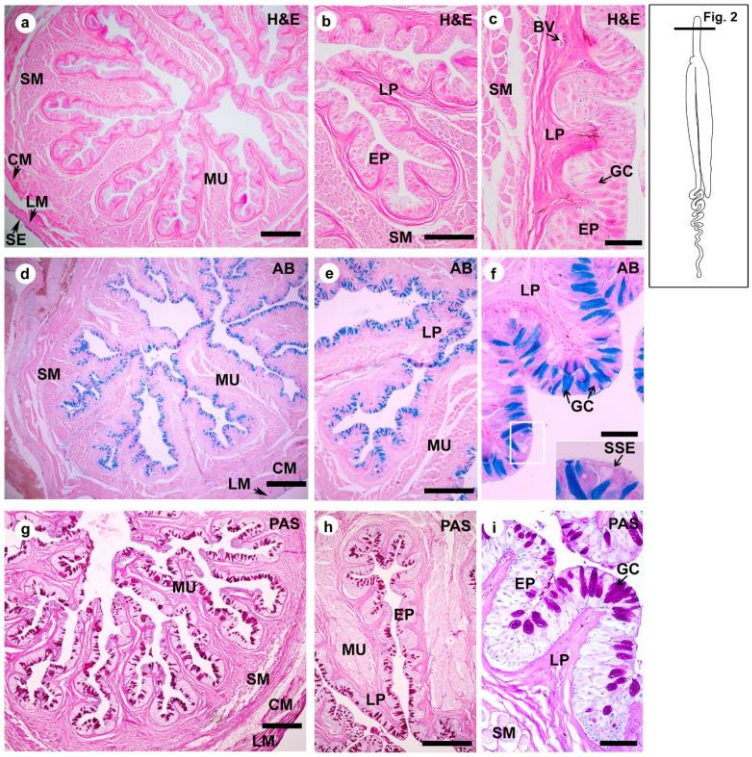

Figure 2. Light photomicrographs of *P. boro* esophagus transverse sections with different histochemical stains. A schematic diagram of the location of the transverse section is shown to the far right. Staining: H&E, Haematoxylin and Eosin (a, b, c); AB, Alcian blue pH 2.5 (d, e, f); PAS, Periodic Acid 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

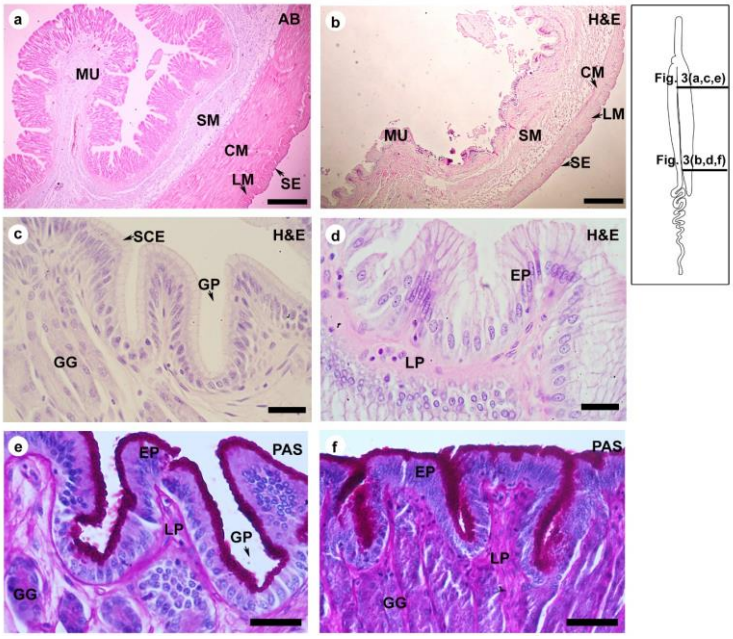

Figure 3. Transverse section of stomach regions of *P. boro*: anterior stomach (a, c, e) and posterior stomach (b, d, f). A schematic diagram of the location of the transverse section is shown to the far right. Staining: AB, Alcian blue stain; H&E, Haematoxylin and Eosin stain; PAS, Periodic Acid Schiff stain. Abbreviations: GG, gastric gland; GP, gastric pit; SCE, simple columnar epitheliums. Scale bars: a and b = 400 µm, c-f = 30 µm

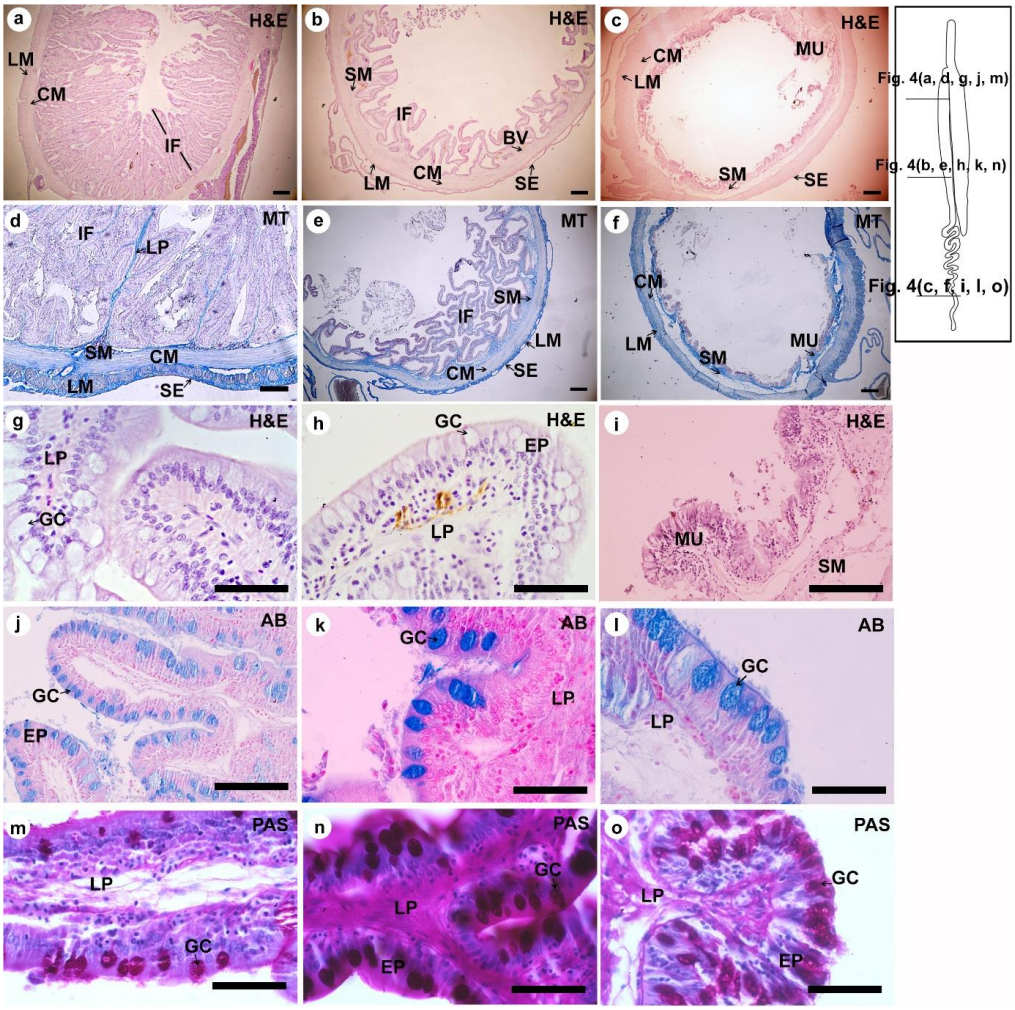

Figure 4. Light photomicrographs of *P. boro* intestines transverse sections with different histochemical stains. A schematic diagram of the location of the transverse section is shown to the far right: anterior intestine (a, d, g, j, m); middle intestine (b, e, h, k, n); posterior intestine (c, f, i, 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). Staining: AB, Alcian blue stain; H&E, Haematoxylin and Eosin stain; MT, Masson's Trichrome stained; PAS, Periodic Acid Schiff stain. Abbreviations: IF, intestinal fold. Scale bars: a-f = 300 µm, g-o = 50 µm

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, respectively (Table 2). The number of goblet cells in the posterior region was significantly higher ($P<0.05$) than the anterior region and higher than the middle region.

3.4 Histol-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 (Figure 5a). The oval nucleus in hepatocyte was surrounded by eosinophilic cytoplasm. Sinusoidal portal blood was present between cords. The central vein was located in the middle area of the liver/of the sinusoids (Figure 5b). Within the liver tissue, the bile duct was also identifiable and was surrounded by 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 (Figures 5c, d). Pancreas cells had a strong basophilic cytoplasm with a basal nucleus. Eosinophilic zymogen granules were also observed.

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 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 6.

Table 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); 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

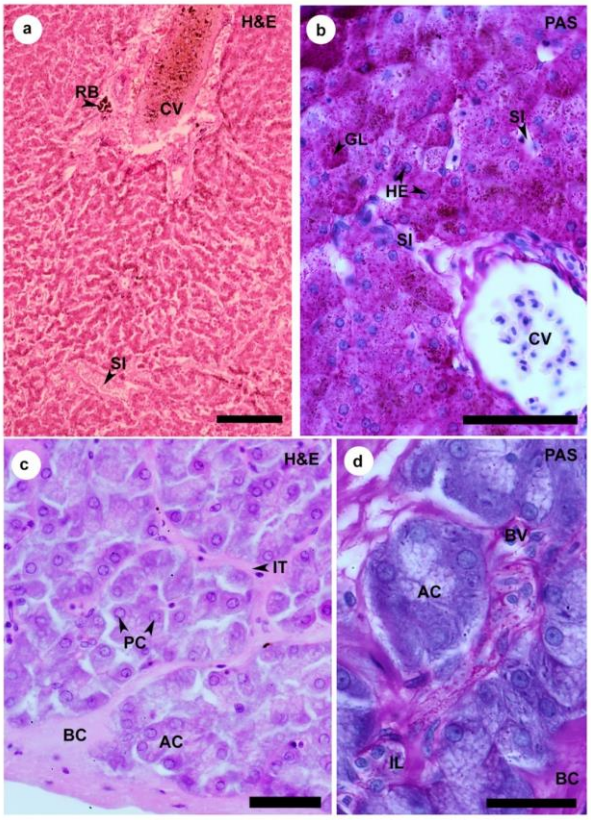

Figure 5. Light photomicrographs of histological structure of liver and pancreas. (a, b) Organization of the liver showing hepatocytes, central vein, and glycogen accumulation; (c, d) Pancreas features showing granular exocrine pancreatic cells with basophilic cytoplasm. Staining: H&E, Haematoxylin and 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

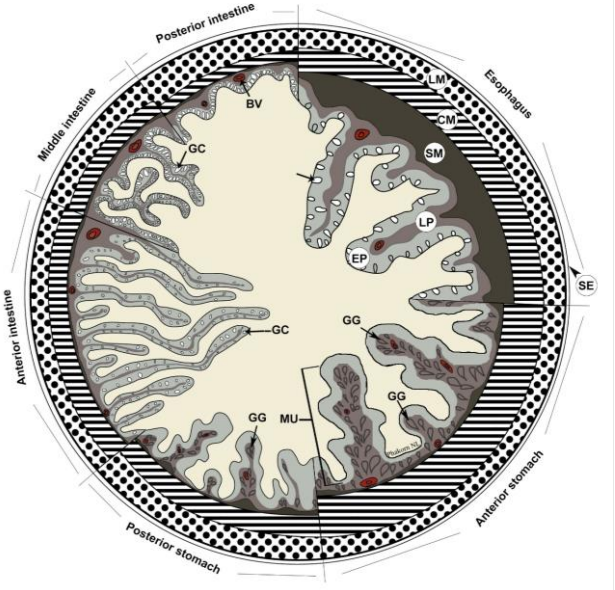

Figure 6. Schematic microscopic organization of the tubular digestive tract in *Pisodonophis boro*. All structures are drawn to appropriate scale in terms of thickness and abundance. Abbreviations: BV, blood vessel; CM, circular layer of muscle; EP, epithelium; GC, goblet cells; LM, longitudinal layer of muscle; LP, lamina propria; MU, mucosa; SE, serosa; SM, submucosa

4. Discussions

4.1 Gut content analysis

Sarmatium germaini was the dominant prey item identified 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 with the best investigation (Townsend & Winfield, 1985). Similar to Gerking (1994) suggested that the pattern of fish feeding is commonly required to optimize benefits of energy and the fitness-related activities of foraging efficiency. Previous observation showed that several crabs i.e. *P. eumolpe, P. bidens* and *M. elegans* were more abundance than *S. germaini* in Pranburi River estuary (Paphavasit *et al.*, 2014). However, based on the field observation, *S. germaini* movement is often sluggish and having slower movement when compared to *P. eumolpe, P. bidens* and *M. elegans*. Therefore, *P. boro* could select on *S. germaini* based on the ease of capture. Similar 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,

Table 3. Histochemical reactions in different regions of the digestive tract of *Pisodonophis boro*

Procedures	Esophagus	Stomach		Intestine		
		Anterior	Posterior	Anterior	Middle	Posterior
AB (pH 2.5)	++	-	-	++	++	++
PAS	++	+++	+++	++	++	++

Note: AB = Alcian blue, PAS = periodic acid Schiff. Symbols: − no reaction, + weak reaction, ++ moderate reaction and +++ strong reaction

which varies their prey among habitats, can also be found in other members of the order Anguilliformes (Casadevall, Matallanas, & Bartolí, 1994; Kaifu *et al.*, 2013). Hence, it is possible 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. We proposed that *P. boro* might be considered as a specialist feeder that feed mainly on *S. germaini* based on the gut content analysis. This eel seemed to select its prey depends on prey availability in the natural habitat.

4.2 Morpho-histology of *P. 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*. The IC value of *P. boro* in this study was similar to previous reported of carnivorous fish (ranges between 0.5-0.8) *Sparus aurata* IC as 0.5-0.6 (Cataldi, Cataudella, Monaco, Rossi, & Tancioni, 1987) and *Glyptosternum maculatum*has IC as 0.8 (Xiong *et al.*, 2011). In contrast, herbivorous fish have IC ranging from 2 to 3 (Senarat, Yenchum, & Poolprasert, 2013). Therefore, we suggest that *P. boro* can be classified within the carnivorous type, 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, Azmai, Ismail, Saad, Daud, & Zulkifli, 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 epithelial lining of the esophagus of *P. boro* was not covered with cilia; in contrast, the esophagus of the *Dentex dentex* (Carrassón, Grau, Dopazo, & Crespo, 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) and *A. bicolor* (Nasruddin *et al.*, 2014). 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, Ferreira, & Caramaschi, 2001). 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 (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, we would expect that it produces gastric fluid with digestive enzymes and plays a major role in secretion of pepsinogen for protein digestion, which is similar to the stomach of *A. bicolor* (Xiong *et al.*, 2011; Nasruddin *et al.*, 2014). Carrassón *et al.* (2006) also suggested that protein secretion from the gastric gland may indicate that nutrient absorption occurs in the stomach. Other observation, the structural analysis of stomach between the two regions of *P. boro* stomach was 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 digest 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 digestive breakdown (Xiong *et al.*, 2011). The pyloric caecum in *P. boro* was absent which is similar to other fishes (Clarke & Witcomb, 1980; Nasruddin *et al.*, 2014). 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 caeca was related to the natural habitat and food habits (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). Hence, it is not surprising that *P. boro*, a carnivorous specialist, would not have a pyloric caeca.

The mucosal epithelium of the *P. boro* intestine was covered by a simple columnar structure which contains several goblet cells. These goblet cells were mostly found throughout the intestine mucosal. This is similar to other eels such as *A. anguilla* (Clarke & Witcomb, 1980) and *C. myriaster* (Takiue & Akiyoshi, 2013). Many studies suggested that a high density of goblet cells in the intestine may serve as a lubrication aid to defecation in fish (Xiong *et al.*, 2011). Moreover, these cells had been reported to play an important role in absorption, transportation, enzymatic cofactors, and also additional role of defense from bacterial injects, which has been reported in *Tilapia* spp. (Scocco, Menghi, & Ceccarelli, 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 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 than the anterior portion. This is similar to that of *A. bicolor* (Nasruddin *et al.*, 2014) and others carnivorous fishes (Dai, Shu, & Fang, 2007; Xiong *et al.*, 2011). It is probably useful for increased mucous production to lubricate food particles, aid fecal expulsion, and also for protecting the epithelium (Purushothaman *et al.*, 2016). The mucosal fold was abundant in the anterior portion of 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 adsorption in *P. boro*, and support the general idea that the middle and posterior intestine are adapted to elimination of prey items (e.g., carapace, chelipeds) which are poorly broken down and/or digested. The liver and pancreas were generally unremarkable in *P. boro* relative to other fish. When compare the degree of food selection of *P. boro* to species with similar digestive tract morphology such as *A. anguilla* and *A. bicolor*, the selective differences between the *P. boro* and the other two species of eel *(A. anguilla* and *A. bicolor*) which does not process such strong degree of selection as in *P. boro* (Barak & Mason, 1992; Morais, 2017) was probably not cause the morphological differences in the digestive tract. The main digestive morphological different between the *P. boro* and the *A. anguilla* were the presence of taste bud. Taste buds function as chemoreceptors. The stimulus from the taste bud determines the decision the prey of the fish (Morais, 2017). Therefore, based on the stated fact about the function of taste bud, the overwhelming load on the IRI of the prey *S. germaini* and the absence of taste bud in *P. boro*, it is unlikely that taste bud was involve in the decision that *P. boro* selected on the *S. germaini*. It is more likely that they selected *S. germaini* not because that they prefer the taste of the crab but because they are the easiest prey to capture.

5. Conclusions

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. The %IRI from the gut content showed that *S. germaini* was close to 100% whereas other prey had less than 1% IRI. The morpho-histology of the gut also support that *P. boro* is well adapted to a carnivorous lifestyle. The adapted morpho-histology traits include digestion and defection of the hard body parts of its crab prey. Base on the IRI analysis and structure of digestive system, we suggest that *P. boro* at Pranburi river estuary is a specialist carnivore that feeds on one crab species, *S. germaini* base on the ease of capture. This available information contributed to the understanding of the basic feeding ecology, which will be applicable to aquaculture and fisheries management of this species.

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References

Albrecht, M., Ferreira, M., & Caramaschi, E. (2001). Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Characiformes; Anostomidae). *Journal of Fish Biology, 58*, 419-430.

Barak, N. A. E., & Mason, C. F. (1992). Population density, growth and diet of eels, *Anguilla anguilla* L., in two rivers in eastern England. *Aquaculture Research, 23*, 59-70.

Braga, R. R., Bornatowski, H., & Vitule, J. R. S. (2012). Feeding ecology of fishes: An overview of worldwide publications. *Reviews in Fish Biology and Fisheries, 22*, 915-929.

Carrassón, M., Grau, A., Dopazo, L., & Crespo, S. (2006). A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). *Histology and Histopathology, 21*, 579-593.

Casadevall, M., Matallanas, J., & Bartolí, T. (1994). Feeding habits of *Ophichthus rufus* (Anguilliformes, Ophichthidae) in the western Mediterranean. *International Journal of Ichthyology, 18*, 431-440.

Cataldi, E., Cataudella, S., Monaco, G., Rossi, A., & Tancioni, L. (1987). A study of the histology and morphology of the digestive tract of the sea-bream, *Sparus aurata*. *Journal of Fish Biology, 30*, 135-145.

Clarke, A., & Witcomb, D. (1980). A study of the histology and morphology of the digestive tract of the common eel (Anguilla anguilla). *Journal of Fish Biology, 16*, 159-170.

Dai, X., Shu, M., & Fang, W. (2007). Histological and ultrastructural study of the digestive tract of rice field eel, *Monopterus albus*. *Journal of Applied Ichthyology, 23*, 177-183.

Evans, D., & Claiborne, J. (2006). *The physiology of fishes*. Boca Raton, FL: CRC Taylor and Francis.

Froese, R., and Pauly, D. (2016). FishBase. World Wide Web electronic publication. *Pisodonphis boro.* Retrieved from www.fishbase.org

Gerking, S. D. (1994). *Feeding ecology of fish*. San Diego, CA: Academic Press.

Guedes, A., & Araújo, F. (2008). Trophic resource partitioning among five flatfish species (Actinopterygii, Pleuronectiformes) in a tropical bay in south-eastern Brazil. *Journal of Fish Biology, 72*, 1035-1054.

Hajisamae, S. (2012). *Fish ecology: Theory and application* (1st ed.). Pattani, Thailand: Mitttrapap Printng.

Kaifu, K., Miyazaki, S., Aoyama, J., Kimura, S., & Tsukamoto, K. (2013). Diet of Japanese eels *Anguilla japonica* in the Kojima Bay-Asahi River system, Japan. *Environmental Biology of Fishes, 96*(4), 439-446. doi:10.1007/s10641-012-0027-0

Machjajib, S. (1973). *Grapsid crabs in the Gulf of Thailand* (Master's thesis, Chulalongkorn University, Bangkok, Thailand).

Melo G, R., Stabille, S. R., Britto Mari, R., Pereira, J. N. B., Faglioni, J. R. S., Neto, M., & Hubner, M. (2014). Morphological characteristics of the *Pterodoras granulosus* digestive tube (Valenciennes, 1821) (Osteichthyes, Doradidae). *Acta Zoologica, 95*, 166-175.

Morais, S. (2017). The physiology of taste in fish: Potential implications for feeding stimulation and gut chemical sensing. *Reviews in Fisheries Science and Aquaculture, 25*, 133-149.

Nasruddin, N. S., Azmai, M. N. A., Ismail, A., Saad, M. Z., Daud, H. M., & Zulkifli, S. Z. (2014). Histological features of the gastrointestinal tract of wild Indonesian shortfin eel, *Anguilla bicolor bicolor* (McClelland, 1844), Captured in Peninsular Malaysia. *The Scientific World Journal, 2014*.

Oliveira Ribeiro, C. A. D., & Fanta, E. (2000). Microscopic morphology and histochemistry of the digestive system of a tropical freshwater fish *Trichomycterus brasiliensis* (Lütken)(Siluroidei, Trichomycteridae). *Revista Brasileira de Zoologia, 17*, 953-971.

Paphavasit, N., Siriboon, S., Jaiperm, J., & Mookui, P. (2014). *Sirinath Rajini mangrove ecosystem learning center. . . . from mangrove plantation to mangrove forest enhancing human development* (1st ed.). Bangkok, Thailand: PTT and Department of Science, Chulalongkorn University.

Purushothaman, K., Lau, D., Saju, J. M., Musthaq, S. K. S., Lunny, D. P., Vij, S., & Orban, L. (2016). Morpho-histological characterisation of the alimentary canal of an important food fish, Asian seabass (*Lates calcarifer*). *PeerJ, 4*.

Rainboth, W. J. (1996). *FAO Species identification field guide for fishesry purposes. Fishes of the Cambodian Mekong*. Rome, Italy: Food and Agriculture Organization.

Raji, A., & Norouzi, E. (2010). Histological and histochemical study on the alimentary canal in walking catfish (*Claris batrachus*) and piranha (*Serrasalmus nattereri*). *Iranian Journal of Veterinary Research, 11*, 255-261.

Scocco, P., Menghi, G., & Ceccarelli, P. (1997). Histochemical differentiation of glycoconjugates occurring in the tilapine intestine. *Journal of Fish Biology, 51*, 848-857.

Senarat, S., Kettratad, J., Jiraungoorskul, W., & Kangwanrangsan, N. (2015). Structural classifications in the digestive tract of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1865) from Upper Gulf of Thailand. *Songklanakarin Journal of Science and Technology, 37*, 561-567.

Senarat, S., Yenchum, W., & Poolprasert, P. (2013). Histological study of the intestine of Stoliczkae's barb *Puntius stoliczkanus* (Day, 1871) (Cypriniformes: Cyprinidae). *Kasetsart Journal (Natural Science), 47*, 247-251.

Subramanian, A. (1984). Burrowing behavior and ecology of the crab-eating Indian snake eel *Pisoodonophis boro*. *Environmental Biology of Fishes, 10*, 195-202.

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2013). *Bancroft's Theory and Practice of Histological Techniques* (7th ed.). Ontario, Canada: Elsevier.

Takiue, S., & Akiyoshi, H. (2013). Histological and scanning electron microscopic examination of the digestive tract in Whitespotted conger, *Conger Myriaster* (Anguilliformes). *Journal of Phylogenetics and Evolutionary Biology, 2014*.

Townsend, C. R., & Winfield, I. J. (1985). The application of optimal foraging theory to feeding behaviour in fish *Fish energetics*. Germany: Springer.

Townsend C.R., Winfield I.J. (1985) The application of optimal foraging theory to feeding behaviour in fish. In P. Tytler, P. Calow (Eds.), *Fish Energetics* (pp. 67-98). The Netherlands, Dordrecht: Springer. doi:10.1007/978-94-011-7918-8_3

Wenner, C. A. (1976). Aspects of the biology and morphology of the snake eel, *Pisodonophis cruentifer* (Pisces, Ophichthidae). *Journal of the Fisheries Board of Canada, 33*, 656-665.

Wilson, J. M., Bunte, R. M., & Carty, A. J. (2009). Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science, 48*, 785-789.

Xiong, D., Zhang, L., Yu, H., Xie, C., Kong, Y., Zeng, Y., . . . Liu, Z. (2011). A study of morphology and histology of the alimentary tract of *Glyptosternum maculatum* (Sisoridae, Siluriformes). *Acta Zoologica, 92*, 161-169.

Young, J. W., Guest, M. A., Lansdell, M., Phleger, C. F., & Nichols, P. D. (2010). Discrimination of prey species of juvenile swordfish *Xiphias gladius* (Linnaeus, 1758) using signature fatty acid analyses. *Progress in Oceanography, 86*, 139-151.