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Anatomical Structure And Histology Of Digestion Organ Of Timpakul Fish At Barito Outfall South Kalimantan, Indonesia.

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ABSTRACT

Timpakul fish (*Periathalmodon scholosseri*) with its digestion organs is classified as carnivore. Micro-anatomical structure of carnivore is different from that of herbivore. *P. schlosseri* fish will experience two important adaptations: food adaptation and adaptation to water loss due to evaporation. The purpose of this research is to study anatomy and histology of digestion organs especially esophagus, stomach, and intestine of Timpakul (*P. Schlosseri)*.To determine the location of sampling, purposive sampling with Line Transect method, a sampling technique based on how much is obtained in one time, is used. The research result shows that digestion organ of Timpakul has short anatomical structures which are connected one another with pale yellow color. The intestine of Timpakul is shorter than its body. The histological structure of digestion organ which consists of esophagus, stomach, and *P. schlosseri* intestine has mucosa, sub-mucosa, muscularis, and serosa layers.
Keywords: anatomy, histology, esophagus, stomach, and intestine.

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INTRODUCTION

Barito Outfall is an outfall with Mangrove forest with more than 30 km width along the West coast and 20 km along the river to the inland. Swamp area of Mangrove forest is rich with fauna that can adapt to the wide mud area condition during the rise or fall of sea level. One kind of fauna found in Mangrove swamp at Barito outfall, South Kalimantan, is Gelodok or Timpakul fish (*Periophalmodon schlosseri*) **[1]**. Some countries another kind of the Timpakul as medicine or source of animal protein because its meat contains important nutrition [2,3]. Besides, the fish meat can be used also as medicine [4] .However, the consumption of this fish is limited by the tradition and local belief [5]. Timpakul mucus contains anti-bacteria [3].

Timpakul has particular uniqueness compared to other fish; that is the way it adapts to environment which is very much like amphibian. Although Timpakul is like amphibian, it is classified as Pisces. This fish, however, tends to spend most of its time outside water and are more active beyond water [6] . Timpakul fish can breathe actively when it is on land, known as air breathing. Its capability is related with the morphological and physiological structure of its body organs. It causes differences in morphology and physiology with other aquatic fish [7].

The rise and fall of sea water level forces living beings to adapt physiologically and anatomically to their environment [8]. Timpakul can last in intertidal area which will affect on how its food hunting behavior and anatomical structure. Timpakul is a predator fish because it has sharp teeth. During high tide, Timpakul climbs up tree root to eat insects around it; during low tide, the fish 'walks' around on the mud to eat small animals like shrimp, crab, Manila clams (*Ucap sp),* and small fish or even small Timpakul fish from another kind; this is why Timpakul is well-known as carnivore [9] .

Micro-anatomical structure of carnivore is different from that of herbivore. *P.schlosseri* experiences two important adaptation: adaptation to food kind and loss of water due to evaporation. Adaptation on the upper skin due to the loss of water is indicated by extracting mucus to keep the body moist and by having dense scales. Alimentary canal also adapts to the loss of water. This is because alimentary canal is one of organs that go through some change. This adaptation is most possibly different from that of true carnivore that live in water, particularly adaptation around alimentary canal close to mouth, such as esophagus, stomach, and intestine.

The uniqueness of this digestion organ is interesting to observe further especially viewed from anatomical and histological aspects in order to explain the function of digestion organs that adapt to the environment. Deep study related to biological aspect, especially digestion organ of Timpakul has never been reported before. Therefore, it is important to conduct study on anatomical and histological structures of Timpakul digestion organs especially esophagus, stomach, and intestine around Barito river, South Kalimantan. Indonesia

MATERIALS AND METHODS

Chemicals

The materials used in this study are Formalin,, alcohol, xilol, a solution of hematoxylin and eosin for dyeing histological preparations

Procedure

The research procedure of microscopic esophagus, stomach, intestine of *P. Schlossreri* is conducted through the following steps:

Experimental (Sampling Location Determination)

Sampling Location Determination is done through purposive sampling under consideration that the Timpakul (*P. Schloseri)* samples spread are not homogeneous. Sampling is done at tide area of Bahagia river bank, Tanipah villange, Tabunganen district, Barito Kuala regency, South Kalimantan. Indonesia. The *P.*

Schloseri sampling is done using Line Transect method, a sample taken based on how much is obtained in one time.

Making Alimentary Canal Preparate

P.Schlosseri was anesthetized and its weight and length are measured then it is torn. The alimentary canal was ripped off then the anatomical and histological organs were measured. The histological digestion organ was made using paraffin method.

RESULTS AND DISCUSSION

Morphological and Anatomical Images of Timpakul *P. Schlosseri*

Five *P. Schlosseri* with morphology displayed in Figure 1 were caught successfully. From the weight measurement *of P.Schlosseri*, average weight 179,6 gram is obtained with average body length 27,7 cm (Table 1). *P. schlosseri* is a carnivore fish. The carnivore attributes are indicated by the mouth and the teeth. The width of the mouth is 2,94±0,13 cm and the height of the mouth is 3,5 ±0,35 cm, the average volume of the mouth is 10,6 ± 1,34 ml (Figure 2). The upper jaw of the fish has two teeth line with the 28 front teeth and 30 back teeth. The lower jaw of the fish has only one line consisting of 28 teeth. *P. schlosseri* has one tongue that sticks on the bae of the mouth with average length 3±0 cm and average width 1±0 cm (Table 1).

Table 1. The average score of measurement on morphology and anatomy of alimentary canal *P. Schlosseri*

No	Measurement parameter	Average score
1	Fish weight (g)	179,6 ±11,4
2	Fish length (cm)	27,7 ±0,96
3	Teeth total number	
	Upper teeth	
	- Front upper teeth	28
	- Back upper teeth	30
	Lower teeth	28
4	Mouth volume (mL)	10,6 ±1,34
5	Tongue length (cm)	3±0
6	Tongue width (cm)	1± 0
7	Mouth width (cm)	2,94 ±0,13
8	Mouth height (cm)	3,5 ±0,35
9	Esophagus length (cm)	2,16+0,23
10	Esophagus weight (g)	0,75±0,13
11	Stomach length (cm)	2,68±0,33
12	Stomach weight (g)	1,01±0,1
14	Intestine length (cm)	6,6±0,38
	Intestine weight (g)	1,28±0,23

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Figure 1.Timpakul fish seen from above (A); Timpakul fish seen from the left side (B).

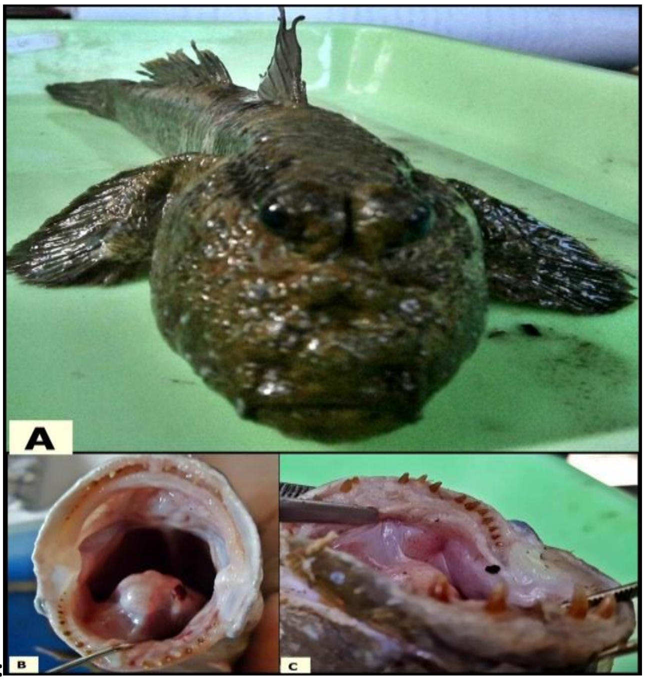


Figure 2. Front part of Timpakul head (A); Mouth wide open (B); Teeth (C)

Timpakul that live around sea water tide area has unique behavior and way of living that are different from common fish that live in brackish water; therefore, they can adapt in anatomical physiology and behavior [5]. Timpakul fish is a carnivore; that is why morphologically, the teeth of Timpakul, upper teeth and lower teeth, are sharp-pointed. The teeth kind owned by Timpakul is sharp pointed (*Canine*). The measurement result shows that Timpakul's jaw has two lines: 28 front teeth and 30 back teeth (Table 1) and the low jaw has 28 teeth (Table 1). Timpakul has different teeth from that of other fish; Timpakul's teeth are close to lip. Timpakul has special teeth which are sharp-pointed (both upper and lower teeth) that enable it to eat many kinds of food, such as crab, Manila clams *(Ucap Sp*), even small fish [10].

Timpakul's food kinds affects its mouth morphology, such as its tongue, which is morphologically different from that of other fish. Timpakul's fish is small and ellipse; the tip of the tongue is round and the color of the tongue located in the basic of the mouth is white and static—cannot move freely. Timpakul tongue is approximately 3±0 cm, as it is shown on Table 1. To measure the volume of Timpakul mouth, the researcher put water inside Timpakul mouth using *sekuit* and obtained that the volume of the mouth is approximately 10,6±1,34 mL.

Timpakul habitat is around sea water tide area which makes it have unique behavior and way of living that are different from common fish that live in brackish water in order to adapt in anatomical physiology and behavior [5]. Timpakul is one of *amphibians Gobi* which can adapts to distinct salinity levels and become the main model of fish that acts like amphibians. Timpakul often leave water (nest) and 'climb up' to the mud to hunt [11]. In this research, *mudskipper* kind being observed is *P. schlosseri.*

Generally total length and weight ratio of fish affect stomach weight ratio [12]. The length of fish stomach is in accordance with its body length, regardless the fish is herbivore, carnivore and omnivore [13]. In this research, Timpakul observed has average length 27,7 ±0,96 cm and average weight 179,6 ±11 gram.

Timpakul during high tide will climb up tree root and during low tide will walk on the mud. During high tide, it will climb up tree root to eat aquatic insects around it and during low tide, it will walk around the mud to eat small animals such as shimps *(Crustacea)* crab, Manila clams *(Ucap Sp*), small fish, or even Timpakul fish from other kinds; that is why Timpakul is also well known as carnivore fish [9] .

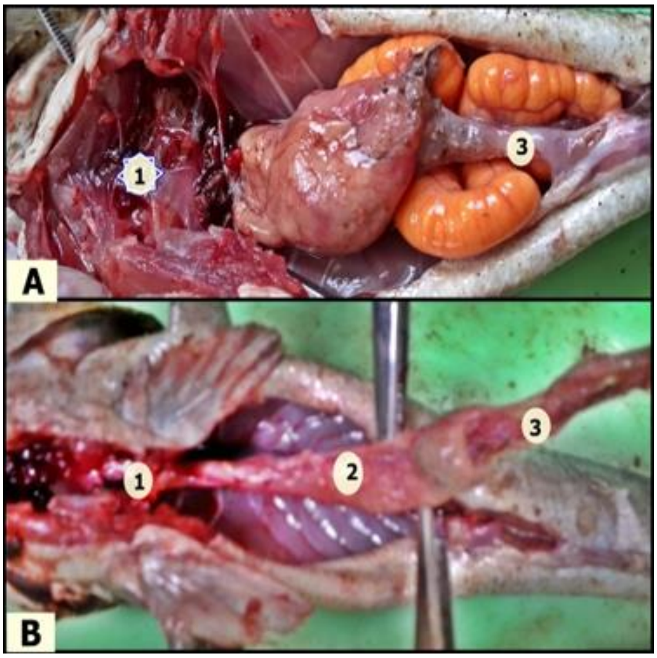
Histology of Alimentary Canal of *P. schlosseri*

Histological structure of alimentary canal of Timpakul *P. Schlosseri* around esophagus, stomach, and intestine has four layers: mucosa, submucosa, muscularis and serosa. Histological measurement of each layer is shown in the table 2.

Based on the observation on organ position, it is obtained that esophagus organ is located behind the gill, right in front of the heart. The stomach is located below the liver, connected to intestine with pale yellow color. The intestine is located next to stomach, with medium length and pale yellow color and the alimentary canal length is 12 cm (Figure 3)

Table 2. Measurement on alimentary canal histology of *P. schlosseri*

No	Parameter	Esophgus,	Stomach,	Intestine
1	Mucosa(µm)	1480,7±369,6	1326,6±1801	273,4±121,2
2	Submucosa(µm)	1001,3±174,1	938,8±897,7	681,6±129,6
3	Muscularis (µm)	1744±367,8	1784,6±614,1	1075,8±427,6
4	Serosa(µm)	1972,3±1341,8	534,9±157,4	784,2±33,6



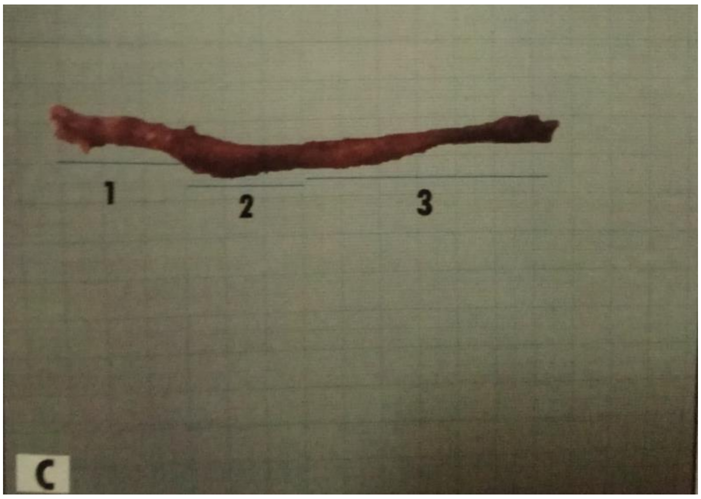


Figure 3. A. Organ anatomy of Timpakul. B. Anatomy of 1 esophagus, 2 stomach and 3 intestine of *P. Schlosseri C. Organ measurement : 1. esophagus, 2 stomach and 3 intestine*

Structure Observation of Microscopic Esophagus, Stomach, and Intestine of Timpakul (*P. schlosseri)*.

Esophagus of *P. schlosseri*

Observatio nresult on esophagus preparat of *P. schlosseri* shows that esophagus histological structure has four layers: mucosa (M), submucosa (SM), muscularis (MU), and serosa (S) (Figure 4). The first layer is mucosa layer (M) with average thickness 1480,7±369,6µm, and has simple columnar epithelium layer. The mucosa structure has many indentations that walk to lamina propia (LP). This mucosa has many epithelial cells, which have mucus cells that spread all over the mucosa. The second layer of histological structure of esophagus consists of submucosa layer (SM) with average thickness 1001,3±174,1µm. Submucosa layer contains connective tissue and elastin fibers.The third layer of histological structure of Timpakul esophagus, named muscularis (MU) with average thickness 1744±367,8µm. Muscularis layers of Timpakul fish contains transverse smooth muscle. The fourth histological structure of Timpakul is serosa (S) layer with average thickness 1972,3±1341,8µm. The serosa layer of Timpakul has thicker layer becaue serosa is a connective tissue that easily moves peristaltically in esophagus so that solid food can be swallowed (Figure 4).

Table.3 Tissue components of alimentary canal of *P. schlosseri*

No.	Compiler Components	Esophagus	Stomach	Intestine
1.	Mucosa			
	- Epitel kolumnar sebais	+	+	+
	- Epitel squamous	+	-	+
	- capillary esophageal	+	-	-
	- capillary gastrikal	-	+	-
	- Goblet cell	-	-	++
	- Vili	-	-	++
2.	Submucosa			
	- Connective tissue	+	+	+
	- Blood capillary	+	+	+
3.	Muscularis			
	- Circular Smooth Muscle	+	+	+
4.	Serosa			
	- Loose Connective Tissue	+	+	+

Information : + : Found ; ++ : Found many ; - : Nothing found

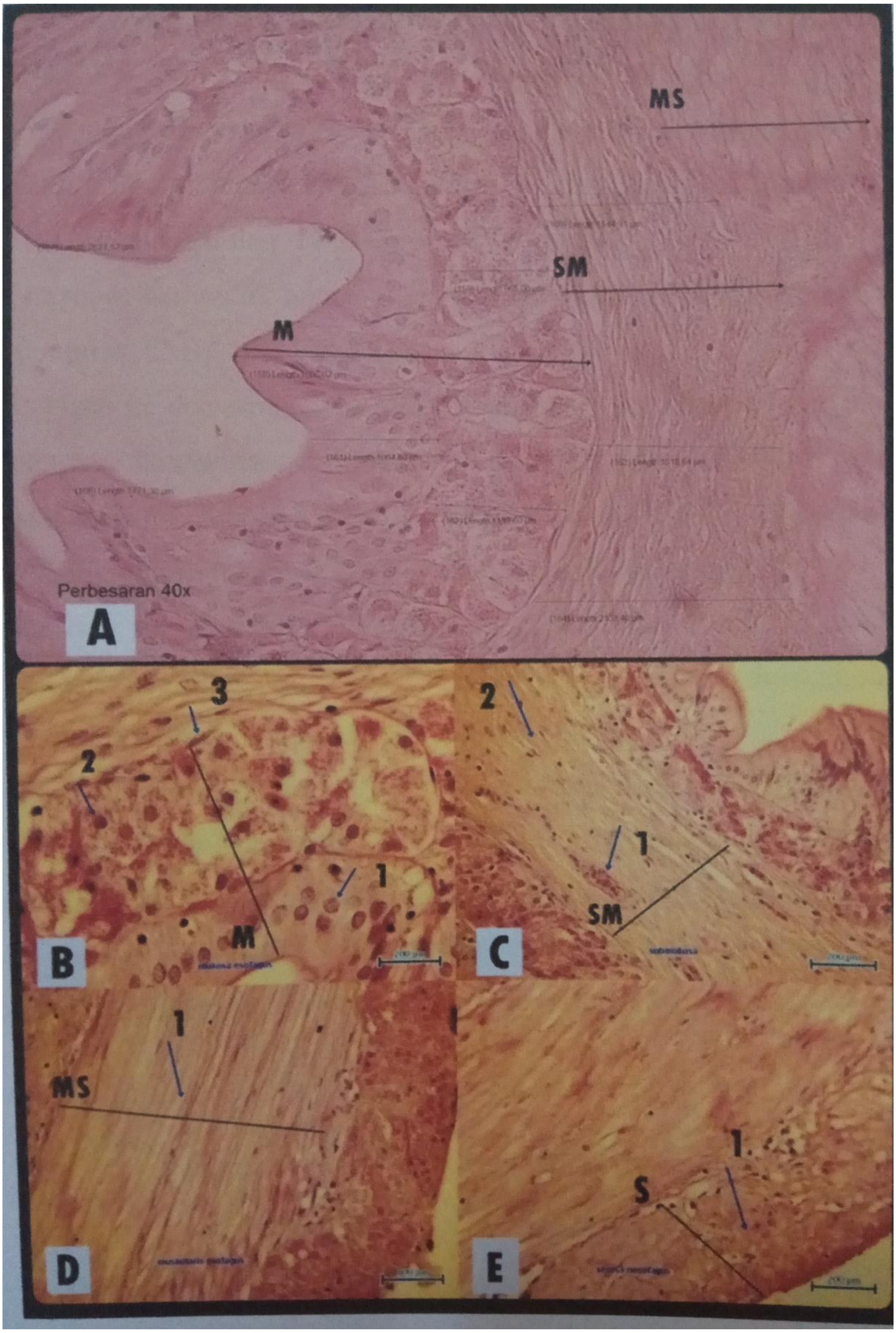


Figure 4. Histological image of esophagus of Timpakul with Haematoxylin Eosin coloring. A. Histology of Esophagus with 10 times magnification. B. Mucosa with 100X magnification. 1. Eitel columnar, 2. Esophageal gland, 3. Epitel squamosa. C. Submucosa with 40X magnification, 1. Blood capillary, 2. Connective tissue. D. Muscularis with 40X magnification, 1. Circular Smooth Muscle. E. Serosa with 40X magnification.

Esophagus of fish has shape like pipe that is suitable with its body shape. Generally, esophagus has four network layers: mucosa, submucosa, mucosa-muscularis, and serosa [14]. Mucosa layer has epithelial that consist of several layers, which are *mukosit* that contains mucus as food lubricant that moves from mouth to the stomach without destroying the esophagus wall [15].

In carnivore esophagus, esophagus epithelium contains mucus which is an important component in osmoregulation process [16]. In the stomach carnivore, there are mucus cells, goblet cells, and pencil cell that are different from that of herbivore. This difference is caused by distinct food kinds [17,18].

Esophagus has mucus cell, which is an important component of osmoregulation process [16]. Mucus layer in sea fish is highly necessary in the process of water absorption at esophagus [19]. Epithelium cell of esophagus, especially mucus cell will adapt to watering condition, in both salinity or chemistry substances. Esophagus layer of fish plays a role in salt absorption through passive dilution. When it is within high water salinity, the amount of mucus cell in fish esophagus is more than the mucus cell in esophagus of fish living in water with low salinity [14]. Epithelium layer of esophagus of fish living at sea is thicker because it absorbed much salt of the sea water that was accidentally drunk; as a consequence, the salt concentration in the stomach decreases.

The unique way of living of Timpakul affects esophagus structure which is unique seen from the color, shape, and size (Figure 2). A research on tuna esophagus (*Thunnus thynnus L*) shows that mucosa layer is overlaid by epithelia [20]. Simple columnar epithelia are owned by esophagus surrounds all mucosa. Based on microscopic research on esophagus, Timpakul fish contains cell structure of mucosa, submucosa, muscularis, and serosa. In this part, there are a lot of simple columnar epithelia that exists in each cell yet the mucosa layer contains the most epithelia to form enzymes and to help digestion process as well as maintain the cell at esophagus.

Histological result of Timpakul esophagus measurement shows that serosa cell has average thickness 1972,3±1341,8µm. This is because serosa cell is a connective tissue that surrounds esophagus. Around esophagus, serosa is thicker, which creates assumption that Timpakul does not chew very well its food. Submucosa of Timpakul is thinner with average thickness 1001,3±174,1µm; this is because submucosa functions as solid connective tissue and elastine fiber (Table 2).

Stomach of *P. schlosseri*

Based on the observation, the stomach histology of Timpakul *P. schlosseri* contains four mucosa layers: mucosa (M), submucosa (SM), muscularis (MU), and serosa *(S)* (Figure 5). The first layer is *mucosa* (M) layer with thickness 1326,6±1801µm. Mucosa has thin indentation aiming to lamina propria. In this layer, there are many simple columnar epithelial that spread on mucosa layer. The second layer of histological structure of Timpakul stomach contains submucosa SM) with different layer and thickness about 938,8±897,7µm. In this submucosa, there are simple columnar epithelial and blood capillary, less than those that are found in esophagus.

The third layer of stomach histology of Timpakul contains muscularis (MU) with average weight 1784,6±614,1µm. In this layer, there is blood capillary called circular smooth muscle. The fourth layer of stomach histology of Timpakul contains serosa (S) with thickness 534,9±157,4µm. The serosa of stomach is thinner than that of esophagus (Figure 5).

Generally, intestine is where the last phase digestion happens with the aid of enzyme of intestine and pancreas and gall of heart. Intestine has mucus that forms villi to ease the absorption process, and has three kinds of cell in digestion epithelium of absorbent cell, goblet cell, and Argentaffin cell. Intestine has complete layers [21]. Generally, intestine contains four layers: mucosa, submucosa, muscularis, and serosa [14] .

Histological structure of Timpakul stomach as average thickness 1784,6±614,1µm because mucosa muscularis in the stomach has stripped muscle layer that transverses regularly and connects to other part of alimentary canal to directly ease digestion process. This smooth muscle cell works unconsciously; therefore, it absorbs a lot of water and food; the food structure of Timpakul are mostly solid. Based on the histological measurement, it is found that serosa has thin wall for about 534,9±157,4µm. This is because serosa functions as a connective tissue at stomach outer cell layer,

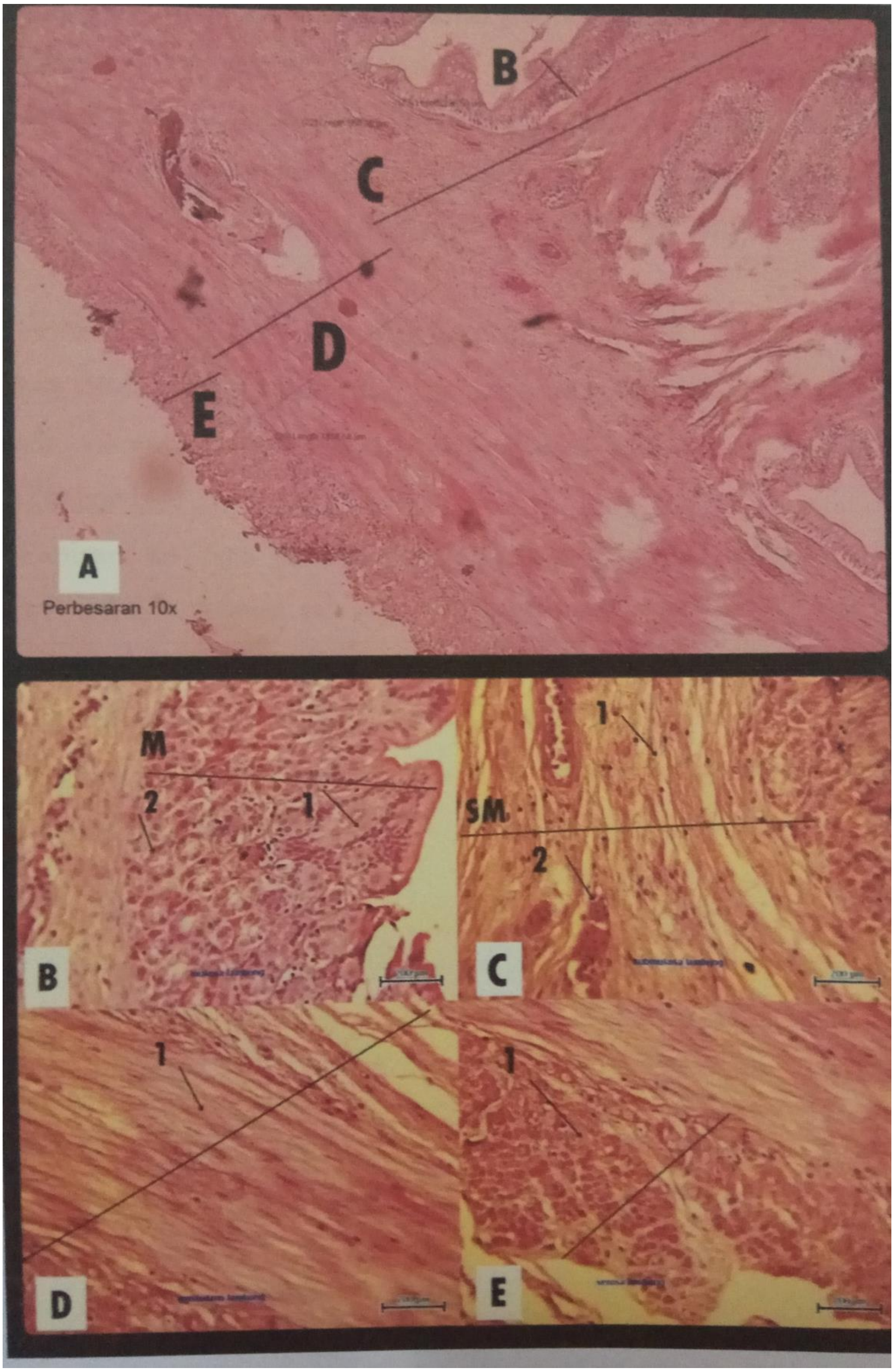


Figure 5. Stomach histology of *P. Schlosseri* with Haematoxylin Eosin coloring. A. Stomach histology. A histology of Timpakul stomach with 10 x magnifications. A. Histology of stomach with 10 X magnification. B. Mucosa with 40X magnification. 1. Eitel columnar, 2. Gastrical gland. C. Submucosa with 40X magnification, 1. Connective tissue 2.Blood capillary, D. Muscularis with 40X magnification, 1. Circular Smooth Muscle. E. Serosa with 40X magnification.

Intestine of *P. schlosseri*

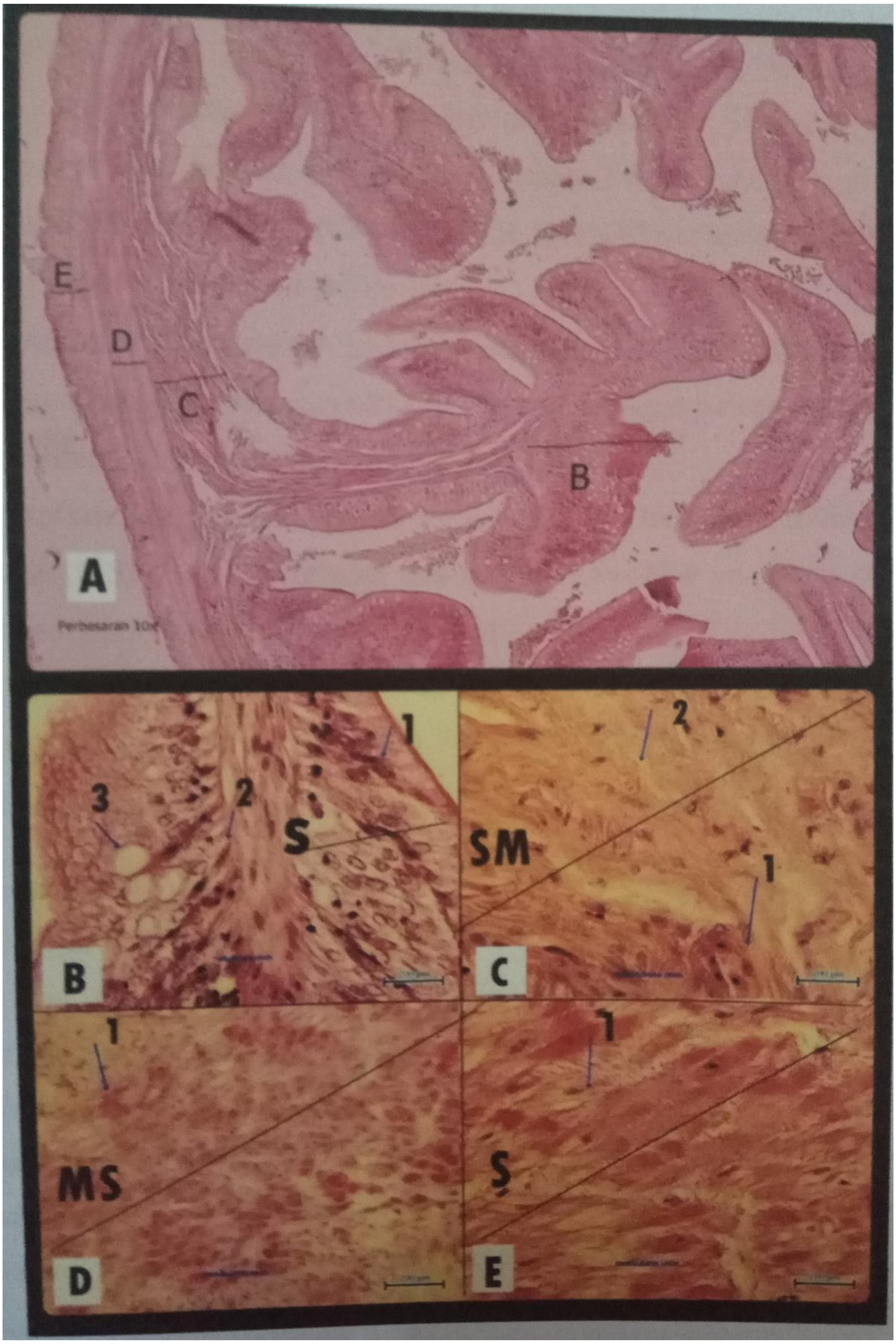


Figure6. Histological figure of Timpakul intestine with Haematoxylin Eosin coloring. A histology of Timpakul intestine with 10 x magnifications. A. Histology of intestine with 10X magnification. B. Mucosa with 100X magnification. 1. Eitel columnar, 2. Epitel squamosa, 3. Goblet cel. C. Submucosa with 100X magnification, 1. Connective tissue 2.Blood capillary, D. Muscularis with 100X magnification, 1. Circular Smooth Muscle. E. Serosa with 100X magnification.

Based on the observation, histology of intestine preparate of Timpakul *P. schlosseri* has four layers: mucosa (M), submucosa (SM), muscularis (MU), serosa (S) (Figure 6). The first layer of histological structure contains mucosa (M) with thickness 273,4±121,2µm. Mucosa structure is in irregular form, thick, and has irregular indentations (some of them are long and the others are short). The inside mucosa aims towards lamina propria (LP). The first layer of histological intestine of Timpakul fish contains submucosa (SM) with average thickness 681,6±129,6 µm. This layer has many circular smooth muscles and a little blood capillaries. The third layer of histological structure of Timpakul intestine contains muscularis (MU) layer with average thickness 1075,8±427,6µm. In this layer, there are circular smooth muscles that has very clear yet thin form where stomach esophagus is located. The fourth layer of histological structure of Timpakul intestine has serosa (S) layer with average thickness 784,2±33,6µm. The serosa layer is thin around Timpakul intestine and has a little circular smooth muscles (Figure 6).

Carnivore intestine is smaller than that of herbivore yet the role is the same with that of herbivore. The carnivore intestine is shorter than that of herbivore with food absorption inside it is helped by mucosa cell and goblet cell for water absorption, ion absorption, and protein absorption [22].

Mucosa muscularis of Puffer fish (*Tetraodon Lunaris*) is the thickest in the middle intenstine and the thinnest in the back intestine[12]. This is because the middle intestine can hold food longer so that the food can be processes and absorbed longer. The research on the histological structure of Timpakul intestine also shows that mucosa muscularis cell is thick for about 1075,8±427,6µm. This is because intestine functinos as food holder so the food can be processed longer.

The histological structure of Timpakul in intestine cell which has thin cell is serosa, which is the same with cell layer in the stomach, which is about 784,2±33,6µm. Serosa in the intestine does not play significant role because it is a connective tissue that surrounds the intestine.

CONCLUSION

Based on the research conducted, it is concluded that:

- Anatomically, digestion organs of Timpakul *(P. Schlosseri)* is the same with common carnivore fish.
- Histological structure of esophagus, stomach, and intestine of *P. schlosseri* contains mucosa, submucosa, muscularis, and serosa layers.

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