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Comparative Analysis of Morphometric Characteristics and Mucous Cell Distribution between *Pangasius hypophthalmus* and *Clarias batrachus*

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ABSTRACT

The morphology characteristics and the mucous cell distribution in two species of commercial catfish (*Pangasius hypophthalmus* and *Clarias batrachus*) were investigated in the present study. To study morphological characteristics, the parameters - such as total length (TL), standard length (SL), body depth (BD), pre-dorsal lengh (PDL), pre-pectoral length (PPCL), pre-pelvic length (PPVL), pre-anal length (PAL), depth of caudal peduncle (DCP), length of caudal peduncle (LCP), head length (HL), eye diameter (ED), caudal fin length (CFL), pectoral fin length (PFL), and length of dorsal fin base (LAFB) were measured. The mucous cell distributions of the dorsal and ventral parts in the head, abdomen, and GI tract were examined using periodic acid Schiff (PAS) and hematoxylin and eosin (H&E) staining dyes. In *P. hypophthalmus*, the tail region had the highest mucous cell count, followed by the abdomen and the head, whereas in *C. batrachus,* the pattern was the opposite. In conclusion, *P. hypophthalmus* was longer than *C. batrachus* in most morphological characteristics. The dorsal region had more mucous cells in both species than the ventral region. Overall, *C. batrachus* was found to have more mucous cell distribution in both ventral and dorsal parts compared to *P. hypophthalmus*, suggesting that *C. batrachus* has a better innate immune system than *P. hypophthalmus.* The sizes of blood cells were also found to be different in both species.

INTRODUCTION

Pangasius hypophthalmus, commonly known as striped catfish, belongs to Animalia (Kingdom), Chordata (Phylum), Actinoptergyii (Class), Siluriformes (Order) and Pangasiidae (Family) and *Pangasius hypophthalmus* (Species) **(Vidthayanon and Hogan, 2013).** This species is native to Cambodia, Lao People's Democratic Republic, Thailand, and Viet Nam. It has features of a long and latterly flattened body without scales, a relatively small head, broad mouth with tiny sharp teeth on the jaw vornerine, and palatal bones. They have large eyes with two pairs of barbels (the upper one is shorter than the lower one) with a dark grey or black fin, and six branched dorsal-fin rays The immature fish has a black strip coated along the later line and another long back stripe under the lateral line, while the adult fish is often characterized by a grey or greenish tint and silvery sides **(Griffiths *et al.,* 2014).** They are omnivores and mainly consume algae, plants, zooplankton, insects, fruits, crustaceans, agricultural by-products like rice bran, soy) and fish **(Rahman *et al.,* 2006; Griffiths *et al*., 2014).** Traditional captured based aquaculture is widely used in Vietnam and also to a lesser extent in Thailand because this species is very fertile, which can produce a vast number of larvae into the flowing river **(Tadpitchayangkoon and Yongsawatdigul, 2009).**

Clarias batrachus, commonly known as walking catfish, belongs to Animalia (Kingdom), Chordata (Phylum), Actinopterygii (Class), Siluriformes (Order), Clariide Bonaparter (Family) and *Clarais batrachus* (Species) **(Ng, 2010; ITIS, 2014).** This species is native to Southeast Asia, and is distributed throughout Bangladesh, India, Indonesia, Malaysia, Myanmar, Pakistan, Philippines, Singapore, Sri Lanka, and Thailand **(Talwar and Jhingram, 1992; ITIS, 2014).** It is characterized by its broad, flat head with an elongated body. The recognizable features are four paired of well-developed barbels with fleshy, papillated lips and its lack of adipose fin. The dorsal fin is extended throughout its back (around two-third of the length of the body), but they lack a dorsal spine. A near-continuous margin is formed by the dorsal, caudal, and anal fins. It has large pectoral spines serrated along the margins **(Ng, 2010).** The body color can be of a wide range of varieties: olive to dark brown or purple to black, blue-green on both sides with whitish underside and rear side coated with white specks **(Ng, 2010).** They are omnivores, benthic, nocturnal, and opportunistic feeder that eats a wide variety of things available in its environments such as aquatic insects, crustaceans, plant materials, tadpoles, and small fish. Their inhabitants include river systems, lowlands fresh and brackish waters like rivers, lakes ponds and reservoirs **(Brogan, 2003).**

The fish immune system comprises both systemic and mucosal immune system. As the majority of the infectious agents start the process of infection in the mucous surface, in the fish mucous immune system is the first-line defense against the encounter of pathogens **(Esteban, 2012).** Immune molecules in fish mucus include lysozyme, immunoglobulins, complement, lectins, agglutinin, calmodulin, interferon, C-reactive protein, proteolytic enzymes, antimicrobial peptides, or vitellogenin. Though the fish skin mucus has been known as a biological barrier because of its ability to produce several substances involved in preventing infection. However, the information of the epidermal mucus of fishes as the defense mechanism is still very limited **(Guardiola *et al*., 2014).**The external mucous gel makes a layer of adherent mucus that covers the living epithelial cells, which is secreted by epidermal goblet cells. The main components of skin mucus are water and glycoproteins containing mucin. Mucin is a mechanical barrier that acts as filters for pathogens and prevents the pathogen to attach to the underlying tissues **(Esteban, 2012).**

There are two different classes of mucin such as membrane-bound mucin and secreted mucin. Membrane-bound mucin is located on the plasma membrane, where it functions as cellular adhesion, pathogen binding, and signal transduction **(Curran and Cohn, 2010).** It has the transmembrane and cystolic domain. Secreted mucin is made into epithelial cells and stored in the intracellular secretory granules **(Roussel and Delmotte, 2004).** The cutaneous mucous layer is the mucosal surfaces of fishes (in gill, skin, and gastrointestinal tract), forming a physical barrier between the environment and the internal parts of the fish. Protective mucous layer overlay covers the host defense mechanisms and their epithelia **(Kumari *et al.*, 2009).** Cutaneous mucus serves as the first-line defense mechanism against infection *via* skin epidermis. Skin mucus is designed to trap and immobilize pathogens before they travel to epithelial surfaces as it is impairment to the majority of bacteria and numerous pathogens **(Guardiola *et al*., 2014).**

The past study on three salmonids to examine the skin and gill mucus in response to amoebic gill disease (AGD) suggested that Atlantic salmon and brown trout, (*Salmon trutta*) had a whole-body mucous response to AGD. However, there is a limited understanding of how fish can regulate mucin gene expression, mucus glycosylation patterns, and mucus composition in responding pathogens **(Gomez *et al*., 2013).** Moreover, mucus is constantly secreted and replaced in most fishes, preventing the invasion of infectious microorganisms and metazoan parasites. The mucous layer can be cast-off or digested. Therefore, pathogens have to move upstream *via* the unstirred mucous layers, which attach to the cells on the epithelium surface or penetrate a mucus "blanket" before it is being cast off **(Kaetzel, 2005).**

P. hypophthalmus and *C. batrachus* lack scales. Unlike fish with scales, they mainly rely on both external and internal mucous production to protect themselves against the infectious agents and the toxins produced by the predators. Regarding the anatomical locations, mucous cells can be found in the mucosa-associated lymphoid tissue, gut-, skin-, and gill-associated lymphoid tissue, and mostly found in the gut in the previous studies **(Uribe *et al*., 2011).**

P. hypophthalmus and *C. batrachus* are important sources of the commodity that have high export value and are in global demand triggering the expansion of aquaculture **(Srivastava *et al.*, 2012; Globefish, 2016).** Reportedly, *P. hypophthalmus* alone has an estimate export value of USD 1.6 billion **(Globefish, 2016).** Aquaculture, considered as one of the main food-producing industries in the world, contributes to the intensification of aquaculture, increasing the risk of disease outbreak, and stressful conditions that impacts its productivity and sustainability of fish farming **(Kennedy *et al.*, 2015).** This, in turn, incentivizes the excessive use of antibiotics and chemotherapeutics to control the disease outbreak. Fish treated with antimicrobial agents cause the occurrence of antibiotic resistance **(Akinbowale *et al*., 2006).** The resistance genes carried by the bacteria is harmful to human health. Thus, modern aquaculture has a massive interest in the fish immune system because of the need to improve health management strategy in supporting the fish aquaculture industry **(Balcázar *et al*., 2006).** More understanding in the mucosal immune system can enhance their natural innate immune system and facilitate the formulation of new vaccination techniques in fish if needed.

The main aims of this study are to investigate the morphological characteristics of *P. hypophthalmus* and *C. batrachus* and to analyze the distribution of mucous cells across their internal body regions to lay the foundation of a better understanding of the mucus system as the innate immune system in these two species of catfish.

MATERIALS AND METHODS

Catfish Sampling

Catfish (*P. hypophthalmus* and *C. batrachus*, n=10 each) were purchased from the Thai commercial market. Both fishes were transferred to the Department of

Pathobiology, Faculty of Science, Mahidol University, Bangkok, Thailand for acclimatization. They were fed with commercial fish food amount 2% of their body weight per day. Before the experiment, all the fishes were acclimatized under the laboratory conditions for at least 7 days. They were fed twice a day with 38%-protein commercial fish food. The amount of food was 2% of their initial body weight per day. They were monitored carefully and detected to see whether they had any signs of diseases, stress, physical damage, and mortality. Any abnormal or dead fish was removed immediately **(APHA, 2005).** The entire stock was also removed if their mortality rate was more than 10%; 16 hours of light and 8 hours of darkness photoperiod were also maintained. This experiment was conducted before the guideline of the aquatic animal ethics was imposed.

Morphometric Characteristics Measurement

The morphometric characteristics, as shown in Table 1, were measured on all fishes using the measurement from 15 landmarks according to **Kosai *et al.* (2014).**

Table 1: Definitions of Morphometric Measurements.

Character	Acronym	Description
Total length	TL	Tip of the snout to the end of tail
Standard length	SL	Tip of the snout to the tail base
Body depth	BD	Maximum depth measured from the base of the dorsal spine
Pre-dorsal length	PDL	Front of the upper lip to the origin of the dorsal fin
Pre-pectoral length	PPCL	Front of the upper lip to the origin of the pectoral fin
Pre-pelvic length	PPVL	Front of the upper lip to the origin of the pelvic fin
Pre-anal length	PAL	Front of the upper lip to the origin of the anal fin
Depth of caudal peduncle	DCP	The least depth of the tail base
Length of caudal peduncle	LCP	From base of the last anal fin ray to middle of caudal fin fold
Head length	HL	Front of the upper lip to the posterior end of the opercula membrane
Eye diameter	ED	The greatest bony diameter of the orbit
Caudal fin length	CFL	From tail base to tip of the caudal fin
Pectoral fin length	PFL	From base to tip of the pectoral fin
Length of dorsal fin base	LDFB	From base of first dorsal spine to base of last dorsal ray
Length of anal fin base	LAFB	From base of first anal spine to base of last anal ray

Blood Collection

Using the blood collection method of **Bain *et al* (2012),** peripheral blood was withdrawn from the mid-ventral line behind the anal fin by a syringe needle. The needle was covered with heparin to prevent blood clotting inside the syringe. The needle was inserted into the musculature until it reached the spinal column. It was required to maintain a constant vacuum inside the syringe and slowly take out the needle until the blood went into the syringe. The needle was carefully removed from the fish and then removed the needle tip from the syringe to empty the blood into an Eppendorf tube. The collected blood samples from both types of fish were used to make blood smear slides. A dropper was used to place a small drop of blood onto a clean and grease-free slide. The slide was placed on a flat surface with a drop of blood facing up. A second slide was held while the edge was placed at a 45-degree angle against the first slide. The second slide was pushed lightly down until it touched the drop of blood. The blood was spread itself along the edge of the slide in a formed angle. Then, the second slide was pushed against the first slides, which draw the blood across the surface. This was performed with constant speed and without wobbling the slide to obtain a nice smear.

The blood smear was placed on a staining rack. It was fixed using alcohol from Wright Instant Stain Set for 1 min. The slides were also immediately stained in the eosin-based and then the Giemsa-based dyes of the Wright Instant Stain Set for one more minute. The slides were washed two times using tap water to rinse the excess stain. It was then allowed to air dry and was examined under a light microscope and photographed by a digital camera. A good smear is the one that is thin with uniform distribution.

Mucous Cell Collection

Small pieces of each part were cut from all fish for mucous cell analysis. These regions were such as tentacle, intestine, tail, and skin with muscle from head dorsal and ventral parts, abdomen dorsal and ventral parts.

Histopathological Analysis

Using the modification of **Humason (1979),** the histological technique was performed. The formalin-fixing organs were washed in water and then dehydrated with a range of alcohol concentration: 70, 80, 95, and 100%, respectively. This was done before it was embedded in paraffin. The paraffin blocks were of 5 µm thickness and stained with hematoxylin and eosin (H&E) dyes and periodic acid schiff (PAS) staining. It was then observed under the light microscope. The picture of five different regions of each section stained with PAS and H&E in the head (including tentacles), the abdomen, the tail, and the gastrointestinal tract were taken from the microscope to count the mucous distribution on ventral and dorsal parts of both species.

RESULTS

Body weight

P. hypophthalmus had a heavier average weight of 11.72 g than *C. batrachus,* which had an average weight of 32.20 g. Among five *P. hypophthalmus*, the heaviest weight was 15.09 g, and the lightest was 9.31 g, whereas in *C. batrachus* the heaviest weight was only around 3.31 g, and the lightest was 3.00 g.

Table 2: Morphometric characteristics of *Pangasius hypophthalmus* and *Clarias batrachus*.

Character	*Pangasius hypophthalmus*		*Clarias batrachus*	
	Mean (cm)	TL (%)	Mean (cm)	TL (%)
TL	12.66	100.00	7.92	100.00
SL	10.36	81.83	7.14	90.15
BD	2.10	16.59	1.22	15.40
PDL	4.70	37.12	2.80	35.35
PPCL	2.80	22.12	1.56	19.70
PPVL	4.88	38.55	3.20	40.40
PAL	6.20	48.97	3.84	48.48
DCP	0.64	5.06	1.12	14.14
LCP	1.40	11.06	0.26	3.28
HL	1.80	14.22	1.46	18.43
ED	0.50	3.95	0.10	1.26
CFL	2.12	16.75	0.96	12.12
PFL	1.58	12.48	0.64	8.08
LDFB	0.66	5.21	4.30	54.29
LAFB	3.00	23.70	3.44	43.43

Morphometric characteristics

P. hypophthalmus had almost twice longer in total length (TL) than *C. batrachus* (Table 2). Moreover, the average characteristics of the other parts such as SL, BD, PDL, PPCL, PPVL, PAL LCP, HL, ED, CFL, and PFL were lengthier in *P. hypophthalmus* than in *C. batrachus*; expect for DCP, LDFB and LAFB.

There was a significant difference in the three characters between them. In *P. hypophthalmus*, it was 11.06% LCP in relative to TL, whereas in *C. batrachus*, it was only 3.28% LCP. The differences were due to the LDFB of *C. batrachus*, which was 54.29%, and the LAFB in relative to TL was 43.43%. However, LDFB and LAFB in *P. hypophthalmus* were 5.21% and 23.70% respectively.

The dorsal section with PAS staining

According to PAS staining, the highest number of mucous cells in the dorsal part of *P. hypophthalmus* was found in the tail (7.2), followed by the head (tentacles) (2.8), the GI tract (2.2), and the head (1.4) respectively. The lowest mucous cell count was seen in the abdomen (6.0). However, the reverse pattern was found in *C. batrachus*, where the highest number of mucous cells was found in the head (68.6), and the least was in the tail (1.8). The remaining ones ranked in this order: the GI tract (28.6), the abdomen (5.0), and the head (tentacles) (2.8) as shown in Table 3.

Overall, there was a significant difference in the average mucous cell distribution in the head region and the GI tract between these species, where *C. batrachus* much higher mucous cell count than *P. hypophthalmus*. However, the mucus production was slightly lower in the abdomen and the tail of *C. batrachus* than in *P. hypophthalmus*. The same amount of mucous cell count was found in the head (tentacles) in both species.

Table 3: Mucous cell distribution in the dorsal part of *Pangasius hypophthalmus* and *Clarias batrachus* with PAS staining.

	Average number of mucous cell (cells)				
	Head	**Tentacle**	**Abdomen**	**Tail**	**GI tract**
Pangasius hypophthalmus	1.4	2.8	6.0	7.2	2.2
Clarias batrachus	68.6	2.8	5.0	1.8	28.6

The ventral section with PAS staining

In the ventral part of *P. hypophthalmus*, the highest mucous cell count was found in the tail (4.8), followed by the abdomen (0.6) and in the head (0.4). Like in the dorsal part, the opposite trend was found in *C. batrachus*, where the highest mucous cell count was in the head (39.4), followed by the abdomen (15.6) and the tail (5.2).

C. batrachus had a significantly high mucous cell distribution in the head and the abdomen compared to *P. hypophthalmus*. Overall, *C. batrachus* was found to have more average mucous cell counts than in *P. hypophthalmus* as shown in Table 4.

Table 4: Mucous cell distribution in the ventral part of *Pangasius hypophthalmus* and *Clarias batrachus* with PAS staining.

	Average number of mucous cells (cells)		
	Head	**Abdomen**	**Tail**
Pangasius hypophthalmus	0.4	0.6	4.8
Clarias batrachus	39.4	15.6	5.2

The dorsal section with H&E staining

According to H&E staining in the dorsal part of *P. hypophthalmus*, the highest mucous cell count was in the tail (2.8), followed by the GI tract (2.6) and the abdomen (0.6) respectively. There was no mucous cell found in the head and the tentacles.

The opposite trend was found in *C. batrachus*, where the head (tentacles) had the highest cell counts (47.0), followed by the head (40.2), the abdomen (26.7), and the GI tract (12.6) respectively. The tail region had none.

In general, *C. batrachus* had a more average mucous cell distribution in the dorsal region than *P. hypophthalmus*. Moreover, there was a significantly high level of average mucous cell distribution in the head and the tentacles in *C., whereas*, none was found in the same regions of *P. hypophthalmus*. *C. batrachus* also had a higher average mucous cell count of 26.7 in the abdomen and 12.6 in the GI tract compared to *P. hypophthalmus*, which had only 0.6 in the abdomen and 2.6 in the GI tract. On the contrary, *C. batrachus* did not have any mucous cells in the tail, whereas *P. hypophthalmus* had 2.8 as shown in Table 5.

Table 5: Mucous cell distribution in the dorsal part of *Pangasius hypophthalmus* and *Clarias batrachus* with H&E staining.

	Average number of mucous cell (cells)				
	Head	**Tentacle**	**Abdomen**	**Tail**	**GI tract**
Pangasius hypophthalmus	0.0	0.0	0.6	2.8	2.6
Clarias batrachus	40.2	47.0	26.7	0.0	12.6

The ventral section with H&E staining

In the ventral part of *P. hypophthalmus*, the highest mucous count was found in the abdomen (11.8), followed by the head (10.2), whilst there was none in the tail region. In *C. batrachus*, the head region had the highest number of mucous cell count (16.2), followed by the abdomen region (14.4) and the tail region (5.6). Overall, *C. batrachus* had more mucous cell distribution in all regions of the ventral part than *P. hypophthalmus* as shown in Table 6.

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Table 6: Mucous cell distribution in the ventral part of *Pangasius hypophthalmus* and *Clarias batrachus* with H&E staining.

	Average number of mucous cell (cells)		
	Head	**Abdomen**	**Tail**
Pangasius hypophthalmus	10.2	11.8	0.0
Clarias batrachus	16.2	14.4	5.6

Blood Cell Count Analysis

In *P. hypophthalmus*, the biggest blood cell was thrombocyte spindle (11 µm), and the smallest was thrombocyte (fragmented) with 6 µm. Thrombocyte (spike and oval) was 8 µm, while the rest had a similar size of 10 µm. In *C. batrachus*, erythrocyte (immature) was the biggest size with14 µm, followed by thrombocyte spike with 11 µm, and erythrocyte with 10 µm respectively. The smallest was thrombocyte (fragmented) with 5 µm. The remaining ones had the same size of 9 µm, as shown in Table 7.

Table 7: Blood cell types and sizes in *Pangasius hypophthalmus* and *Clarias batrachus*.

Blood cell types	***Pangasius hypophthalmus* (µm)**	***Clarias batrachus* (µm)**
Erythrocyte (immature)	10	14
Erythrocyte (mature)	10	10
Thrombocyte (fragmented)	6	5
Thrombocyte (spindle)	11	9
Thrombocyte (oval)	8	9
Thrombocyte (spike)	8	11
Eosinophil	10	9
Lymphocyte	10	9
Monocyte	10	9
Neutrophil	0	9

DISCUSSION

Regarding morphological characteristics in the present study, overall, *P. hypophthalmus* was bigger, longer in body length, and had more body mass than *C. batrachus*. The mean total length of *P. hypophthalmus* was 12.66 cm, and the average weight was 11.72 ± 2.20 g. According to **Griffiths *et al.* (2014),** the mature fish of this species can be 130 cm long as maximum length and 44 kg in weight. However, *C. batrachus* was significantly smaller than *P. hypophthalmus*, with an average weight of 3.24 ± 0.32 g and a total length of 7.92 cm. This can be explained by the fact that *C. batrachus* used in this study was immature one. Most importantly, adult fish of this species do not eat every day. It remains dormant during cold dry months and can survive many days without eating. To compensate for the dormant period, it consumes a lot when it has the opportunities **(Brogan, 2003)**. From **Allen (2013),** the length of *C. batrachus* falls within the range of 8 to 47 cm and the maximum weight up to 1.2 kg, with similar shape and size in males and females. The only difference is that female may have a wider belly than a male, which is most apparent during mating season **(Ng and Kottelat, 2008).** The total length of *C. batrachus* used in this study was slightly below the normal range as suggested by the previous study, which may be due the fact they were immature. The present study focused more on comparing the two different species of catfish such as *P. hypophthalmus* and *C. batrachus* regarding the overall differences in their morphology, mucous cells in various regions as the first-line defense mechanism, and its specific distributions that contribute as the major sites of the innate immune system on their encounter to triggers like infectious micro-organisms, parasites, traumatic events, etc. To compare these factors between males and females in the given species, there is an additional need to meet the standard of comparable sample sizes, which in and of itself could be the focus of another study.

In the present study, mucous cell distribution was assessed by H&E and PAS staining method for the two catfish species, *C. batrachus* and *P. hypophthalmus.* Overall, there were more mucous cell counts in *C. batrachus* in both types of staining. According to **Kanchanakhan (2019)**, the prominent mucus production is fundamental to *C. batrachus* due its preferential habitat in the bottom zones of swampy waters, where the risk of infections is high as the bacterial population is 10-20 times more than in the water column. Therefore, it is likely that *C. batrachus* is required to be equipped with a more competent innate immunity system than *P. hypophthalmus* for survival. Based on the results of the current study, it also suggestive that *C. batrachus* has a better mucosal immune system than *P. hypophthalmus*.

The dorsal section in both species displayed more mucous cell counts than in the ventral region in the sections stained with PAS, while the ventral region in *P. hypophthalmus* showed more mucous cells in the H&E staining sections. The exposure to infection in the water is potentially high with the dorsal segment and may be the reason for the increased mucous cell production in that particular region. The result obtained from the current study is similar to the studies on the mucous cell distribution in the dorsal and the ventral regions of Japanese flounder *Paralichthys olivaceus* by **Yamamoto *et al.* (2011),** and the mucous cell distribution in the intestinal tract using PAS stain on *Silurus asotus* by **Qiao *et al.* (2007),** where mucous cell counts were more profound in the dorsal side than in the ventral side. On the other hand, a study on channel catfish, *Ictalurus punctatus*, showed that the concentration of mucous Ig production was most elevated in the anal and caudal fins, followed by the ventral skin between gill covers and pectoral fin **(Zilberg and Klesius, 1997).**

The head region in *C. batrachus*, displayed the most profound mucus production, followed by the abdomen, the GI tract, and the tail both dorsally and ventrally with both H&E and PAS. This suggests that the head is the region with most potential to encounter pathogens or stressors, and thus produces more mucus than other regions. The GI tract came as the second-highest segment of mucus production, which is also indicative of an important role in the mucus defense system. The explanation of this significant number of mucous cells counts could be similar to channel catfish, which has the most abundant mucous cells found in the distal rectal gut segment. The mucin production in that segment attributes to coating antigens randomly and makes binding to the epithelial surface more challenging **(Xie *et al*., 2019).** On the contrary, in *P. hypophthalmus*, the pattern was the opposite of *C. batrachus* as the tail region had the highest mucous cell count, followed by the abdomen and the head, which was in agreement with the finding of **Qiao *et al.* (2007)** on *Silurus asotus*. In Japanese flounder, *Paralichthys olivaceus*, the lower jaw has the most mucous cell count compared to other regions, and the decrease in density and size of mucous cells were seen after bacterial infection **(Yamamoto *et al.*, 2011).** However, the ventral part of *P. hypophthalmus* with H&E staining in the present study had a similar mucous cell distribution pattern as *C. batrachus,* except for the tail in *P.hypophthalmus*, which had no mucous production. This implies that in P.hypophthalmus the tail region does not play a role in the innate immune defense mechanism, while other parts are important as the initial protective system.

Inevitably, fish needs a robust host defense system to survive in a microbe-rich environment due to the constant exposure to waterborne organisms. Unlike mammals and amphibians, the adaptive immune system does not provide adequate protection **(Pasquier, 2001).** Therefore, fish is presumed to have a heavy reliance on their innate immune system like skin as their first-line defense against waterborne microbes **(Ellis, 2001).** For instance, skin mucus in gilthead seabream, *Sparus aurata*, displayed a better bactericidal activity against fish pathogen bacteria than the serum activity, unlike human bacteria which grows increasingly in the presence of mucus. It has mucus components comprised of IgM (low level), lysozyme, alkaline phosphatase, proteases, and high level of esterase, peroxidase, and antiprotease activities than serum against opportunist fish pathogens (*Vibrio harveyi*, *V. angillarum,* and *Photobacterium damselae*) and non-pathogenic bacteria (*Escherichia coli,* and *Bacillus subtilis*) **(Guardiola *et al*., 2014**). In the channel catfish, *Ictalurus punctatus*, the skin mucus content like IgM antibody, lysozyme, and lymphocytes gives rise to agglutinating antibody and bactericidal activity as a response to the invasion of *Salmonella paratyphi* as first line response **(Ourth, 1980).**

Bacterial pathogens are evolved in terms of detection, adherence, and initiation of infection in their finfish hosts. Though the mucosal system as the front-line defense against the bacterial invasion is paramount, there has been a limited study on the molecular events that occur upon the first encounter of bacterial pathogens. A study on the consequences of a virulent *Aeromonas hydrophila* challenge in the skin of blue catfish, *Ictalurus furcatus*, elucidated that 1155 unique genes were altered on the first encounter of pathogens and that *A. hydrophila* attempted to adjust various cellular pathway of mucosal factors to increase its adherence to infect the host **(Li *et al.*, 2013).** These include the genes that are mainly responsible for antioxidant responses, apoptosis, cytoskeletal rearrangement, immunity, and extracellular matrix protein diversity and regulation **(Li *et al.*, 2013). Subramanian *et al.* (2008)** also found out that fish skin mucus has a collection of antibacterial substances like antimicrobial peptides (AMPs).

Nutrition is an important factor that influences the immune system and mortality in fish. A study led by **Nhu *et al.* (2019)** demonstrated that 8 weeks of a diet with the extracts of *P. guajava* at 0.2 and 1.0% or *M. pudica* at 2.0% was found to boost striped catfish's immune system and reduced the mortality against *E. ictalurid* infection. Temporary feed deprivation is not an uncommon issue for both wild and farmed fish species due to reproductive processes, temperature change secondary to seasonal variations, and an outbreak of disease. Fasting can impact fish physiologically and biologically, resulting in an alteration of host susceptibility to pathogens. A previous study on the blue catfish (*Ictalurus furcatus*) and the channel catfish *(Ictalurus punctatus)* suggests that fasting disturbs arginine synthesis and metabolism pathways in such a way that it alters macrophage activation and immune readiness **(Li *et al.*, 2013).**

Concerning the blood cell analysis, different types of blood cells (erythrocyte, thrombocyte, eosinophil, lymphocyte, monocyte, and neutrophil) were obtained in this study. A similar result of blood cells was reported by a previous study on the lymphoid tissues on *C. batrachus*, which showed that the peripheral blood of this species was constituted of an increased number of nucleated erythrocytes, total leucocyte count (TLC), and leucocytes that resemble mammalian white blood cells (WBCs). Moreover, several lymphocytes, monocytes, and nucleated red blood cells (RBCs) along with hemosiderin-containing macrophages in the spleen and pronephros were also seen **(Dash *et al*., 2003).** Generally, there are three types of granulocytes in fish such as neutrophils, eosinophils, and basophils. In our study, neutrophil was absent in *P. hypophthalmus.* None of the species had basophil, which was similar to what was reported by **Esteban and Cerezuela (2015),** suggesting that basophil is found rarely. Secondly, the sizes of blood cell types in both species were varied in this study. The number of granulocytes varies significantly in fish. Generally, it may be around 4-60%. For example, 1-9% of granulocyte may be found in juvenile Rainbow Trout, *Oncorhynchus mykiss*. Lymphocytes are approximately 85% in some fish species. In young Rainbow Trout (*Oncorhynchus mykiss*), there are 89% to 98% lymphocytes in total. However, because of their similarity to thrombocytes, it is difficult to count the approximate number of lymphocytes in fish **(Arnold, 2009).**

Regarding the size of blood cell in *P. hypophthalmus*, the biggest was thrombocyte (spindle), 11 µm, and thrombocyte (fragmented), 6 µm, being the smallest, whereas in *C. batrachus,* they were erythrocyte (immature), 14 µm, and thrombocyte (fragmented), 5 µm, respectively. In the present study, two types of erythrocyte-mature and immature were found. Erythropoiesis occurs in the kidney in more advanced bony fishes like *Osteichthyes*, primitive jawless fishes like *Agnatha,* and cartilaginous fishes like *Chondrichthyes* **(Arnold, 2009).** Erythrocytes are the main blood cell type in fish, and its number varies, depending on the species of fish and the health status. For instance, in Rainbow Trout (*Oncorhynchus mykiss*), erythrocyte count maybe around 0.77 to 1.58 x 106 cells/mm^3 **(Morera and MacKenzie, 2011).** Overall, the present study demonstrated the four morphological forms of thrombocytes that were oval, spindle-shaped, spiked and fragmented, which was consistent with the finding of **Zinkl *et al.* (1991).** In another study on six species of fish including catfish, the oval or spindle-shaped cells were considered a normal form of thrombocyte; in addition the shape of thrombocyte tends to be confused with lymphocytes due to their similarity **(Zinkl *et al*., 1991).**

CONCLUSION

Generally, *P. hypophthalmus* had lengthier morphometric characteristics than *C. batrachus.* However, *C. batrachus* had higher mucous cells distribution both dorsally and ventrally compared to *P. hypophthalmus*, suggesting that the overall innate immune system of *C. batrachus* is superior to that of *P. hypophthalmus*. In general, the dorsal section had more mucous cells in both species than the ventral section. In *P. hypophthalmus*, the highest mucous cell count was in the tail region and the lowest was in the head, whereas the pattern was the opposite in *C. batrachus.* Moreover, the sizes of blood cells were found to be different in both species, despite their similarity in the blood cell types.

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