Thesis for the Degree of Master of Fisheries Science

Histopathological Health Evaluation of Cultured Olive Flounder*, Paralicthyes olivaceus*, with Different Size

By

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Histopathological Health Evaluation of Cultured Olive Flounder, *Paralicthyes olivaceus*, with Different Size

양식 넙치(*Paralicthyes olivaceus*)의 크기에 따른 병리조직학적 건강도

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By

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A dissertation

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Abstract

A histopathological health evaluation study for olive flounder, *Paralicthyes olivaceus,* with different size based on total body length and body weight was carried out. Two groups of fishes were administered for the study from the same culture system and age group-the big sized fishes (fast growing) and the small sized fishes (slow growing) in terms of total length and body weight. Samplings were done two times and six fishes were taken for each group in each sampling. For sampling I, total length and body weight were 27.58±2.10cm and 221.83±23.72g for big sized fishes (fast growing) and 22.58±0.58cm and 125.83±11.51g for small sized fishes (slow growing) respectively. In case of sampling II, total length and body weight

were 26.70±1.64cm and 404.00±58.26g for big sized fishes(fast growing) and 22.03±0.82cm and 215.00±38.89g for small sized fishes (slow growing) respectively. Clinical record, blood chemistry; total protein (Tp), hemoglobin (Hb), hematocrit (Ht), functional indices (HSI and HHI) and histological study of different organ (liver, stomach, intestine, spleen, kidney and heart) were done for each individual of each group for the experiment. Moderate to severe fatty change of liver were observed in both groups of fishes in case of sampling I. Moderate to severe atrophy of liver were clearly observed in both groups in sampling II. Focal epicarditis were observed in sampling I for big sized fishes and vacuolative changes of gastric gland of big sized fishes were clearly observed in sampling II under light microscopic examination. Tp, Ht, and HHI were recorded significantly higher for big sized fish group in sampling I. In case of sampling II only HHI was recorded significantly higher in big sized fish group. So it can be concluded fast growing individuals are more vulnerable to disease in terms of histopathological evaluation.

Keyword: Fatty change. Epicarditis. Vacuolative change. Olive flounder. *Paralichthyes olivaceus.*

Introduction

Fish disease is a common problem in aquaculture. A basic understanding of the nature of fish disease is important for the farm operator. So farm operators should be well equipped to prevent and handle diseases outbreak. Fish like other animals are prone to a variety of diseases.

Fish also can suffer from environmental and nutritional diseases. Fish disease is the result of interaction between fish, pathogen and stressful environment (Snieszko, 1974). Disease outbreaks occur when there are adverse physiological changes; they can have either infectious or noninfectious causes. Pathological alterations in the body are important evidence of physiological changes. A clear understanding of histopathology and path physiology is necessary to determine the real causes of diseases. For the above mentioned cases histological changes occur comparing to the normal situation. That is the interest of the histopathological study. Previously, animal histology aimed primarily to morphologically clarify the fine structures of the body. However, more recently, the main aims of histology changed to focus on the study of the functions of the body at the tissue level and the clarification of the physiological functions from the

view point of cellular correlation. The main purpose of histopathology is to diagnose disease from pathological changes in tissue level (Patino& Takashima, 1995). Histopathological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organ. A histological investigation may therefore prove to be cost effective tool to determine the health of fish population, hence reflecting the health of an entire aquatic ecosystem (Velkova-Jordanoska&Kostoski, 2005).

Limited researches have been conducted regarding histology and histopathology of fishes especially in terms of health evaluation. The aim of the present study is to investigate histopathological health status of olive flounder of the same age and culture system with different sized fishes in terms of total length and body weight.

Liver is the main focus of the present study. Liver is the most functional organ of fish as well as other animals, as nutrients are stored, absorbed and processed for the use of other organ through liver. It also plays a pivotal role for gathering; transforming and accumulating metabolites. Liver has an important role in maintaining body's metabolic homeostasis

that includes processing of carbohydrate, protein, lipids and vitamins. The liver also plays a key role in detoxification and in the synthesis of serum protein like albumin, fibrinogen, complement factors and acute phase proteins (Patino& Takashima, 1995, Akiyoshi& Inoue, 2004). Stomach and intestine are also focused for the study as they have strong relation with liver. Other organs (kidney, heart and spleen) and blood chemistry are also histopathologically considered as biomarkers for evaluating the susceptibility of disease.

Materials and methods

Two categories of fishes were chosen from same age group and culture system according to their body weight and total length. The big sized fish group (fast growing) and the small sized fish group (slow growing). Liver and stomach were chosen as focus organ for the study. Other organ such as, intestine, spleen, heart, body kidney, gill and functional indices (Hb, Tp, Ht, HSI and HHI) were also taken in consideration for clear clarification. To pursue the experiment and processing of tissue for Histopathological study, the following chronological steps were followed.

Sample collection:

Fishes were collected from a local fish farm named Dongji fish farm, Gijang in Busan and subsequently carried to the laboratory in polyethylene bag with oxygen in live condition. Samplings were done two times. First one (sampling I) was on 4th October/2010 and the second one (Sampling II) was on 25th January/2011.

History taking:

General history regarding culture system, feeding, stocking density and other information were also collected from the farm operators.

Clinical record and blood chemistry:

By examining each individual's clinical information were recorded in data sheet. Blood chemical parameters such as, total protein, haematocrit and hemoglobin were also taken after collecting blood from caudal vein.

Dissecting of the fish:

Operculum and body cavity were opened by dissecting fishes with sharp scissors and forceps. After opening photos of the internal organ of each individual were taken with a digital camera system (OLYMPUS E-P2, Japan).

Fixation and refixation:

Subsequently after dissection, samples were fixed in Bouin's solution. After 24 hours samples were cut into suitable pieces with sharp

blade and put in labeled cassettes (Categorized as organ and individuals). Then these samples were refixed in 10% buffered formalin solution.

Tissue processing:

All fixed tissues were passed through a series of solvents before finally being embedded fully with paraffin wax. In the present study tissues were washed and dehydrated through alcoholic grades (70%, 80%,90%, 95%, 100%, 100% and 100%) and cleaned in xylene.

Embedding:

Tissues were embedded with paraffin wax at 58-62^{o}C.

Sectioning:

Embedded blocks were cut at 5 micron in thickness by rotatary type microtome (Reichert-jung820, Leica LTD). The sectioned ribbon was floated on warm water bath (54^{o}c) to flatten out the section. The sections were carefully collected on to a glass slide, allowed to dry fully before proceeding to H&E staining.

Staining:

Hematoxylin and eosin (H&E) staining methods were followed to stain the prepared slides. The following consecutive steps were followed to perform-

1. XyleneI,-3minutes
2. XyleneII,-3minutes
3. XyleneIII,-3minutes
4. Alcohol, 100%-1minutes
5. Alcohol, 95%-1minutes
6. Alcohol, 90%-1minutes
7. Alcohol, 80%-1minutes
8. Alcohol, 70%-1 minutes
9. Washing with flowing tap water-10minutes
10. Hematoxylin-3minutes
11. Washing with tap water-1minutes
12. HCl (Acid alcohol)-2 dipping
13. Washing with tap water-1minutes
14. Ammonia water-4 dipping

15. Washing with flowing tap water-15 minutes

16. Eosin-2minutes

17. Alcohol,70-80%-4 dipping

18. Alcohol, 90%-1minutes

19. Alcohol, 95%-1 minutes

20. Alcohol, 100%-1minutes

21. Alcohol, 100%-1minutes

22. Xylene+Alcohol-2minutes

Mounting:

The stained samples were mounted with Canada balsam for permanent preservation.

Photography:

Photos of the prepared slides of different organ were taken by using the software DP2-BSW (Olympus, Japan).

Calculation of HHI:

HHI, hepatohypertrophic index was calculated by using the image analyzer (Image pro-plus3, Media cybernetics). At first the number of nucleus in 1,000μm^{2}were counted by using the software and then values were put in the following formula and HHI were calculated.

HHI= *1/Log (Number of nucleus count in 1,000μm^2)*

Results

Table 1.Information about collected samples of sampling I

Group	No. of individuals	Length (cm)	Weight (g)
Small sized fish	6	22.58±0.58	125.83±11.51
Big sized fish	6	27.58±2.10	221.83±23.72
Difference	-	5.00	96.00

Table 2. Information about collected samples of sampling II

Group	No. of individuals	Length (cm)	Weight (g)
Small Sized fish	06	22.03±0.82	215.00±38.89
Big sized fish	06	26.70±1.64	404.00±38.26
Difference	-	4.67	189.00

Necropsy findings:

Necropsy of the collected samples was done for small and big sized fish groups for sampling I and sampling II. Ascites, liver congestion, kidney enlargement, kidney membrane rupture, spleen enlargement, gill anemia and presence of parasites were taken in consideration. Ascites were found in big sized fish group in sampling I and sampling II. Liver congestion is pronounced in big sized fish group of sampling II. Four individuals were found with liver congestion in case of big sized fish group for sampling II but not any for small sized fish group. Protozoan parasite *Trichodina* was found in all individuals for the both sampling. Other observations were more or less same. All these observation were summarized in Table 3 and Table4.

Blood chemistry:

Blood chemistry parameters; Hb, Tp, and Ht were taken in consideration for the present study. Mean observed values for Hb, Tp and

Ht were 4.46±0.69g/dl, 2.59±0.059g/dl and 34.83±5.91% for big sized fish group and 5.028±1.19g/dl, 1.44±0.19g/dl and 26.5±5.68% for small sized fish group respectively in case of sampling I. For sampling II observed values for Hb, Tp and Ht were 5.96±0.88g/dl, 2.45g/dl and32.33±3.01 for big sized fish group and 4.61±1.58g/dl, 2.43g/dl and 30.16% for small sized fish group respectively. Mean value of Tp and Ht were significantly higher in big sized fish group of sampling I. In case of sampling II mean values of Tp and Hb were observed a little bit higher in big sized fish group comparing to small sized fish group (Table 5, 6, 7 and 8).

Functional indices:

Functional indices such as hepato somatic index (HSI) and hepato hypertropic index (HHI) were taken in consideration for the present study. Mean observed values for the HSI and HHI were 0.87±0.15 and3.07±0.38 for big sized fish group and 0.86±0.09 and 2.40±0.18 for small sized fish group, respectively for sampling I. In case of sampling II mean observed values for HSI and HHI were 1.35±0.19 and 3.14± 0.53 for big sized fish group and 1.46±0.75 and 2.27±0.22 for small sized fish group, respectively. Mean HSI was found more or less same between groups in both samplings.

But significant differences were found between groups in both samplings in case of HHI (Table 11, Fig 3 and 4).

Table 3. Necropsy findings of fish in sampling I

Findings	No. of individuals	
	The big sized	The small sized
Ascites	2	0
Liver congestion	0	2
Irregular color of liver	3	6
Kidney enlargement	0	0
Spleen enlargement	0	0
Paleness of gill	1	0
Presence of parasites (Trichodina)	6	6

*No. of individuals in each group is 6.

Table 4. Necropsy findings of fish in sampling II

Findings	No. of individuals	
	The big sized	The small sized
Ascites	3	1
Liver congestion	4	0
Irregular color of liver	1	2
Kidney enlargement	1	2
Spleen enlargement	1	3
Paleness of gill	0	2
Presence of parasites (Trichodina)	6	6

*No. of individuals in each group is 6.

Table 5.Functional indices for big sized fish group of sampling I

Sample No.	Length(cm)	Wt(g)	Hb(g/dl)	Tp(g/dl)	HSI (%)	Ht (%)	HHI
1	27	220	4.95	3.74	0.95	33	2.97
2	25	193	4.04	2.15	1.14	35	2.63
3	27	205	5.44	2.5	0.73	46	3.32
4	31	254	4.62	2.36	0.75	35	3.67
5	26.5	213	4.29	2.64	0.85	30	3.10
6	29	246	3.46	2.18	0.85	30	2.72
Mean ± SD	27.58±2.10	221.83±23.72	4.46±0.69	2.59±0.59	0.87±0.15	34.83±5.91	3.07±0.38

Table 6.Functional indices for small sized fish group of sampling I

Sample No.	Length (cm)	Wt (g)	Hb (g/dl)	Tp (g/dl)	HSI (%)	Ht (%)	HHI
1	22.5	129	6.76	1.66	1.01	36	2.35
2	23	140	5.44	1.46	0.86	26	2.51
3	23	115	3.62	1.66	0.87	19	2.29
4	23	114	3.71	1.35	0.88	25	2.48
5	22.5	138	5.44	1.14	0.72	24	2.12
6	21.5	119	5.2	1.42	0.84	29	2.63
Mean ± SD	22.58±0.58	125.83±11.51	5.028±1.19	1.44±0.19	0.86±0.09	26.5±5.68	2.39±0.18

Table 7. Functional indices for big sized fish group of sampling II

Sample No.	Length (cm)	Wt (g)	Hb (g/dl)	Tp (g/dl)	HSI (%)	Ht (%)	HHI
1	27.6	395	6.43	2.71	1.34	36	3.50
2	25.8	371	6.69	3.59	1.37	30	2.87
3	29.3	493	6.69	2.37	1.4	35	2.72
4	24.7	360	4.66	2.33	1.22	28	3.92
5	27.1	457	5.048	1.49	1.68	32	3.32
6	25.7	348	6.26	2.22	1.1	33	2.48
Mean ± SD	26.7±1.64	404±58.26	5.96±0.88	2.45±0.68	1.35±0.19	32.33±3.01	3.14±0.53

Table 8.Functional indices for small sized fish group of sampling II

Sample No.	Length (cm)	Wt (g)	Hb (g/dl)	Tp (g/dl)	HSI (%)	Ht (%)	HHI
1	21.8	184	7.36	2.1	0.68	26	2.21
2	22.7	175	4.18	2.1	1.14	33	2.09
3	22.6	212	5.08	1.38	1.14	28	2.05
4	22.8	258	2.62	2.25	2.53	35	2.48
5	20.7	267	4.66	2.94	1.02	32	2.18
6	21.6	194	3.81	3.81	2.28	27	2.59
Mean ± SD	22.03±0.82	215±38.89	4.61±1.58	2.43±0.83	1.46±0.75	30.16±3.65	2.27±0.022

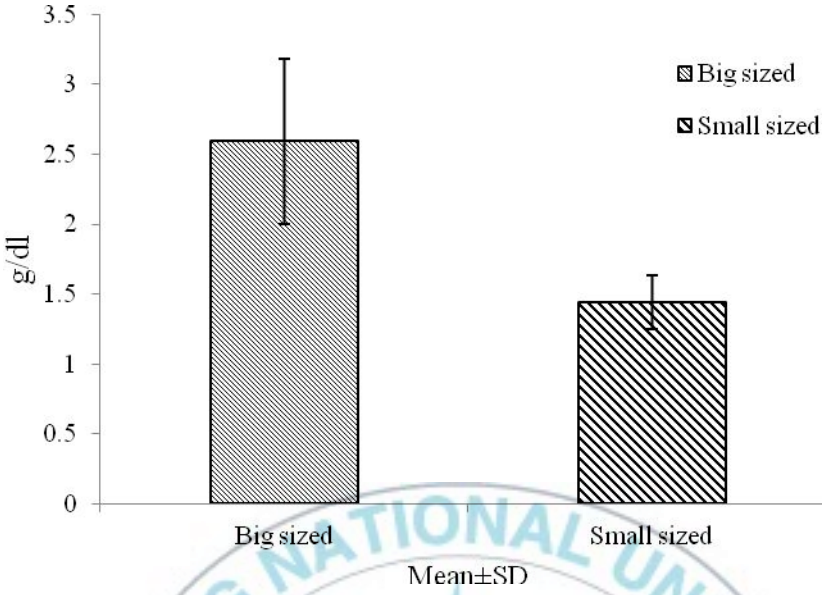


Fig.1. Comparison between big sized and small sized fish for Tp in Sampling I.

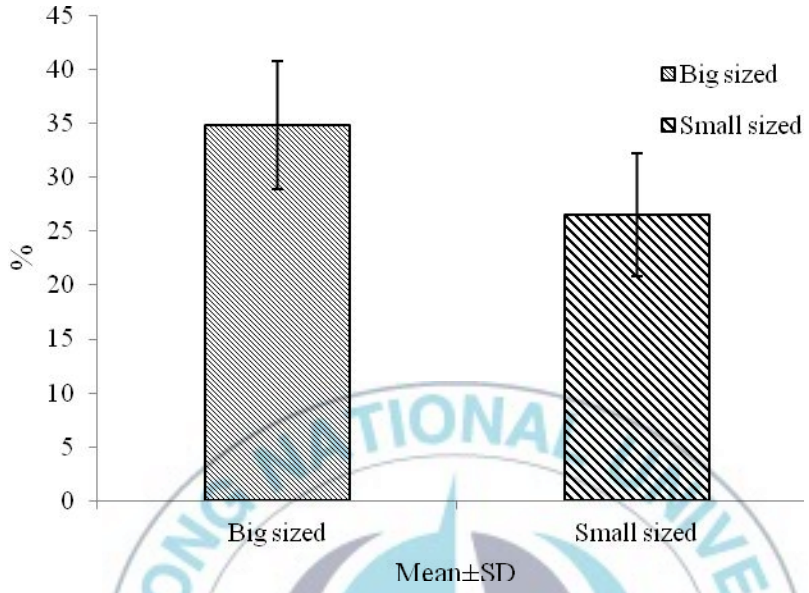


Fig.2.Comparison between big sized and small sized fish group for Ht in sampling I.

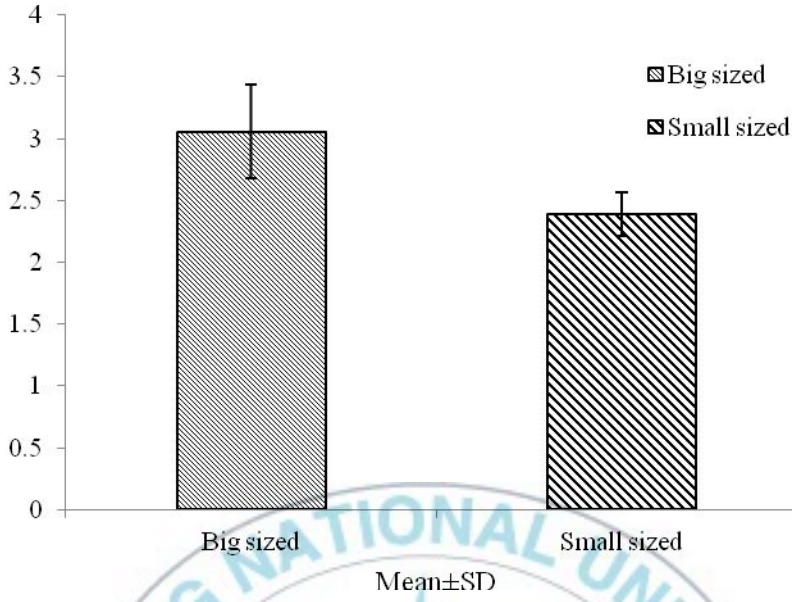


Fig. 3. Comparison between big sized and small sized fish group for HHI in sampling I.

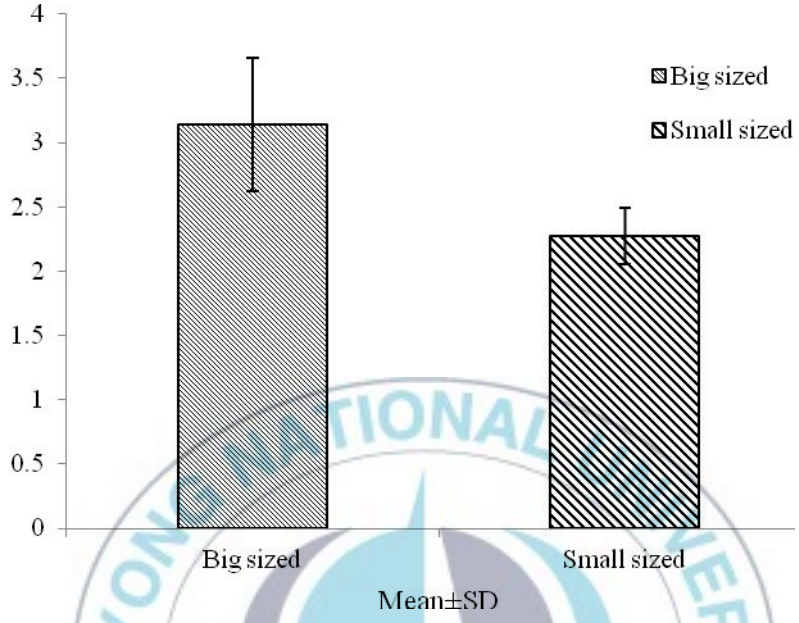


Fig.4. Comparison between small sized and big sized fish group for HHI in sampling II.

Histopathological features under light microscope:

Liver:

In the present study histological slides prepared from liver tissue were observed under light microscope for both samplings. The main alterations observed were atrophic hepatocyte, moderate to severe fatty change in sampling I for small and big sized fish groups. There was no clear difference between groups (Table 9 andFig.5). In case of sampling II, the main feature was mild to moderate atrophy of hepatocycte. No clear difference was observed between groups (Table 10 andFig.6).

Stomach:

In case of sampling I, stomach was observed almost normal except one individual with vaculative change and atrophy of gastric gland and two individuals with immunological activation for big sized fish group. But for small sized fish group, all individuals were observed normal (Table 9). In case of sampling II, vacuolative changes of gastric gland were conspicuous in big sized fish group with pearl like white round space in all individuals. All individuals of small sized fish group were observed almost normal.

Clear and distinct difference was observed in stomach between two groups in case of sampling II (Table 10 and Fig.7).

Intestine:

Histological slides prepared from intestine were observed under light microscope. No alterations were observed except two individuals with presence of inflammatory cell in big sized fish group in case of sampling I (Table9). For sampling II all individuals were normal for both groups (Table 10). No distinct difference was observed between groups regarding intestine for both samplings.

Kidney:

MMCs were increased both in size and number for all individuals of both samplings for small and big sized fish groups. Two individuals from sampling I and one individual from sampling II for big sized fish group were observed with hydrophic degeneration (Table9 and 10).

Heart:

Epicarditis is the main alterations in heart observed for the present study. For sampling I three individuals from big sized fish group were found with epicarditis but not any from small sized fish group. For sampling II, five individuals from big sized and four individuals from small sized fish group were found with epicarditis. So rate of epicarditis is higher in big sized fish group comparing to the small sized (Table 9 and 10).

Spleen:

Activation of ellipsoids increased MMCs both in size and number, lymphocytic infiltration were the main observation for both sampling for both groups of fishes. No clear distinction was observed between groups for both samplings (Table 9 and 10).

Table 9. Microscopic findings of fish in sampling I

Organ examined	Histological feature	No. of individuals	
		The big sized	The small sized
Liver	Atrophic	5	5
	Fatty change	6	5
	Increase of MMC	2	0
Stomach	Vacuolative change	1	0
	Lymphocytic infiltration	2	0
Intestine	Edemateous laminapropriya	3	0
	Lymphocytic infiltration	2	0
	Dialation of blood vessel	0	5
Kidney	Increase of MMCs	6	6
	Hydrophic degeneration	2	0
Heart	Epicarditis	2	0
Spleen	Enlargement of ellipsoid	5	3
	Increase of MMCs(Size/number)	5	6
	Lymphocytic infiltration	1	0

*No. of individuals in each group is 6.

Table 10.Microscopic findings of fish in sampling II

Organ examined	Histological feature	No. of individuals	
		The big sized	The small sized
Liver	Atrophic	4	4
	Fatty change	1	0
	Increase of MMC	0	1
Stomach	Vacuolative change of gastric gland	6	0
	Lymphocytic infiltration	0	0
Intestine	Edemateous laminapropriya.	0	0
	Lymphocytic infiltration	0	0
	Dialation of blood vessel	0	0
Kidney	Increase of MMCs	4	4
	Hydrophic degeneration	1	0
Heart	Epicarditis	5	4
Spleen	Enlargement of ellipsoid	1	1
	Increase of MMCs (Size/number)	2	3
	Lymphocytic infiltration	1	3

*No. of individuals in each group is 6

Table 11.Comparative analysis of blood chemistry and functional indices (Hb, Tp,Ht, HSI and HHI)

Sampling	Parameters	Big sized (Mean±SD)	Small sized (Mean±SD)	P-value
I	Hb (g/dl)	4.46±0.69	5.028±1.19	0.342
	Tp (g/dl)	2.59±0.59	1.44±0.19	*0.001
	Ht (%)	34.83±5.91	26.5±5.68	*0.032
	HIS (%)	0.87±0.15	0.86±0.09	0.839
	HHI (%)	3.068±0.38	2.39±0.18	*0.003
II	Hb (g/dl)	5.96±0.88	4.61±1.58	0.099
	Tp (g/dl)	2.45±0.68	2.43±0.83	0.961
	Ht (%)	32.33±3.01	30.16±3.65	0.28
	HIS (%)	1.35±0.19	1.46±0.75	0.72
	HHI (%)	3.14±0.52	2.27±0.22	*0.004

*Asterisks indicate the significant data (P-Value<0.05)

Discussion

In the present study total protein in blood was found significantly higher in big sized fish group in case of sampling I (Table11 and Fig.1).This may be due to overfeeding of artificial feed by big sized fishes. Previous research reported that blood Tp was positively correlated with feeding level in rainbow trout, *Onchorhynchus mykiss* (Storebakken *et al.*, 1991),while other studies have shown declines in fish plasma Tp during fasting (Navarro & Gutierrez, 1995, Wagner & Congleton, 2004). On the contrary, Coz-Rakovac et al., (2008) found no correlation between Tp values and the feeding regime. This observation was consistent with the present study for sampling II. For sampling II, Tp value is almost same for big and small sized fish group (Table11).

The haematocrit is used to measure the ratio of erythrocytes to plasma, and effectively measure the packed cell volume of the erythrocytes contained in the blood (Blaxhall, 1972). Without the knowledge of the normal range of hematological parameters, it is difficult, if not impossible, to differentiate between the normal and pathological state (Barnhart, 1969).

However as a part of the quantitative health assessment index for rapid evaluation, a normal range of 30-45% is considered for fish in general (Adams *et al.*, 1993). In the present study mean Ht values for big and small sized fish groups were 34.83±5.91 and 26.5±5.68 respectively in case of sampling I. In case of sampling II, Ht values were 32.33±3.01 and 30.16±3.65 for big and small sized fish groups respectively. All these values consistent with the above mentioned range except small sized fish group in sampling I. In sampling I, Ht was significantly higher in big sized fish group comparing to the small sized (Table11 and Fig.3).

The mean HSI value is species- specific and correlates with the amount of fat deposition (Chiba *et al.*, 1976, Oguri, 1985, Anelo *et al.*, 1993, Brusle *et al.*, 1996). In osteichthyes, the HSI is normally calculated to be between 1-2 % (Brusle *et al.*, 1996). It has also been found that HSI is highly sensitive to the nutritional status of the fish and correlates with the quantity and quality of feed (Hung *et al.*, 1990). In the present study, the mean values for HSI for both groups (small and big sized) in both sampling were calculated. Those values consistent with the normal range although a little bit minimum values were calculated lower than 1% in case of sampling

I (Table 11). But for sampling II, all values fall in normal range. No significant differences were observed between groups (Table11).

Another functional index known as hepatohypertrophic index (HHI) was used for the present study. This index was first introduced in fish and shellfish pathology laboratory, in the Department of Aquatic Life Medicine, Pukyong National University. Actually, this is a parameter used to describe the hepatic function. Limited references were found regarding HHI. Previous research found that incidence rate and severity of green liver syndrome were increased with the increasing of HHI values (Lee, 2008). In present study HHI values were found significantly higher in big sized fish group comparing to the small sized in both samplings (Table11, Fig. 3, 4, 5 and 6). Severe increase of HHI values obliterate the Disse space and sinusoids which may lead to hypertrophy, fatty change and necrosis of liver. Finally it may lower the immunity of the fish and make more vulnerable to disease.

In the present study fatty change and atrophy of liver were observed in most of individuals of big and small sized fish groups for sampling I (Fig.5). For sampling II both groups were found with atrophic liver (Fig. 6).

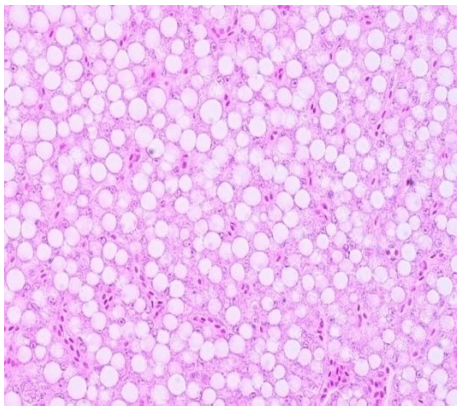
This may be due to intake of excess artificial feed. It has been reported that cytoplasmic vacuolation of liver of the Saddled bream, *Oblada melanura* in cage associated specimens with artificial feed was pronounced (J. Ferri 2011). Similar results also found in populations of mullets (Coz Rakovac *et al.*, 2008). Fatty degeneration has documented as a significant problem in cultured fish especially in sea bream (Benedito Palos *et al.*, 2008).

In the present study vacuolative changes in the gastric gland in big sized fish group in case of sampling II is the most important observation from the view point of health evaluation of olive flounder (Fig.7). Vacuolative changes in gastric gland may lead to critical pathological state and could have contributed to increase mortality (Mobin *et al.*, 1999). Histological alterations in gastrointestinal tract caused by high stocking density and resulting social stress have been reported in the case of Elvers (Willemse *et al.*, 1984) and adult European eels, *Anguilla anguilla* (Peters, 1982). In the present study stocking density and social stress were the same because both small and big sized fishes were taken from same age group and culture system. Vacuolative changes were observed only in big sized fish group. So this was not the cause of the histological alterations. Blebbing

and necrosis of gastric cells, vacuolative changes of gastric glands and necrosis of enterocytes and intestinal wall were clearly found to increase in severity as the feeding level increased in case of Japanese juvenile olive flounder (Mobin *et al.*, 2000). This was the probable cause of vacuolative changes in gastric glands in big sized fish group for the present study. Because big sized fishes take more feed comparing to the small sized in competition. Vacuolative change and atrophy of gastric gland is the indicator of high feeding regime. High feeding regime causes hyper secretory activities of gastric gland and leading to vacuolative changes and atrophy of gastric gland. Overfeed leading to dysfunction of liver by mechanically obliterating the microcirculation of hepatic parenchyma.

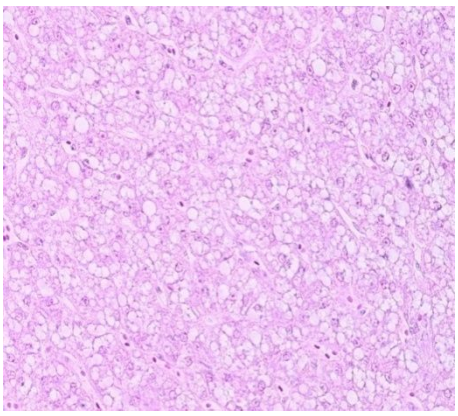
In the present study MMCs were increased both in size and numbers in all individuals of both groups and samplings in case of kidney and spleen tissue (Table 9 and 10). Kidney is the major lymphoid organ in teleosts in addition to the thymus, spleen and mucosa-associated lymphoid tissue. MMCs are distinctive population of pigment containing cells present in the hematopoietic tissue of spleen and kidney (Roberts, 1975, Wolke, 1992). So it is normal to observe MMCs in kidney and spleen tissue.

The big sized



HHI=2.97

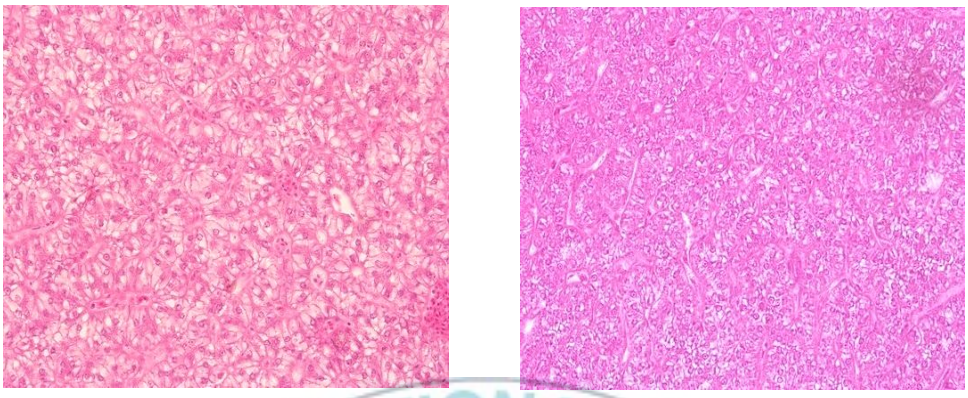
The small sized



HHI=2.12

Fig.5.Comparison of liver tissue between big sized and small sized fish group (X400) in sampling I

The big sized The small sized



HHI=3.32 HHI=2.59

Fig.6. Comparison of liver tissue between big sized and small sized fish group (X400) in sampling II.

The big sized The small sized

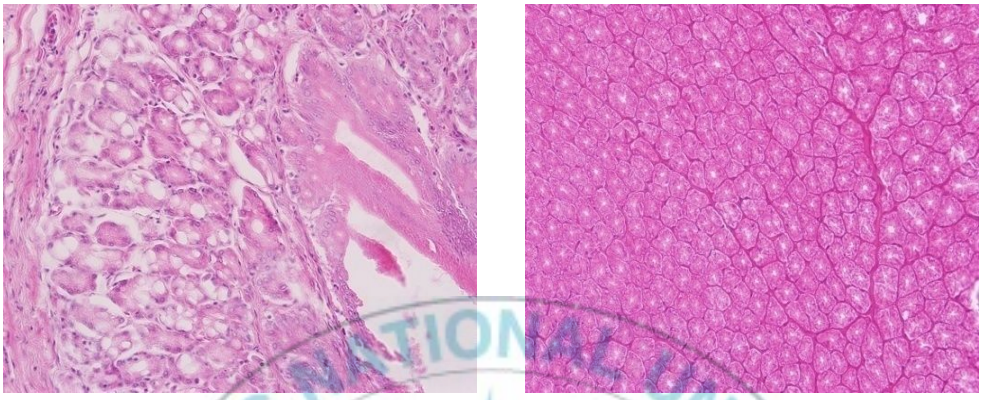


Fig.7. Comparison of stomach tissue between big sized and small sized fish group (X400) in sampling II.

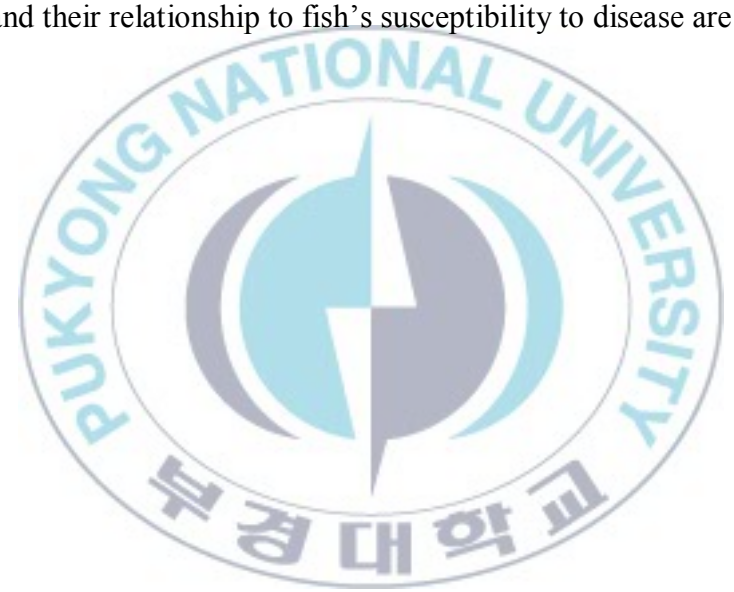
Conclusion

In the present study clear histopathological difference between big and small sized fish group were observed in case of stomach with vacuolative changes in gastric gland in big sized fish group for sampling II. HHI values were also found significantly higher in big sized fish groups in both samplings. Some significant higher values were also observed for Tp and Ht in sampling I for big sized fish group. Vacuolative changes in gastric gland for big sized fishes are an important observation for the present study. Vacuolative changes in gastric gland may lead to critical pathological state and could have contributed to increase mortality. Vacuolative changes and atrophy of gastric gland related to the higher feeding regime. High feeding regime causes hyper secretory activities of gastric glands and leading to vacuolative change and atrophy of gastric glands. Overfeeding, leading to dysfunction of liver by mechanically obliterating the microcirculation of hepatic parenchyma. The visceral organs are important component of the defense system (Fange, 1984).

Higher HHI values in big sized fishes implicated over nourished state. Severe increase of HHI values obliterate the Disse space and sinusoids

which lead to hypertrophy, fatty change, atrophy and necrosis of liver. Finally it may lower the immunity of fish and cause more vulnerable to disease.

The present study is only based on fish size from same age group and culture system. Impacts of different feeding regimes are also important for health evaluation of fishes. Further studies with different feeding regimes and their relationship to fish's susceptibility to disease are required.



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