Thesis for the Degree of Master of Fisheries

Histopathological health of cultured Pacific oyster, *Crassostrea gigas*

by

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KOICA-PKNU International Graduate Program of Fisheries Science

The Graduate School

Pukyong National University

February, 2013

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Pacific oyster,

Crassostrea gigas

참굴의 병리조직학적 건강도

Advisor: Prof. Min Do HUH

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Fisheries

in KOICA-PKNU International Graduate Program of Fisheries Science,

The Graduate School,

Pukyong National University

February, 2013

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A dissertation

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February 22, 2013

텟 모우윈의 수산학 석사 학위 논문을

인준함

2013 년 2 월 22 일

주심	농학 박사	김창훈	인
위원	이학 박사	김현우	인
위원	농학 박사	허민도	인

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Abstract

Histopathological health of Pacific Oyster (*Crassostrea gigas)* was evaluated between different sizes based on the body length and body weight. The samples were collected in an oyster production facility located in Tongyeong City of the southern coastal area of South Korea. For the histological examination, six (big sized) and six (small sized) one-year old oysters and six (big sized) and six (small sized) two-year old oysters were collected for sampling I. For sampling II oysters were divided into three groups, six (big sized) and six (small sized) oysters for

each group for sampling II. For sampling III, oysters were collected from two different farms and nine (big sized) and nine (small sized) from each farm were sacrificed in each group. The total length and body weight were11.36 ±1.29 cm and 21.92 ± 5.31 g for big sized oysters and 7.98± 0.82 cm and 9.97 ± 3.24 g for small sized oysters .In the case of sampling II, 12.4± 1.47 cm and 17.51 ± 4.22 g for big sized and 8.18 ± 0.71 cm and 10 .23 ± 2.68 g for small sized . In the sampling III, 12.07± 1.16 cm and 22.10± 5.03 g for big sized and 9.10 ±0.84 cm and 13.24 ± 3.8 g for small sized oysters respectively. Significantly, the hemocytic infiltration in Leydig's tissue, atrophic changes of digestive gland and parasitic infestation were recognized in the sampled animals. The hemocytic infiltration were detected in the Leydig's tissue in body, mantle and beneath gastrointestinal tracts with the various intensiveness. The absorptive epithelium of digestive glands showed various degrees of atrophic changes The 3rd sample had protozoan parasitic infestation. The morphological characteristic of parasites revealed the parasites had several points of similarity to *Bonamia*, causative agent of oyster mass mortality. Although hemocytic infiltration were detected and observed with various degree of severity by using histopathological routine analysis method, there were no significant differences in severity of the lesions between small sized and big sized oyster groups during this examining periodoftheyear(JanuarytoMarch,12).

Introduction

The Pacific oyster (*Crassostrea gigas*) is a species of considerable economic importance, with among the highest global production of any cultured aquatic animal species (Evans et. al., 2009). Despite the sharp increase in price of Pacific oyster (*Crassostrea gigas*), the demand has mounted rapidly both in domestic as well as international seafood market. Oyster's flesh is called milk of sea, uncleaned rice of sea, medical drug of sea and is very nutrition high food. Oyster has been top health food since the dawn of history and used regularly as popular edible food. The stimulated consumption of oyster in response to increasing interest in healthy human foods together with high commercial value has attracted the attention of researchers from worldwide to encourage stock enhancement and aquaculture programs for oyster. Oysters are produced in many countries of the world. A variety of species are cultured using many innovative methods. Oyster culture has been practiced since ancient times in Asia as well as Korea.

The Pacific oyster is native to Japan. It was imported to the West Coast of the United States early in the 20th century where it has been the basis of the oyster industry. The Pacific oyster has been introduced in such countries as Australia (intentionally introduced in the1940's), England (1960's), France (1970's), and New Zealand (accidentally introduced in the 1970's). It was first introduced in British Columbia in 1912 or 1913 for culturing purposes. Significant introductions occurred in British

Columbia between 1929 and 1932 when approximately four million seed oysters were imported from Japan and the Pacific. The first attempt to introduce the Pacific oyster into Washington State occurred sometimes between 1900 and 1902. By 1928, it was successfully established. By 1939, it was cultivated in Ketchikan, Alaska, and it is now reared in Prince William Sound, Kachemak Bay and other locations in Alaska. This oyster has adapted well to a wide range of environmental conditions and is now considered the most widespread and ubiquitous oyster species in the world. As marine invertebrates, oysters have an immune system that resembles the innate immune system of vertebrates (for review see Schmitt et al., 2012b). In oysters, both hemocytes (circulating blood cells) and surface epithelia display immune functions, expressing both non-self-recognition receptors and immune effectors involved in the control of the resident/pathogenic micro flora. Oysters possess a semi-open circulatory system where the blood, called hemolymph, flows largely through a system of sinuses. The hemolymph bathes the organs directly providing oxygen and nutrients, and is composed of fluid plasma and hemocytes. The circulatory system contributes to the haemocytes ability to migrate toward connective tissues .They migrate by diapedesis from blood sinuses and vessels to the surface body, particularly to surface epithelia such as mantle and gills (Cheng, 1996).

Summer mortality of Pacific oysters is known in several countries (Samian et al., 2007). However no specific pathogen has been systematically associated with this phenomenon. A complex combination of environmental and biological parameters has been suggested as the cause and is now starting to be identified. According to Koganezawa (1974), the phenomenon known as *Crassostrea gigas* “summer mortality” first began along the Japanese Pacific coast in 1945. Features common to mortality episodes were elevated temperature, full maturation or spawning and high atrophic conditions.The relationship between gonadal maturation, energy metabolism,and the mortalities was investigated (Koganezawa, 1974; Mori, 1979) and it was concluded that summer mortality in Japan was due to a “physiological disorder and metabolic disturbance derived by heavy gonad formation and massive spawning under high water temperature and eutrophication” (Koganezawa, 1974). In the late 1950s, mortalities of *C. gigas* were first noted on the west coast of USA (Glued, 1975; Cheney et al., 2000). Experimental studies further implicated gonadal maturation and loss of carbohydrate reserves (Perdue et al., 1981). Lipovsky and Chew (1972) showed experimentally that rising temperature (up to 21°C) or high levels of microalgae significantly increased mortality.

Different pathogens were isolated but none of them could explain summer mortality (Elston et al., 1987; Friedman et al., 1998, 1991, 2005). In France, oyster production has experienced periodical mass mortalities for the last 20 years (Renault et al., 1994; Goulletquer et al., 1998; Soletchnik et al., 1999). Results have confirmed that summer mortality is the result of multiple factors including elevated temperature, physiological stress associated with maturation, aquaculture practices pathogens, pollutants, etc. (Soletchnik al., 1997, 2006; Goulletquer et al., 1998; Le Roux et al., 2002, 2004; Gay et al., 2004).

Although the Pacific oyster provides an economic opportunity to local fishermen in Korea as well and continued export generate considerable foreign exchange. Apart from mortality, soft shell is a major barrier in further expansion of Pacific oyster aquaculture. Visual perception of food products is known to affect consumer preference and, therefore, product value (Kahn and Wansink, 2004). For example, the level of red pigmentation in salmon flesh is positively correlated with consumers' enjoyment of the product (Sylvia et al., 1995) and their willingness to pay a premium price (Alfneset al., 2006). Similarly, consumer preference for Pacific oysters (*Crassostrea gigas*) may also be influenced by visual cues including shell and meat color (R. Jacobsen, pers. comm.; Nell, 2001). Bacteriological and epidemiological study of these outbreaks indicated that the cause was not infectious, and no association could be found with artificial pollutants or pesticides from equipment within the bay or from agricultural or other activities upstream (Elton, 1999). Histological examination of oyster tissues can be used to determine if the animal is stressed. Seasonal changes occur during tidal cycles in digestive gland tubules of the Pacific oyster. Histopathological health evaluation has gained momentum in fish and shell fish pathology and the potential of histopathology has been well recognized as a basis of developing effective control measures. It is strongly recommended that we should monitor early parasitic invasion and simultaneously find and evaluate the pathological relationship between the infection and possible precursor lesions suggesting health deviation of the host oyster. Therefore, the present study was conducted to understand the hemocyte infiltration and degeneration of Leydig's tissue as precursor lesions suggesting functional damage of Pacific oyster by using routine histopathological examination.

Materials and Methods

Two categories of sampling oysters were chosen randomly with same age group and culture system but differences in size according to their body weight and total length. Mainly were compared and evaluated with the big sized and small sized group of samplings. The present study was mainly focused on the hemocyte infiltration and degeneration of Leydig tissues, gills, mantle tissues, mid gut, digestive gland, gonadal tissue atrophy were examined for lesions under a light microscope. In order to get the significant histopathological health deviations between the individual oysters, the following chronological procedures were used.

1. Sample collection

Oysters were collected from the Seuhon Susan production company, Tongyeong City located in southern coastal area of South Korea and subsequently carried to the laboratory in polyethylene bag with oxygen in live condition. Samplings were done three times. Sampling I, II and III were conducted on January, February and March in 2012, respectively.

1. Dissection of the oysters

Each oyster's shell was carefully opened and dissected with the scalpel or knife gently in order to prevent damage of any tissue.

2. Fixation and refixation

Subsequently after dissection, samples were fixed in Bouin's solution. After 24 hrs samples were cut into suitable pieces with sharp blade and put in labelled cassettes (categorized as organ and individuals). And then, these samples were refixed in Bouin's solution.

4. Tissue processing

All fixed tissues were passed through a series of solvents before finally being embedded fully with paraffin wax. Tissues were washed and dehydrated through alcoholic grades (70%, 80%, 90%, 95%, 100%, 100% and 100%) and cleaned in xylene for 19 hrs.

5. Embedding

Tissues were embedded with paraffin wax at 58 - 62°C.

6. Sectioning

A portion of embedded blocks of each oyster were sliced into five µm using a rotary type microtome (Reichert –Jung 820, Leica Ltd). The sectioned ribbon was floated on warm water bath (540c) 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.

7. Staining

In this study, Analyses are conducted on paraffin-embedded tissues sectioned at a 5-µm thickness and stained using staining H&E procedures. The following consecutive steps were followed.

1. Xylene I, -3min
2. Xylene II,- 3 min
3. Xylene III, -3 min

4. Alcohol, 100% - 1 min

5. Alcohol, 95%- 1 min

6. Alcohol, 90%- 1 min

7. Alcohol, 80%- 1 min

8. Alcohol, 70%-1min

9. Washing with flowing tap water -10 min

10. Haematoxylin -3 min

11. Washing with tap water – 1min

12. HCL (acid alcohol)- 2 dipping

13. Washing with tap water – 1min

14. Ammonia water – 4 dipping

15. Washing with flowing tap water – 15 min

16. Eosin- 2 min

17. Alcohol, 70-80% - 4 dipping

18. Alcohol, 90%- 1 min

19. Alcohol, 95%- 1min

20. Alcohol, 100%- 1min

21. Alcohol, 100%-1min

22. Xylene +Alcohol – 3min

23. Xylene I, - 3 min

24. Xylene II, -3 min

25. Xylene III,- 3min

8. Mounting

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

9. Photography

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

Results

Gross findings

There were no external abnormalities in gross examination of all samples because we selected only non-diseased, seemingly healthy individuals. As shown in Table 1~3, oysters were divided into 2 groups which were big-sized and small sized. The differences in body length between 2 groups were ranged 2.97 to 3.38 cm and those in body weight were between 8.86 and 11.95g in their mean values.

Histopathological findings

Leydig's tissue

In the case of study, hemocytic infiltration was occurred in the Leydig's tissue with the severities of normal to severe stages (Fig. 4). In the sampling I and II, normal to severe conditions were found. For sampling III, only normal and mild conditions were found. Severe conditions were not observed. According to the total grading scored, we can evaluate the health status of sampled oysters between both sized. Outcome results were 77, 93 for year one and 93, 70 for year two oysters in sampling I . For sampling II, grading scored were observed 260 for big sized and 243 for small sized .In the sampling III, 96 and 87 for v-island and 98 ,100 grading scores for Sam Cheam Po were examined for both sized sampled oysters respectively ,Table (4,8,12),Fig (1,2,3).

Digestive gland

In present study, all of individuals of digestive gland were mostly normal to mild in severity of hemocytic infiltration in both big and small sized groups in the sampling I(Table 5). For sampling II, one individual from a big-sized group III showed moderate infiltration of hemocytes (Table 9). In the case of sampling III, moderate infiltrations were found in many small oysters sampled from Sam Chean Po area. The rest of them were normal to mild in condition of infiltration (Table 13).

From group health based on the total scores of sample I, there were no difference between two groups in one-year old group but those of 2-year old big and small-sized groups were 90 and 100, respectively (Table 5, Fig. 1). In group II and III of sample II, small-sized oysters had good scores in group (Table 9, Fig. 2). For oysters from the v-island in sampling III, small-sized group had good group health scores (Table 13, Fig. 3).

Nearly normal and low cuboidal epithelia with a large circular lumen suggest that availability of food was similar to each other. There was no significant difference in severity among different groups but results obtained from digestive gland enabled us to categorize in three distinct groups depending upon the intensity of infiltration.

Mantle

The clear histological features on the severities of hemocytic infiltration in the mantle were observed. In the case of sampling I, most of them were observed normal and some were mild in severity. Group health was similar between big and small sized groups (Table 6 and Fig. 1).

In the sampling II, only 2 of 18 individuals were moderate infiltration. Group health was also similar to each other. Also in the sampling III, only four of 18 individuals were found to be mild in severity (Table. 15, Fig. 3). The other 14 oysters were normal. Total group health of the mantle tissues was similar between 2 sized groups despite of sampling times and sampling sites (Fig. 1, 2, and 3).

Midgut

Hemocytic infiltration was observed around the midgut and also tips of the mantle. In the first and second samples, normal to severe conditions were found (Table 6, Fig. 6).But for third sample, severe conditions were not observed in both sized. Total group health was good in one year-old, small sized group of sample I, and in small sized group of sample II, and in small sized group of sample III from Sam Chean Po area compared to that of big sized groups (Fig. 1, 2 and 3).

But there were no big differences in total health scores between the small and big sized groups.

Table 1. Body weight and length of oysters in sampling I

Group of Oysters	No of individuals	Length(cm) Major axis	Weight(g)
Big sized	12	11.36±1.29	21.92±5.31
Small sized	12	7.98±0.82	9.97±3.24
Differences		3.38 ± 0.47	11.95±2.07

Table 2. Body weight and length oysters in sampling II

Group of Oysters	No of individuals	Length(cm) Major axis	Weight(g)
Big sized	18	12.4±1.47	17.51±4.22
Small sized	18	8.18±0.71	10.23±2.68
Differences		3.96±0.76	7.28±1.54

Table 3. Body weight and length of oysters in sampling III

Group of oysters	No of individuals	Length(cm) Major axis	Weight(g)
Big sized	18	12.07±1.16	22.10±5.03
Small sized	18	9.10±0.84	13.24±3.8
Differences		2.97± 0.32	8.86± 1.23

Table 4. The health scores of Leydig's tissue in sampling I

Sample No	Leydig tissue of body							
	1year				2years			
	Big		Small		Big		Small	
1	moderate	10	normal	17	normal	17	mild	13
2	normal	17	mild	13	mild	13	mild	13
3	moderate	10	normal	17	normal	17	moderate	10
4	normal	17	normal	17	normal	17	severe	7
5	moderate	10	mild	13	mild	13	mild	13
6	Mild	13	normal	17	normal	17	mild	13
Total		77		93		93		70

Table 5. The health scores of digestive gland in sampling I

Sample No	Digestive gland							
	1year				2years			
	Big		Small		Big		Small	
1	Mild	13	normal	17	mild	13	normal	17
2	Mild	13	normal	17	normal	17	normal	17
3	normal	17	mild	13	normal	17	normal	17
4	normal	17	normal	17	normal	17	normal	17
5	normal	17	mild	13	mild	13	normal	17
6	normal	17	normal	17	mild	13	normal	17
Total		93		93		90		100

Table 6. The health scores of mantle in sampling I

Sample No	Mantle							
	1year				2years			
	Big		Small		Big		Small	
1	normal	17	normal	17	normal	17	mild	13
2	normal	17	mild	13	mild	13	normal	17
3	Mild	13	normal	17	mild	13	mild	13
4	normal	17	normal	17	normal	17	mild	13
5	normal	17	mild	13	normal	17	normal	17
6	normal	17	normal	17	normal	17	normal	17
Total	97		93		93		90	

Table 7. The health scores of midgut for sampling I

Sample No	Midgut							
	1year				2years			
	Big		Small		Big		Small	
1	Severe	**7**	moderate	10	mild	13	mild	13
2	moderate	10	mild	13	moderate	10	mild	13
3	moderate	10	moderate	10	mild	13	severe	**7**
4	Mild	13	mild	13	mild	13	severe	**7**
5	moderate	10	moderate	10	mild	13	severe	**7**
6	Mild	13	mild	13	mild	13	moderate	10
Total	63		70		77		57	

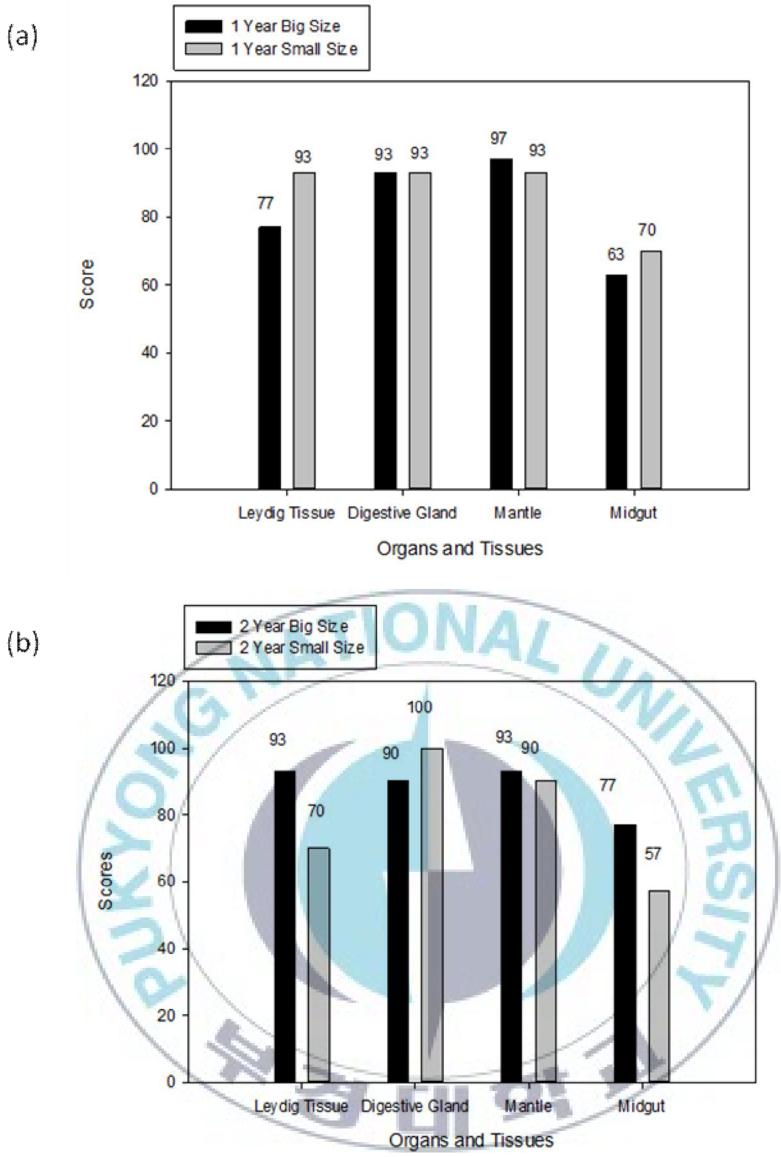

Fig. 1. Histopathological health of one year (a) and two years (b) big sized and small sized oysters in sampling I

Table 8. The health scores of Leydig's tissue in sampling II

Sample No	Leydig tissue											
	Group I				Group II				Group III			
	Big		Small		Big		Small		Big		Small	
1	Mild	13	severe	7	mild	13	mild	13	normal	17	Mild	13
2	Mild	13	moderate	10	normal	17	normal	17	moderate	10	Normal	17
3	Normal	17	normal	17	mild	13	normal	17	normal	17	Severe	7
4	Normal	17	normal	17	mild	13	normal	17	moderate	10	moderate	10
5	Normal	17	moderate	10	mild	13	normal	17	mild	13	Normal	17
6	Mild	13	normal	17	normal	17	mild	13	normal	17	moderate	10
	90		77		87		93		83		73	
	Big						Small					
Total	260						243					

Table 9. The health scores of digestive gland in sampling II

Sample No.	Digestive gland											
	Group I				Group II				Group III			
	Big		Small		Big		Small		Big		Small	
1		N/A	mild	13	mild	13	normal	17	normal	17	normal	17
2	normal	17	mild	13	mild	13	normal	17	moderate	10	mild	13
3	normal	17	normal	17	mild	13	mild	13	mild	13	normal	17
4	normal	17	normal	17	mild	13	mild	13	normal	17	normal	17
5	normal	17	mild	13	mild	13	normal	17	normal	17	normal	17
6	normal	17	normal	17	normal	17	mild	13	mild	13	normal	17
	N/A		90		83		90		87		97	
	Big						Small					
Total	N/A						277					

Table 10. The health scores of mantle in sampling II

Sample No	Mantle											
	Group I				Group II				Group III			
	Big		Small		Big		Small		Big		Small	
1	normal	17	mild	13	normal	17	normal	17	normal	17	Normal	17
2	moderate	10	normal	17	normal	17	normal	17	normal	17	Normal	17
3	mild	13	normal	17	normal	17	normal	17	normal	17	moderate	10
4	normal	17	mild	13	mild	13	normal	17	normal	17	Mild	13
5	normal	17	mild	13	normal	17	normal	17	normal	17	Mild	13
6	normal	17	normal	17	mild	13	normal	17	normal	17	Normal	17
	90		90		93		100		100		87	
	Big						Small					
Total	283						277					

Table 11. The health scores of midgut in sampling II

Sample No	Midgut											
	Group I				Group II				Group III			
	Big		Small		Big		Small		Big		Small	
1	mild	13	mild	13	moderate	10	moderate	10	mild	13	Mild	13
2	severe	7	mild	13	mild	13	mild	13	mild	13	Mild	13
3	mild	13	mild	13	mild	13	severe	7	mild	13	Mild	13
4	mild	13	mild	13	moderate	10	mild	13	moderate	10	Mild	13
5	mild	13	mild	13	mild	13	mild	13	severe	7	Mild	13
6	mild	13	mild	13	mild	13	mild	13	mild	13	Mild	13
	73		80		73		70		70		80	
	Big						Small					
Total	217						230					

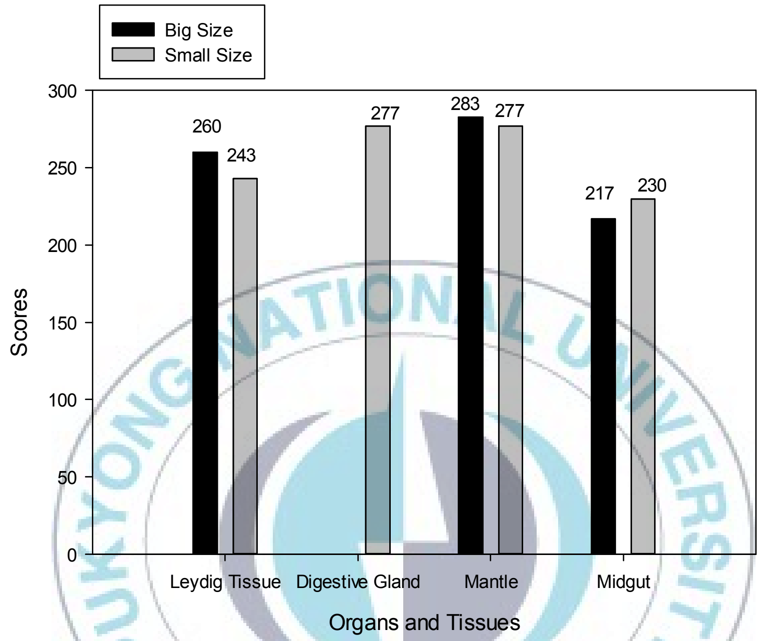

Fig. 2. Histopathological health of big sized and small sized oysters in sampling II

Table 12. The health scores of Leydig tissue in sampling III

Sample No	V- island				Samples No	Sam Cheam Po			
	Leydig's tissue					Leydig's tissue			
	Big		Small			Big		Small	
1	normal	11	mild	9	1	normal	11	normal	11
2	normal	11	mild	9	2	normal	11	normal	11
3	normal	11	mild	9	3	normal	11	normal	11
4	normal	11	mild	9	4	normal	11	normal	11
5	normal	11	normal	11	5	normal	11	normal	11
6	normal	11	mild	9	6	mild	9	normal	11
7	Mild	9	mild	9	7	normal	11	normal	11
8	normal	11	normal	11	8	normal	11	normal	11
9	Mild	9	normal	11	9	normal	11	normal	11
Total		96		87	Total		98		100

Table 13. The health scores of digestive gland in sampling III

Sample No	V- island				Samples No	Sam Cheam Po			
	Digestive gland					Digestive gland			
	Big		Small			Big		Small	
1	Mild	9	mild	9	1	mild	9	normal	11
2	Mild	9	mild	9	2	mild	9	mild	9
3	Normal	11	mild	9	3	mild	9	moderate	7
4	moderate	7	mild	9	4	mild	9	mild	9
5	Mild	9	moderate	7	5	mild	9	moderate	7
6	Normal	11	normal	11	6	mild	9	moderate	7
7	Mild	9	mild	9	7	normal	11	moderate	7
8	moderate	7	mild	9	8		N/A	mild	9
9	Mild	9	normal	11	9		N/A	mild	9
Total		80		82	Total		N/A		73

Table 14. The health scores of midgut for sampling III

Sample No	V- island Midgut				Samples No	Sam Cheam Po Midgut			
	Big		Small			Big		Small	
1	Mild	9	mild	9	1	mild	9	mild	9
2	Mild	9	mild	9	2	mild	9	mild	9
3	Mild	9	mild	9	3	mild	9	mild	9
4	Mild	9	mild	9	4	mild	9	mild	9
5	Mild	9	mild	9	5	mild	9	normal	11
6	Mild	9	mild	9	6	mild	9	mild	9
7	Mild	9	moderate	7	7	mild	9	mild	9
8	Mild	9	mild	9	8	mild	9	mild	9
9	Mild	9	mild	9	9	moderate	7	mild	9
Total	80		78		Total	78		82	

Table 15. The health scores of midgut for sampling III

Sample No	V- island Mantle					Sam Cheam Po Mantle			
	Big		Small			Big		Small	
1	Normal	11	mild	9	1	normal	11	normal	11
2	Normal	11	normal	11	2	normal	11	normal	11
3	Normal	11	normal	11	3	normal	11	normal	11
4	Normal	11	normal	11	4	normal	11	normal	11
5	Normal	11	normal	11	5	normal	11	normal	11
6	Normal	11	mild	9	6	normal	11	mild	9
7	Mild	9	normal	11	7	normal	11	normal	11
8	Normal	11	normal	11	8	normal	11	normal	11
9	Normal	11	normal	11	9	normal	11	normal	11
Total	98		96		Total	100		98	

(a)

(b)

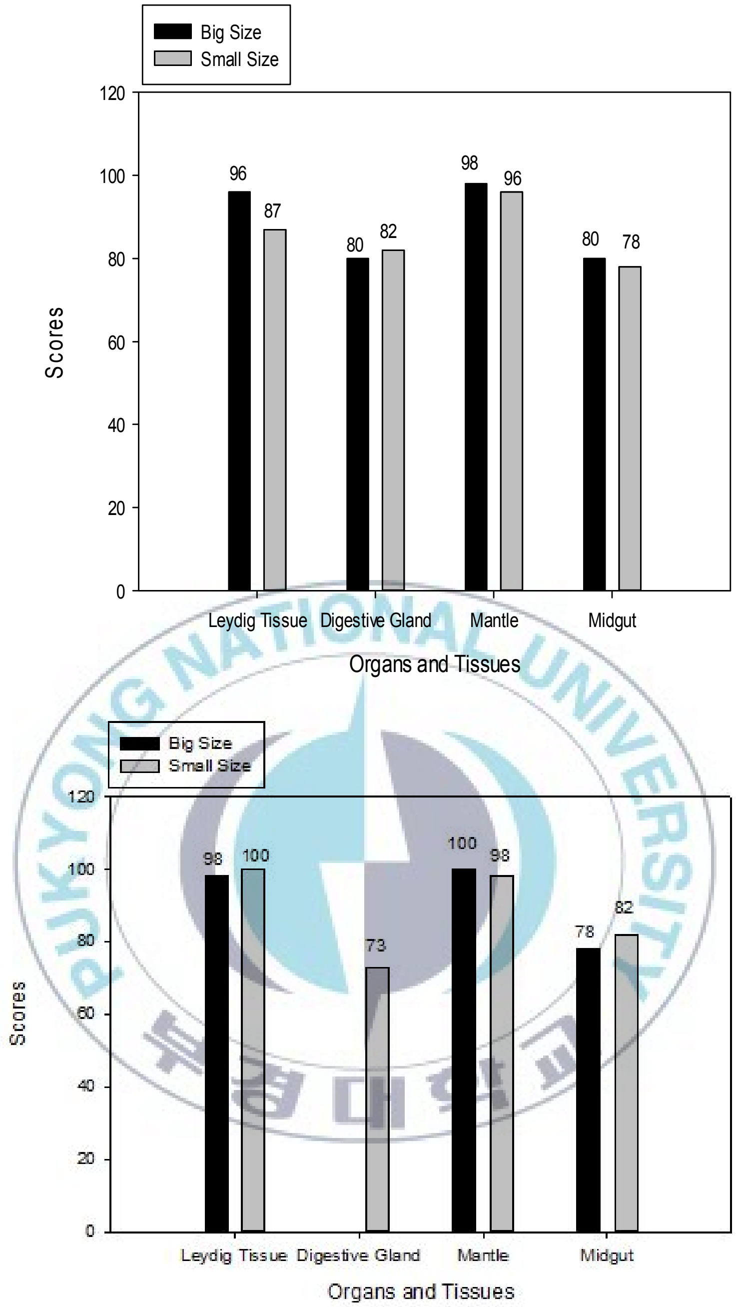

Fig. 3. Histopathological health of big sized and small sized oysters from v-island (a) and Sam Cheam Po (b) farms for sampling III

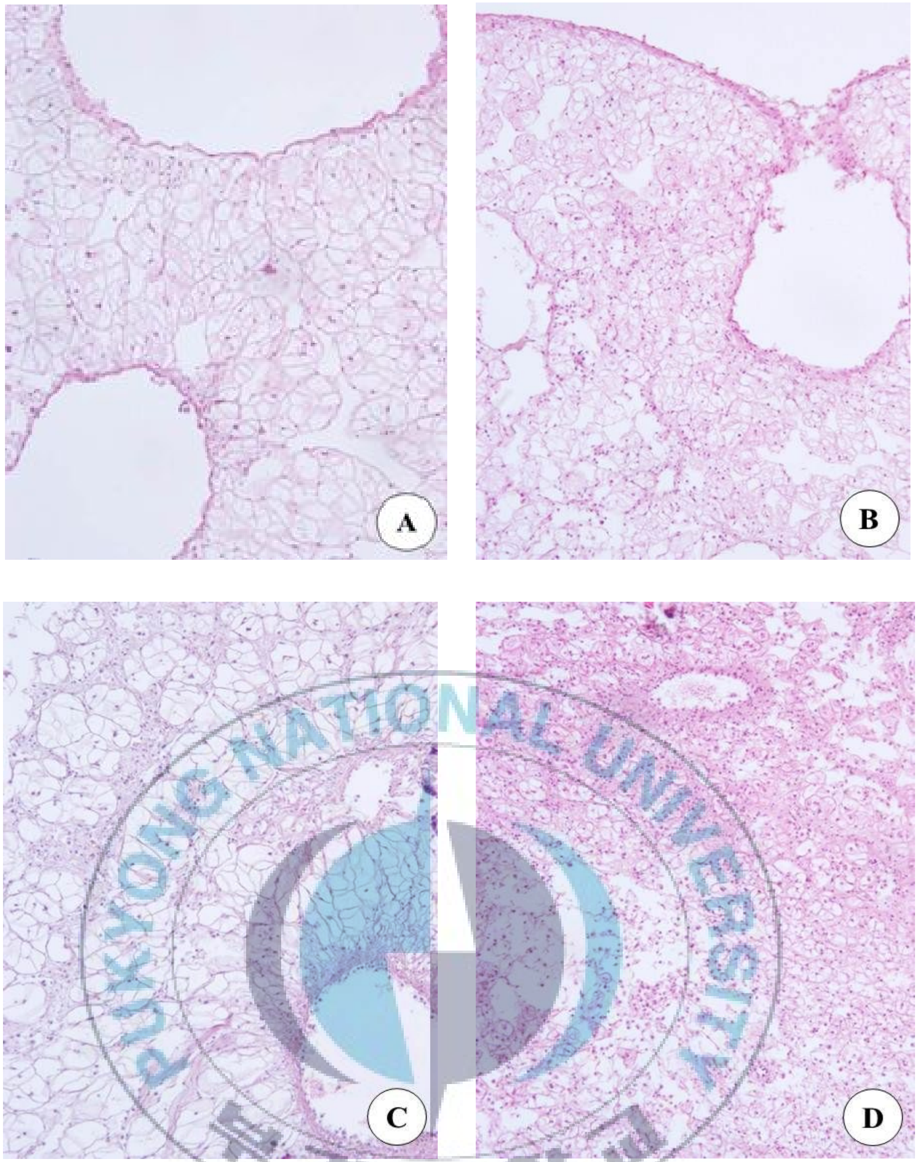

Fig. 4 Histopathological severity of hemocytic infiltration in Leydig's tissue.
A: normal, B: mild, C: moderate, D: severe. X200, H&E

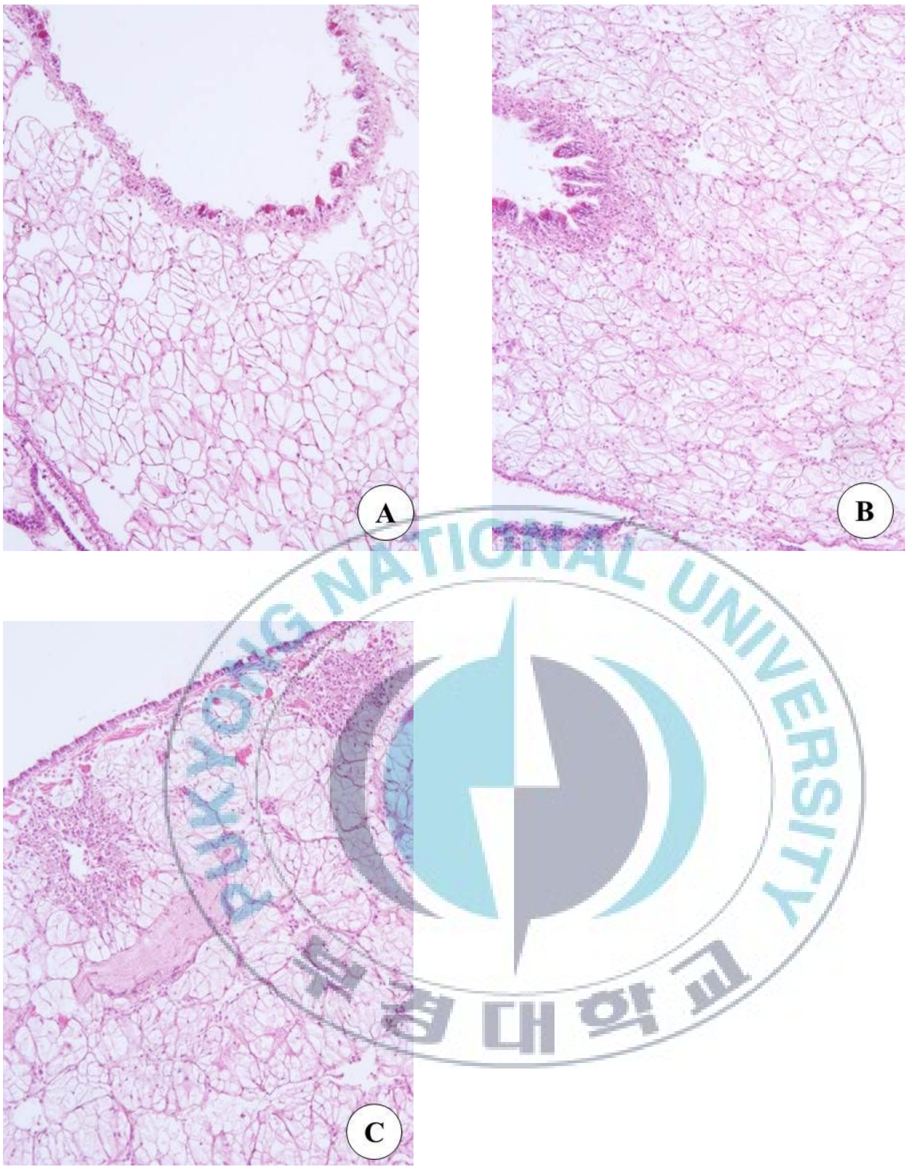

Fig. 5. Histopathological severity of hemocytic infiltration in mantle. A: normal, B: mild, C: moderate, X200, H&E.

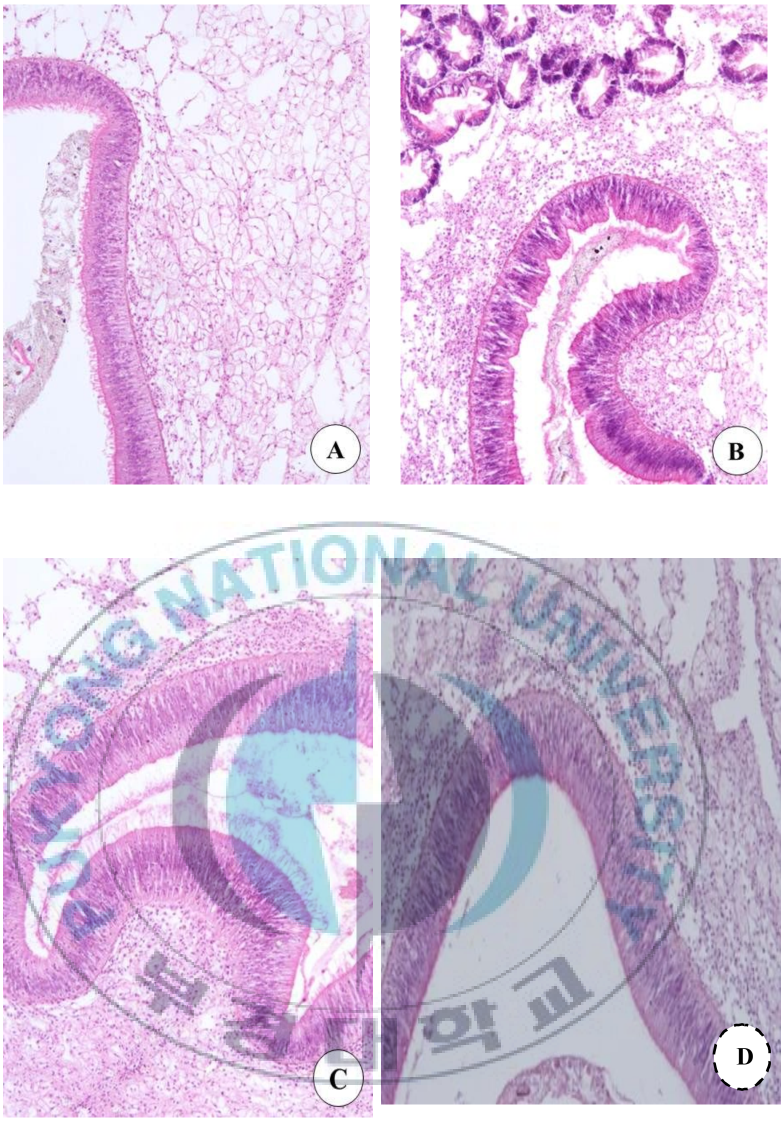

Fig. 6. Histopathological severity of hemocytic infiltration beneath the midgut. A: normal, B: mild, C: moderate, D: severe, X200, H&E

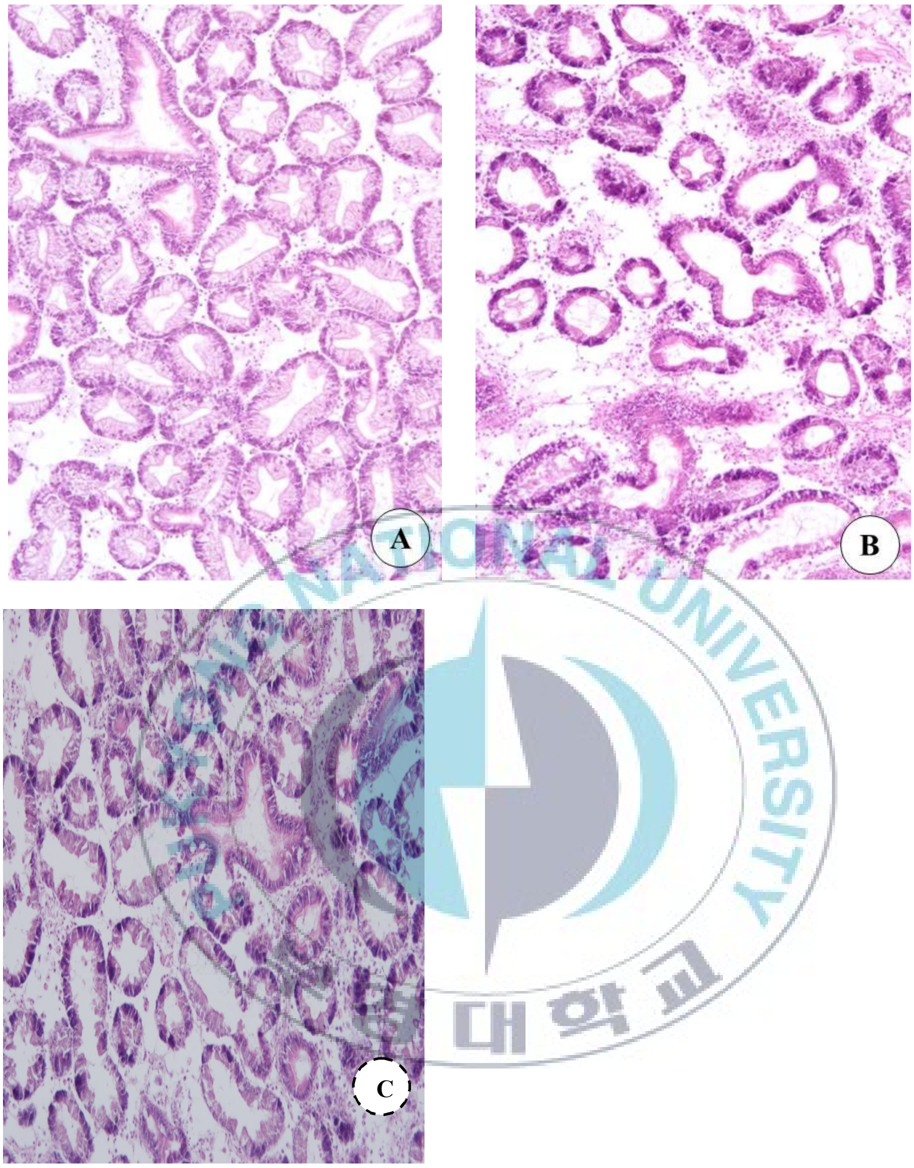

Fig. 7. Histopathological severity of atrophy in digestive glands.
A: normal, B: mild. C: moderate, X200, H&E

Discussion

In the present study histopathological analysis revealed different degrees of severity and intensiveness of hemocytic infiltrations in Leydig's tissues, mantle, midgut as well as digestive gland. Digestive gland is the vital immune organ, which secrets various enzymes to hydrolyze microorganisms and involved in digestive and defense functions. Digestive glands include the salivary glands, gastric glands, intestinal glands, liver, and pancreas. Among important secretions produced by different digestive glands are hydrochloric acid, bile, mucus, and various enzymes.

It is known that the digestive gland of bivalves is subjected to a number of daily and annual cyclic changes caused by digestive and reproductive cycles and by tidal rhythm (Leibson et al., 1979). The midgut is the intestine which lies between the rectum and the stomach. The entire length of the inner wall is made up or a typhlosole. The intestine is filled with ciliated epithelium, where many leucocytes and an abundance of mucous cells. In molluscs, the epithelial cells lining tubules of the gland, pass from 3 up to 5 various phases of the digestive cycle. The four phases of the cycle and the following types of digestive tubules, I—initial normal or holding; II—absorptive and digestive; III—disintegrating and IV—reconstituting, were distinguished in mussels including C. *grayanus* (Kristoforova et al., 2001).

At the same time, it is necessary to note that the availability of granulocytes of different sizes and their varying locations in the digestive gland are usual and often recorded pathological change in bivalve molluscs living in conditions of anthropogenic pollution experimentally subjected to the impact of various pollutants, such as oil carbohydrates, chlorinated pesticides, and heavy metals.

In present study normal cuboidal epithelia of the digestive-gland tubules transforms into simple, low cuboidal epithelia with a large circular lumen however similarity was observed in availability of food materials in the digestive gland of the both sized. According to the grading scores, all of individuals were mostly normal to mild stages in the sampling I. In sampling II, moderate severity was observed only in the big oysters. In the case of sampling III, severity of moderate conditions was found in small oysters. The rest of others were normal and mild stages. However, there was no statistically significant difference in severity among different groups but results obtained from digestive gland enabled us to categorize in three distinct groups depending upon the intensity of infiltration.

Nevertheless, histopathological changes in the digestive gland of mussels such as Gray's mussel have been reported to be applied as an indicator of pollution of the marine environment under bio monitoring. But the changes have been reported to be due to combined effect of complex factor, such as pollution, food, environment etc. Therefore, severity and intensity of histopatholgical changes could not be significant in all the condition. Mantle is the thin layer of the tissues that lines the inner part of each valve. It contains glands that extract elements from the water and convert them to compounds that make up each valve. The principle role is the formation of the shells and the secretion of the ligament as well as playing a part in other biological functions (sensory reception, egg dispersal, respiration, reserve stores, and excretion). In this study histological slides prepared from mantle tissues were observed under a light microscope for all samples. The clear histological features on the severities of hemocytic infiltration in the mantle were observed .In the case of sampling I almost are observed normal and some were mild between big and small sized groups of the oysters .While, In the sampling II, conditions were also about the same and severe conditions were not found. In the sampling III, most of them were normal or in severity. Slight degrees of hemocytic infiltration were found in the mantle .The grading scores in the mantle were nearly the same among two groups of the sampling I, II and III. There were no differences among three samplings .Table (4, 5, and 6) and Fig (1, 2, 3, and 4).

The organs that are associated with food intake, digestion and elimination are: the mouth, oesophagus, stomach, crystalline style sac, digestive diverticula, midgut, and rectum. The midgut is the intestine which lies between the rectum and the stomach. It starts at the stomach′s ventral wall and continues along the sac, then turns around and runs parallel to its former course. The entire length of the inner wall is made up or a typhlosole. This intestine is filled with ciliated epithelium, where many leucocytes are wandering about and where an abundance of mucous cells are present. A muscular layer is not present in the midgut. In the case of this study, hemocytic infiltration was observed around the midgut and also tips of the mantle. In the first and second samples, normal to severe conditions were found .But for third samples, severe stages were not observed in both the sized. In the sampling I, hemocytic infiltration were occurred in the Leydig tissue with the severity of normal to severe stages. In the sampling II, normal to moderate and for sampling III, normal and mild conditions were found.

Changes in histological appearance of Pacific oysters including changes in the Leydig tissue, the types and degree of infiltration of haemocytes and atrophy of digestive tubules show some seasonal trends and are correlated to the gonadal stage of the oyster. Also, digestive tubule atrophy and abundance of brown cells are correlated with lower salinity.

It is clear that oysters have an effective defence mechanism against solid, nontoxic particles; they localize the particles by forming granulomas similar to those observed in insects and vertebrates. However summer mortality of Pacific oysters is known in several countries. But no specific pathogen has been systematically associated with this phenomenon. A complex combination of environmental and biological parameters has been suggested as the cause and is now starting to be identified. Moreover, parasitic invasion has been also revealed in molluscs. However, it is known that bivalves living in conditions of chronic pollution have been reported to be less sustainable to parasitic infestation than individuals living in clear areas. As for example, the extremely high extent of interstitial parasite infestation (100% frequency of occurrence) in the digestive gland of Gray's mussel from Sivuchya Bay has been reported which arose from chronic pollution of that bay. In the present study, unknown protozoan parasites infection among the big sized oyster, clearly indicated that bigger size oyster are more vulnerable to disease in terms of histopatholgical evaluation.

Conclusion

The demand of pacific oyster in domestic as well as international seafood market has rapidly increased due to growing interest in healthy human food and improved purchasing power. The phenomenon known as *Crassostre gigas* " summer mortality" first began along the Japanese Pacific coast in 1945 and The Republic of Korea has also experienced the same frequently. However, a complex combination of environmental and biological parameters has been suggested as the cause and is now starting to be identified .Successful fish and shellfish health management begins with prevention of disease rather than treatment.

Therefore, a clear understanding of histopathology and path physiology is necessary to determine the real causes of diseases. In present study, clear histological features on the severities (normal, mild, moderate, severe. X200, H&E.) of hemocytic infiltration in body, mental, digestive gland were observed. However, there was no significant difference among in these parameters among sample I, II and III .Unidentified protozoan parasites infection among the big sized oyster, clearly indicated that bigger size oyster are more vulnerable to disease in terms of histopathological evaluation. Therefore, the results obtained from present study indicated that, growing individuals of Pacific oyster should be taken more care to prevent disease and mortality. Histopathological health analysis could be a vital and cost effective tool in scientific health management of Pacific oyster.

Acknowledgment

First of all, I would like to express my heartfelt thanks to my supervisor, Professor Min Do Huh who gave me an opportunities to study at Aquatic life medicine department with his kind supports , guidance and encouragement. I also would like to give my sincere gratitude to Dr Mu Kun Lee, who gave me invaluable guidance and provided the comments for preparing and improving this thesis.

My special thanks to Professor Yong Ki Hong for his kindness and caring during the study period. I also would like to extend the special thanks to Dr. Kyoungmi Kang, I appreciated her honest love, mental support, encouragement and all that she has done for me. And also sincere appreciations are extend to my laboratory's members, especially Kim Bo Sung and all of laboratory friends for their friendly help and care for my study, they accompanied and made my life smooth during the time I was in Korea.

A word of thank and highly gratitude to KOICA for offering this scholarship program and gave me a chance to study and to get a good life in Korea .

My deep gratitude is to my beloved parents Mr. Win Maung and Mrs. Mya Mya Win bringing me into this world and raising me till this day , I will always do my best to meet their expectations .

Finally, I would like to thank to my beloved friends who gave me suggestions and kind cooperation in my research and great friendship . The friendship between us could last forever .I will never forget that and always keep in my good memory .

References

Alfnes, F., Guttormsen, A.G., Steine, G., Kolstad, K., 2006. Consumers' willingness to pay. Aquat. Org. 63, 33−41.

Cheney, D.P., MacDonald, B.F., Elston, R.A., 2000. Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. J. Shellfish Res. 19, 353−359.

Elston, R.A., Beattie, J.H., Friedman, C., Hedrick, R., Kent, M.L., 1987. Pathology and significance of fatal inflammatory bacteraemiain the Pacific oyster, *Crassostrea gigas* Thunberg. J. Fish Dis. 10, 121−132.

Evans, S., Camara, M.D., and Langdon, C.J., 2009. Heritability of shell pigmentatio n in the Pacific oyster, *Crassostrea gigas*. Aquaculture. 286. 211-216

Friedman, C.S., Beaman, B.L., Chun, J., Goodfellow, M., Gee, A., Hedrick, R.P., 1998. *Nocardia crassostreae* sp. nov., the causal agent of nocardiosis in Pacific Oysters. Int. J. Syst. Bacteriol. 48, 237−246.

Friedman, C.S., Beattie, J.H., Elston, R.A., Hedrick, R.P., 1991. Investigation of the relationship between the presence of a gram positive bacterial infection and summer mortality of the Pacific Oyter, *Crassostrea gigas* Thunberg. Aquaculture. 94, 1−15.

Friedman, C.S., Estes, R.M., Stokes, N.A., Burge, C.A., Hargove, J.S., Barber, B.J., Elston, R.A., Burreson, E.M., Reece, K.S., 2005. Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes.

Gay, M., Renault, T., Pons, A.M., Le Roux, F., 2004. Two *Vibrio splendidus* related strains collaborate to kill *Crassostrea gigas*: taxonomy and host alterations. Dis Aquat Organ. 23; 62(1-2):65−74.

Glude, J.B., 1975. A summary report of the Pacific Coast oyster mortality investigations 1965−1972. Proceedings of the Third U.S−Japan meeting on Aquaculture at Tokyo, Japan, October15−16, 1974, 28.

Goulletquer, P., Soletchnik, P., Le Moine, O., Razet, D., Geairon, P., Faury, N., Taillade, S., 1998. Summer morality of the Pacific cupped oyster *Crassostrea gigas* in the Bay of Marennes – Oleron (France). ICES Mariculture Committee CM 1998/CC, pp. 14−21.

Goulletquer, P., Soletchnik, P., Le Moine, O., Razet, D., Geairon, P.,Faury, N., Taillade, S., 1998. Summer morality of the Pacific cupped oyster *Crassostrea gigas* in the Bay of Marennes-Oleron(France). ICES Mariculture Committee CM 1998/CC, pp. 14−21.

J.F. Samain, L. Dégremont , P. Soletchnik , J. Haure , E. Bédier, M. Ropert ,J. Moal , A. Huvet , H. Bacca , A. Van Wormhoudt , M. Delaporte, K. Costil, S. Pouvreau, C. Lambert, V. Boulo, P.

Soudant, J.L. Nicolas, F. Le Roux, T. Renault, B. Gagnaire, F.Geret, I. Boutet, T. Burgeot and P. Boudry. 2007. Genetically based resistance to summer mortality in the Pacific oyster (*Crassostrea gigas*) and its relationship with physiological, immunological characteristics and infection processes. Aquaculture. 298.pp227-243.

Kahn, B.E., Wansink, B., 2004. The influence of assortment structure on perceived variety and consumption quantities. J. Consum. Res. 30, 519−533.

Koganezawa, A., 1974. Present status of studies on the mass mortality of cultured oysters in Japan and its prevention. In: (Ed.), Third U.S.−Japan Meeting on Aquaculture, Tokyo, Japan.

LeRoux,F.,2004.Taxonomieetvirulencedevibrionspathogensd'huîtrescreuses*Crassostreagigas.*Habilitation à DirigerdesRecherchesUniversité Paris VI.

Lipovsky,V.P.,Chew,K.K., 1972.Mortality of Pacific oysters, *Crassostreagigas*: the influence of temperature and enriched seawater on survival. Proc. Natl. Shellfish. Ass. 62, 72−82.

Mori, K., 1979. Effects of artificial eutrophication on the metabolism of the Japanese oyster *Crassostrea gigas*. Mar. Biol. 53, 361−369.

Nell, J.A., 2001. The history of oyster farming in Australia. Mar. Fish. Rev. 63,14−25.

Perdue,J.A.,Beattie,J.H.,Chew,K.K.,1981.Some relationships between gameto genic cycle and summer mortality phenomenon in the Pacific oyster (*Crassostreagigas*) in Washington state. J.Shellfish Res. 1, 9−16.

Renault, T., Le Deuff, R.M., Cochennec, N., Maffart, P., 1994.Herpesvirus associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France—comparative study. Rev. Méd. Vét.145 (10), 735−742.Resour. 10, 177−185.

Resour. 12, 131−143.

Soletchnik, P., Le Moine, O., Faury, N., Razet, D., Geairon, P., Goulletquer, P., 1999.Summer mortality of the oyster in the Bay Marennes-Oleron: spatial variability of environment and biologyusing a geographical information system (GIS). Aquat. Living

Soletchnik,P.,Razet,D.,Geairon,P.,Faury,N.,Goulletquer,P., 1997.Ecophysiology of maturation and spawning in oyster (*Crassostrea gigas*): metabolic (respiration) and feeding (clearance and absorption rates) responses at different maturation stages. Aquat. Living

Sylvia, G., Morrissey, M.T., Graham, T., Garcia, S., 1995.Organoleptic qualities of farmed and wild salmon. J. Aquat. Food Prod. Technol. 4, 51−64.