Thesis for the Degree of Master of Fisheries Science

Temperature effect on the growth and gonad development of the Pacific Oyster *Crassostrea gigas*

by

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KOICA-PKNU International Graduate Program of Fisheries Science

Graduate School of Global Fisheries

Pukyong National University

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참굴의 성장과 생식소발달에 미치는 온도의 영향

Advisor: Prof. Wongyu Park

by

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Abstract

Growth and gonad development of the Pacific Oyster *Crassostrea gigas* was investigated in El Salvador and Korea. *C. gigas* was kept at three different water temperatures, 28 °C (normal temperature in tropical areas), 24 °C (optimal temperature for *Crassostrea gigas*) and 15 °C (normal temperature in winter in Korea). Oyster seeds and adults were set in three tanks at three different temperatures. Oysters were maintained during two months and checked fortnightly. A mixture of phytoplankton (*Isochrysis galbana* and *Tetraselmis sp.*) at the proportion of 50:50 was provided every day during the experiment. Growth of oyster increased at 15 °C while it

decreased at 24 °C and 28°C. Growth rate was lowest at 28°C (ANOVA, p=0.002).. Experiment results were compared with data studied in El Salvador. For the oyster sampled randomly in El Salvador, the condition index trough the months sampled fluctuated from 7.4 % reported for the month of January 2013 being the lowest value, to the highest value reported with 18.1 % in November 2013. Condition index were significantly different by months ($p = 0.000$).

Introduction

Physiological processes of bivalves are mainly affected by temperature, foods, and environmental factors such as sediment types and tidal rhythms. In a latitudinal scale, latitudinal differences of growth rate in bivalves have been frequently related to latitudinal gradients in temperature (Gilbert, 1973; Bachelet, 1980; Appeldoorn, 1983, 1995; Beukema and Meehan, 1985; Hech et al., 2002; Fiori and Morsán, 2004). At a regional scale, growth and reproduction of bivalves are influenced by other environmental factors, such as food quality and quantity, tidal level and sediment types (Newell and Hidu, 1982; De Montaudouin, 1996; Beukema and Cadée, 1997; Honkoop and Beukema, 1997; Beukema et al., 2002; Carmichael et al., 2004).

Crassostrea gigas is a cosmopolitan species, being recorded from Japan, Korea, Siberia, Australia, North America from the southeastern Alaska to Baja California, and Europe from the southern British Isles to Portugal in the Mediterranean (http://www.fao.org/fishery/species/3514/). The commercial fishery for this species has grown rapidly in their distributional ranges. *C. gigas* is a dominant shellfish in aquaculture industry along the Pacific Coast. Because of its commercial importance, *C. gigas* have largely been harvested from many countries along the Pacific coasts.

The reproductive cycle of *C. gigas* is principally regulated by the amount and quality of the food, as well as by temperature (Imai and Sakai, 1961; Dinamani, 1987). Gametogenesis in oysters is directly correlated with water temperature (Muranaka and Lannan, 1964). Water temperature influences gonadal development while food enhanced fertility and gamete quality (Loosanoff and Davis, 1963; Lannan et al., 1980; Muranaka and Lannan, 1984; Utting and Millican, 1997). In temperate regions, *C. gigas* exhibited a seasonal reproductive cycle, clearly related to temperature with (1) initiation of gametogenesis usually observed in winter when water temperature was low; (2) active phase of gametogenesis (growing stage) in spring when water temperature increased; (3) maturity and spawning in summer, when temperature was above 19 °C (Mann, 1979); (4) resorption in autumn (degenerating stage).

Since early 2000's, *C. gigas* has been introduced to El Salvador at Jiquilisco Bay and Fonseca Gulf, as an alternative specie for aquaculture by fishermen (Vasquez, et al.; 2009). Since its introduction, the oyster culture has been an important economic activity in the fishermen's communities. However, seed production and maturation process have not been successfully conducted.

The aim of this study is to observe the temperature effects on the growth and gonad development of *C. gigas*. This research will be helpful of applying the knowledge and tools for the development of others native bivalves species for aquaculture in El Salvador.

Hypothesis:

Ho: The water temperature is not related with the gonad development of *C. gigas*.

Ha: The water temperature is related with the gonad development of *C. gigas.*

Materials and methods

1. Sampling area

The site is located at the eastern part of El Salvador in Conchaguita island and Fonseca Gulf (13° 14´ 16.64" N, 87° 45´ 13.02" W), which waters are shared with Nicaragua and Honduras (Figure 1).

Figure 1. From left to right, El Triunfo Port and Conchaguita island, locations where *Crassostrea gigas* has been introduced in El Salvador since 2001 to 2013.

Long line systems were used to culture the oysters. Rainfall patterns in the area of the Gulf of Fonseca are irregular; the average annual rainfall in the region varies from 1,500 mm in the northwest to more than 2,000 mm in the southwest. The wettest month is usually September and the driest one is January. The rainy season lasts about five months (from mid-May to mid-July and mid-August to late October). The dry season continues six months, beginning in November and ending in April. There is a short period in the summer rainy season (July and August). Mean water temperatures range from 27°C to 30°C with the annual average of 30 °C. Mean salinities range from 28 to 34‰ with the lowest salinity for the period of rainy season and highest salinity in summer season (MARN, 2014).

1.1 Gonadic development of *Crassostrea gigas*

C. gigas seeds were produced in the mollusk laboratory of the Fisheries and Aquaculture Development Center in El Triunfo Port and then transported to Conchaguita island in a cooler to protect them against the environment. The batches correspond to those spawned in the months of April and October in 2012.

C. gigas was randomly sampled about 20 individuals from December 2012 to March 2014, except April, July and December 2013, due to the lack of transportation and equipment. Then, they were transported to the mollusk laboratory in Port El Triunfo in a cooler to protect them against the environment.

Total length (TL), total weight (TW), meat weight (MW), visual observation (VO), sex (S) and maturation stage (MS) were measured. Shell length (SL) was measured using a Vernier Caliper, brand Mitutoyo, series 530-105 Standard Model with accuracy of 0.0001 mm. TW and MW were weighed with a digital precision scale (Shimadzu, model BL 2200H) up to 0.01 gram. The color of gonads was classified with naked eyes with categories of transparent, spots (white spots), complete (complete white covered the gonad area) and channels (white channels observed). Sex and maturation stage were identified under a microscope at 40 X. Sexes were categorized into three categories of male, female or unidentified. Maturation stages were classified to undefined, matured, partial spawn, total spawn and post spawn.

2. Temperature effect on growth, weight and condition index.

2.1 Experiment designs

Growth, total weight, meat weight, condition index and gonad tissue in *C. gigas* were investigated under three temperature gradients: 28°C, simulating the conditions in El Salvador; 24°C, the optimal temperature for oyster culture; and 15°C (control), mean temperature in the month of January and February in the Fisheries Research Institute of Pukyong National University, Korea.

C. gigas seeds and adults were brought from the seed production site (35° 4'25.08"N and 128°50'31.50"E). Oyster seeds and adults were reared in three tanks from January and February 2014. For the beginning of the experiment, oysters were collected at 15°C and then transported to the laboratory. They were kept in the tanks and then acclimated to designed water temperatures at 24°C and 28°C. The temperature was raised 1°C each day until the designed temperature was reached.

Ten oyster seeds were sampled fortnightly. TL, TW and MW were measured at each condition. In the same tanks with different temperature treatment, 20 adult oysters were reared.

Each of the tanks was fed with phytoplankton, *Isochrysis galbana* and *Chaetoceros* sp. at a proportion of 50:50 of the concentration of 150,000 cel/ml every day during the experiment period.

For each group of oysters, condition index was calculated using the formula:

$$CI = \frac{\text{Meat weight (MW)}}{\text{Total weight (TW)}} \times 100$$

3. Statistical analysis

To evaluate significance of differences among the 3 treatments of temperature and different parameters recorded, one way ANOVA analysis were used with the software SPSS (ver. 14, IBM).

Results

1. Gonadic development of *Crassostrea gigas* in El Salvador

The Condition Index (CI) during the research period fluctuated from 7.4 %, the lowest value in January 2013, to 18.1%, the highest value in November 2013. CI was significantly different between months ($p = 0.000$) (Figure 2).

In sex definition, the proportion of undefined was the highest in March through May in 2013 and there were no observations of undefined specimens in January, February, September, and October 2013, January and March 2014 (Figure 3).

The proportion of female was the lowest in January 2013 and the highest in July 2013. The proportion of male was the lowest in June 2013 and the highest in December 2012 and August 2013 (Table 1). The proportion of male was relatively higher in February 2013 and January to March 2014 with a average of the 70% of total individuals while that of female was reached up to 40 % of individuals in March to November 2013.

Higher proportion of undefined organisms was also found from February to August in 2013.

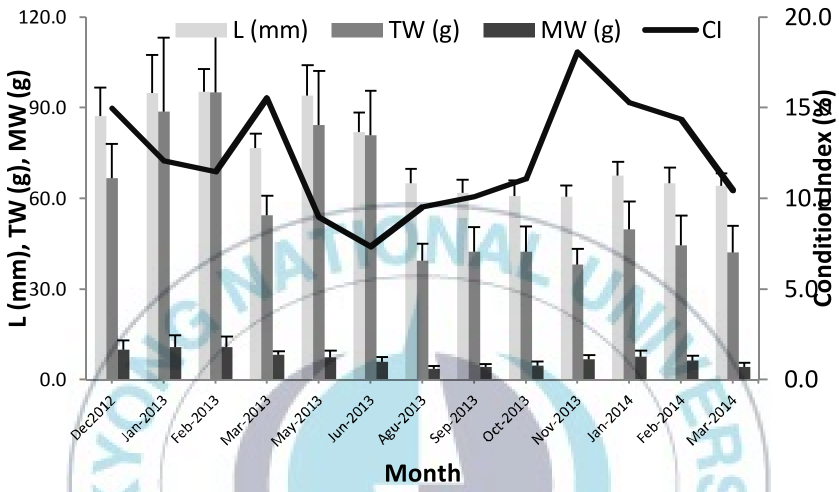

Figure 2. Monitoring of Pacific oyster *Crassostrea gigas*: Shell length, total weight, meat weight and condition index in Fonseca Gulf, La Union, El Salvador.

Figure 3. Proportion of sex in the Pacific oyster *Crassostrea* gigas from Fonseca Gulf, La Union, El Salvador from December 2012 to January 2014.

The proportion of female was the lowest in January 2013 and the highest in July 2013. The proportion of male was the lowest in June 2013 and the highest in December 2012 and August 2013 (Table 1). The proportion of male was relatively higher in February 2013 and January to March 2014 with a average of the 70% of total individuals while that of female was reached up to 40 % of individuals in March to November 2013. Higher proportion of undefined organisms was also found from February to August in 2013.

In gonad color analysis, transparent gonads were observed from March to September 2013, which matches the highest rates of rain of the year. Spot gonad was the most fluctuated among the categories, peaked in March, August, November and February 013. Complete gonad was observed only for the months of January and February of 2013 while channel gonad which is related with full maturity of gonads was observed in January and October in 2013, which matches dry season (Figure 4, 5).

The highest amount of rain recorded for the period was in September 2013 in the middle of winter (rainy season) while no precipitation was recorded in summer from December 2012 to March 2013, and December 2013 to April 2014 (Figure 6).

Table 1. Sex ratio of the Pacific oyster *Crassostrea gigas* from Fonseca Gulf, El Salvador during December 2012 to March 2014

Date	Total	Female	Male	Sex Ratio
D-2012	24	3	21	1:7.0
J-2013	16	1	15	1:15.0
F-2013	20	2	18	1:9.0
M-2013	21	2	19	1:9.5
M-2013	16	9	7	1:0.8
J-2013	19	14	5	1:0.4
A-2013	29	8	21	1:2.6
S-2013	24	6	18	1:3.0
O-2013	24	11	13	1:1.2
N-2013	23	11	12	1:1.1
J-2014	24	5	19	1:3.8
F-2014	22	4	18	1:4.5
M-2014	24	6	18	1:3.0
Total	286	82	204	1:2.5

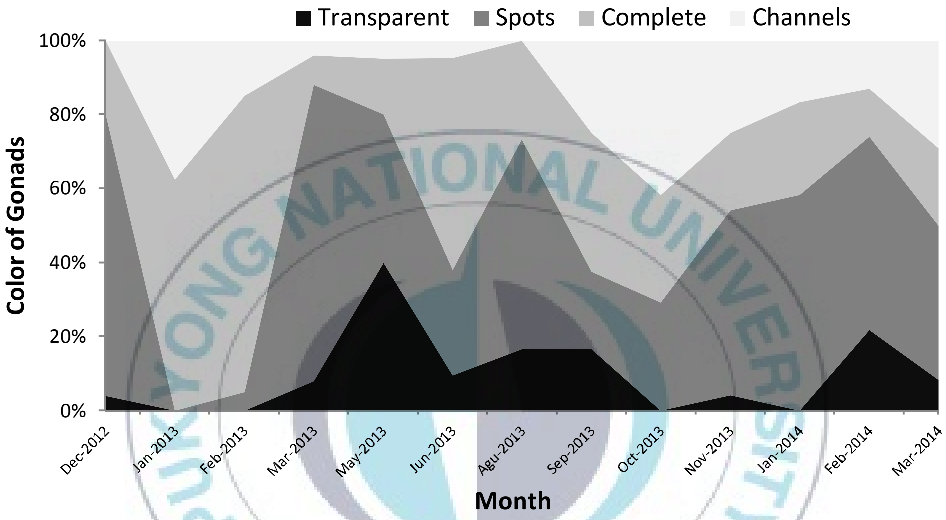

Figure 4. Gonad color of the Pacific oyster *Crassostrea gigas* from Fonseca Gulf, La Union, El Salvador from December 2012 to January 2014.

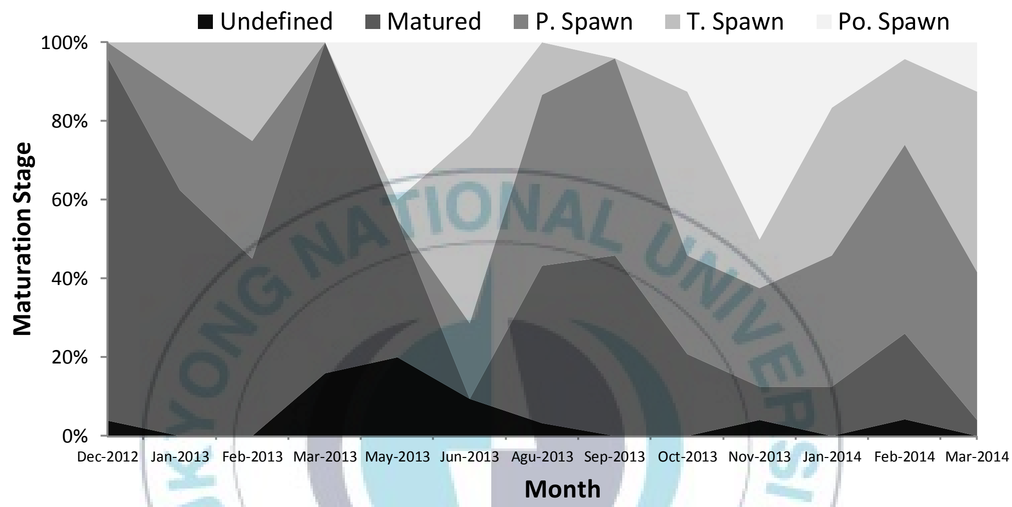

Figure 5. Maturation stages: undefined, mature, partial spawn, total spawn, post spawn in the Pacific oyster *Crassostrea gigas* from Fonseca Gulf, El Salvador from December 2012 to January 2014.

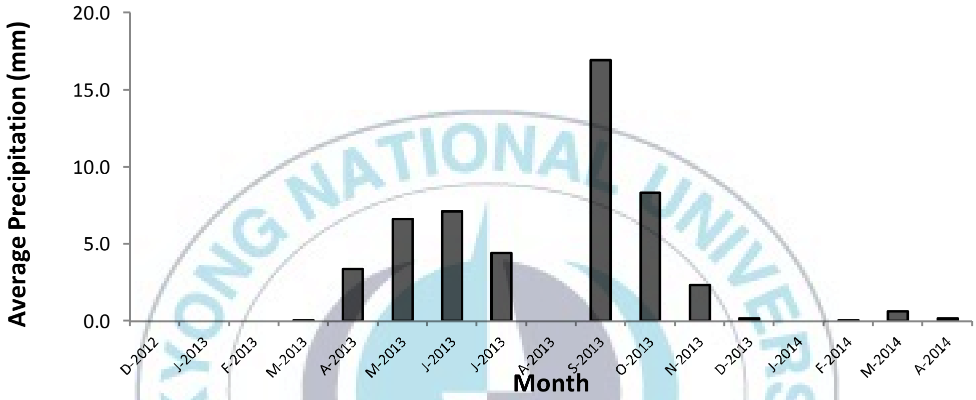

Figure 6. Average precipitation (mm.) in Fonseca Gulf, La Union, El Salvador from December 2012 to April 2014. (Source: Direccion General de Meterologia, Ministry of Environment and Natural Resources, El Salvador, 2014)

2. Temperature effect on growth, weight and condition index

2.1 Growth and condition index: juvenile (seed) specimens

The total length varied with 3 different treatments. The Control showed a decreasing in shell length. On the other hand, treatments at 24 °C and 28°C showed a increasing tendency in all the sampling dates (Figure 7). The total weight and meat weight indicated the same tendency with total length. In this case the temperature could affect the increasing in total weight for the last two treatments. (Figure 8, 9).

The condition index varied with 3 temperature treatments. Individuals at the control increased while ones at 24 °C and 28°C decreased with the lowest at 28°C treatment (one way ANOVA, $p = 0.002$) (Figure 10).

2.2 Growth and condition index: Adults specimens

Condition index was higher at lower temperatures while that was lower at 24°C and 28°C (Figure 11).

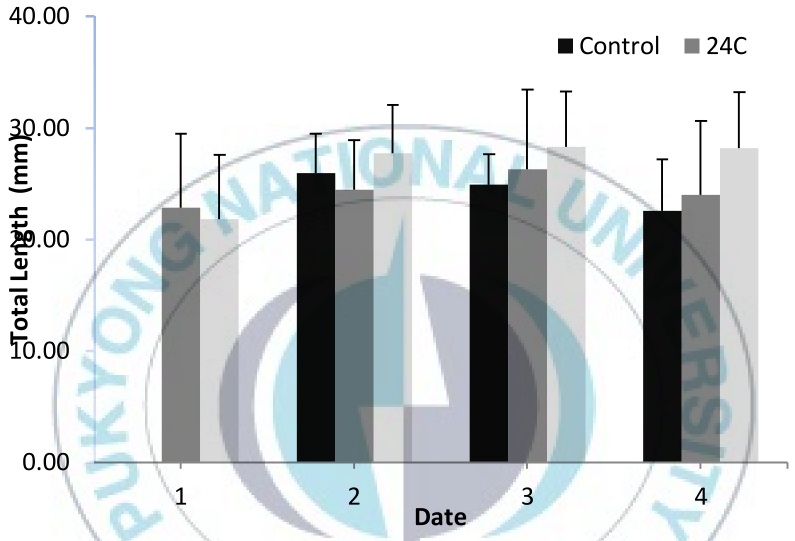

Figure 7. Total length of the Pacific oyster *Crassostrea gigas* at three different temperatures: Control, 24 °C and 28°C.

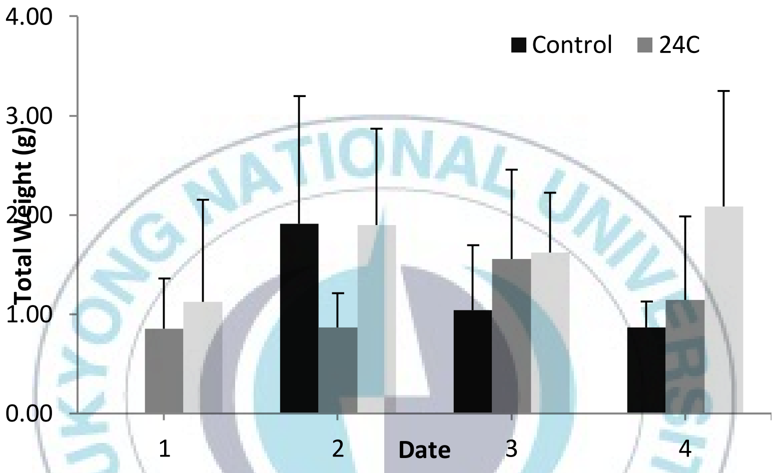

Figure 8. Total weight of the Pacific oyster *Crassostrea gigas* at three different temperatures: Control, 24 °C and 28°C.

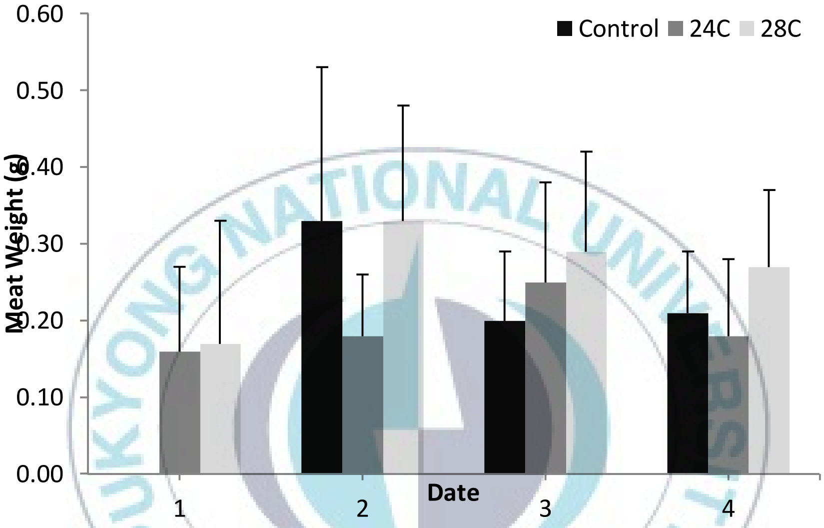

Figure 9. Meat weight of the Pacific Oyster *Crassostrea gigas* at three different temperatures: Control, 24 °C and 28°C.

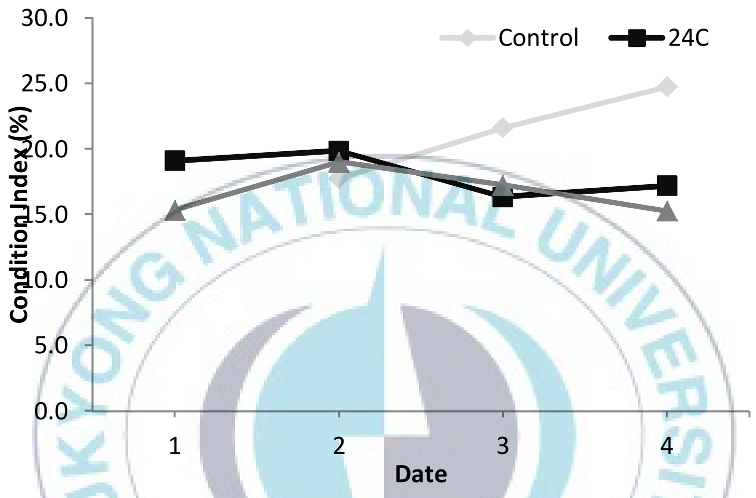

Figure 10. Condition index in the Pacific oyster *Crassostrea gigas* at three different temperatures: Control, 24 °C and 28°C.

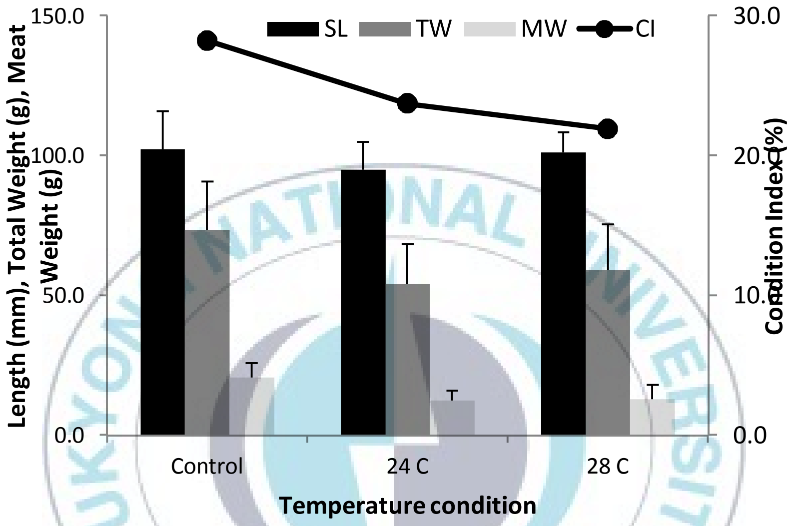

Figure 11. Condition index, total length, total weight and meat weight of the Pacific oyster *Crassostrea gigas* at three different temperatures: Control, 24 °C and 28°C.

Discussion

Considerable attention has been devoted to understanding the role of water temperature on reproduction (Thompson et al., 1996). Gametogenesis in oysters is directly correlated with water temperature (Muranaka and Lannan, 1984). This knowledge has been used in hatcheries by keeping animals at elevated temperatures with a suitable food ration to accelerate gamete maturation (Utting, 1993). However, many aspects of the effect temperature on the gonadal development of *C. gigas* remained unknown.

One of the most important biological processes for cultivating this organism is the reproductive cycle which is principally regulated by the amount and quality of the food, as well as by temperature (Imai and Sakai, 196 1; Dinamani, 1987).

In regarding to the data of gonad observation in El Salvador, there were significant differences among the months (Dec. 2012 to march 2014), with a values of $p < 0.000$ for each parameter, showing an evidence of the development of Crassostrea gigas in a tropical environment. (Vasquez, *et al.*, 2009).

Comparing the condition index of *Crassostrea gigas* through the period sampled Dec-2012 to March-2014, there were found big variations in condition index, this matches the rainy season in El Salvador. Two factors could be evolved: the lowest values of salinity and the production of phytoplankton which is related with the amount of nutrients that could be founded in the environment.

Literature does point to the fact that the proportion of males is higher at the early stage, with increase in the proportion of females arising trough sex reversal as the oyster gets older (Orton, 1933; Galtsoff, 1964; Thompson et al., 1996; Guo et al., 1998). According with Park et al., 2012, sex reversal pattern of oyster, *C. gigas* appears to go from male→female→male, and the sex is rhythmical hermaphroditism.

This study was coincided with the protandric hermaphrodite behavior of *C. gigas* in which individuals mature first as males changing later to female. One finding about this result was when comparing data obtained in El Salvador with the one observed in Korea (Park et al., 2012) the sex ratio it is considerable higher 1:1.0 in Korea vs. 1:2.5 in El Salvador; suggesting that the water temperature conditions in El Salvador might affect and enhance the proportion of males against females in the same population.

When comparing shell length of the 3 temperature treatments for adult oysters group, no big differences were observed. The same behavior was also observed in seeds oyster group. For the case of meat weight and total weight, one way ANOVA was conducted and show significant differences with p values of 0.000 and 0.004 respectively. This tendency was also observed in the case of small seeds group of *Crassostrea gigas*.

In the case of shell length for small oyster group, no significant differences ($p \geq 0.219$) were founded, meanwhile meat weight was marginally significant with p value of ≥ 0.052 and total weight p was ≤ 0.020 which has significant differences among the water temperature treatments.

In the case of Condition Index (CI) there were recorded significant differences among the treatment when applying one-way ANOVA test, with a p value of 0.003.This also match the results obtained for the small seeds group with a p value of 0.002.

Comparing the results obtained with the water temperature experiment and gonad observations in El Salvador, there were some important findings that concern with temperature. It seems that meanwhile the temperature rise, the condition index decrease as shown in the results for

28 °C (Figure 9, 10). It appears to be a misbalance in energy allocation when oyster of different groups (seeds and adults) are maintained in high temperature (28°C) considering that Crassostrea gigas is originally from temperate waters. This matches the results obtained by Chavez-Villalba et al. (2002), and Bourgrier et al. (1995), that signaled that filtration rates decreased while respiration increases at 25 °C.

In El Salvador the survival rate of *Crassotrea gigas* has been reported in 20 % at the end of 8 months of cultivation in Fonseca Gulf, La Union. (Vasquez et al., 2009). The survival rate is considerable low when compared with 70 % survival rate, reported in Korea and Japan with temperate waters where *Crassostrea gigas* is originally founded.

As a conclusion, taking in consideration the findings in this investigation, the average temperature of 28°C directly affects the allocation of energy into the development process of *Crassostrea gigas* in tropical conditions, when taking in consideration the temperature as main factor; rejecting our null hypothesis.

Further research must be done in El Salvador to monitoring the process of adaptation of this specie to tropical conditions to be able to replicate this initiatives in others locations or countries with similar water temperature conditions.

For detail information and basic knowledge, histological techniques must be applied in future research, to be capable of taking more accurate decisions over the future of *Crassostrea gigas* in locations with tropical water conditions. One concern about the gonads maturation of *Crassostrea gigas* in natural conditions (28°C) in El Salvador is that the Pacific Oyster is not an endemic species and has been reported as invasive species, this could be a problem for the environment causing damage to the surrounding ecosystem where the culture sites are located.

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Appendices

	Comercial importance(Edible) marine shellfish in El Salvador			Korean Shellfish, related with Class and Family.		
	Class	Family	Specie	Family	Specie	Book #
1	Bivalvia	Arcidae	Anadara grandis (Broderip y Sowerby, 1829)	Arcidae	Scapharca broughtonii (Schrenck, 1867), Sacpharca inaequivalvis (Bruguiere, 1789), Scapharca satowi (Dunker, 1882), Sacpharca subcrenata (Lischke, 1869)	1241 - 1243
2	Bivalvia	Arcidae	Anadara similis (C. B. Adams, 1852)			
3	Bivalvia	Arcidae	Anadara tuberculosa (Sowerby, 1833)			
4	Bivalvia	Mytilidae	Modolius capax (Conrad, 1837)	Mytilidae	Modiolus sp. (7 species), Mytillus galloprovincialis (Lamarck, 1819), Mytilus coruscus (Gould, 1853)	1190 - 1204
5	Bivalvia	Mytilidae	Mytella guyanensis (Lamarck, 1819)			
6	Bivalvia	Ostreidae	Crassostrea iridescens (Hanley, 1854)	Ostreide	Crassostrea gigas (Thumberg, 1793)	1291
7	Bivalvia	Spondylidae	Spondylus calcifer (Carpenter, 1857)	Spondylidae	S. varius (Sowerby I, 1827), S. balbatus cruentus (Lischke, 1868)	1327-1329
8	Bivalvia	Corbiculidae	Polymesoda inflata (Philippi, 1851)	Corbiculidae	C. japonica , C. colorata	1501 - 1506
9	Bivalvia	Veneridae	Chione subrugosa (Sowerby, 1835)	Veneridae	Protothaca (Notochione) jedoensis (Lischke, 1874), Protothaca (Novathaca) euglypta (Sowerby III, 1914)	1517 - 1518
10	Bivalvia	Veneridae	Protothaca asperrima (Sowerby, 1835)			
11	Bivalvia	Donacidae	Donax dentifer (Hanley, 1843)	N/A		
12	Gastropoda	Strombidae	Strombus galeatus (Swainson, 1823)	Gastropoda	Doxander vittatus japonicus (Reeve, 1851)	294
13	Gastropoda	Melongenidae	Melongena patula (Broderip y Sowerby, 1829)	Melongenidae	(Semifusus) tuba (Gmelin, 1971), Pugilina (S.) crassicaudus (Philip	682-683
14	Gastropoda	Muricidae	Plicopurpura pansa (Gould, 1853)	Muricidae		540 - 575
15	Gastropoda	Muricidae	Plicopurpura columellaris (Lamarck, 1822)			
	Barraza, J. E. . 2006. Identificación de moluscos marinos comestibles de El Salvador. Dirección de Patrimonio Cultural. 17 p. Ministry of Envrironment and natural resources.			Min, D-K. 2004. Mollusks in Korea. Min Molluscan Research Institute.		
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Table 1. Comparisson of comercial importance marine shellfish by Class and Familly from El Salvador related with Korea.