2010

Growth, Reproductive Condition, And Digestive Tubule Atrophy Of Pacific Oyster Crassostrea Gigas In Gamakman Bay Off The Southern Coast Of Korea

DH Kang

Fu-Lin E. Chu
Virginia Institute of Marine Science

HS Yang

CH Lee

CH Lee

See next page for additional authors

Journal of Shellfish Research, Vol. 29, No. 4, 839–845, 2010.

GROWTH, REPRODUCTIVE CONDITION, AND DIGESTIVE TUBULE ATROPHY OF PACIFIC OYSTER *CRASSOSTREA GIGAS* IN GAMAKMAN BAY OFF THE SOUTHERN COAST OF KOREA

DO-HYUNG KANG,[1] FU-LIN E. CHU,[3] HYUN-SUNG YANG,[2] CHANG-HO LEE,[2] HYEONG-BEOM KOH[4] AND KWANG-SIK CHOI[2]*

[1]*Marine Living Resources Research Department, Korea Ocean Research & Development Institute, Ansan PO Box 29, 425-744 Republic of Korea;* [2]*Faculty of Marine Biomedical Sciences and Marine and Environmental Research Institute, Jeju National University, 66 Jejudaehakno, Jeju 690-756, Republic of Korea;* [3]*Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062;* [4]*Jeju Special Self-Governing Province Fisheries Resources Research Institute, Jeju Republic of Korea*

ABSTRACT Spat of Pacific oysters (*Crassostrea gigas*) were collected from Gamakman Bay, Korea, and raised in a spat hardening facility located in the low intertidal zone of the bay for a ''hardening/stunting'' period of 10 mo. Seasonal changes in growth, reproductive condition, and digestive tubule atrophy (DTA) of these ''hardened/stunted'' oysters were monitored for more than a year after transplanting to a suspended longline system in a grow-out area in the bay. After transplantation, the hardened/stunted oysters showed a logarithmic increase in shell size for the first 4 mo, from June to October, and growth remained stable from late fall to early spring. During the 12 mo of the grow-out, the shell size of the hardened/stunted oysters increased from 15.4–74.2 mm, and tissue weight increased from 0.49–12.85 g. Histological analysis revealed that gametogenesis of hardened/stunted oysters commenced as early as February when water temperature remained at 10°C, and spawning occurred from July to September when water temperature reached 25–27°C. DTA assessed from histological analysis was higher from September to February, when the chlorophyll a level in the bay was lower. These data suggest that seasonal fluctuations in water temperature and food availability in the water column are the 2 main environmental parameters governing reproduction and growth of oyster in Gamakman Bay, and DTA could be a useful biomarker for monitoring the nutritional condition of oysters.

KEY WORDS: oyster, *Crassostrea gigas*, hardening, reproduction, digestive tubule atrophy, Gamakman Bay, Korea

INTRODUCTION

The Pacific oyster *Crassostrea gigas* has been widely cultivated in small bays off the southern coast of Korea using longlines suspended with numerous buoys. In 2004, Korea produced 239,270 tons of oysters, and more than 95% of the total production came from small bays in the south. In this region, oyster seeds (spat) are collected from low intertidal and/or shallow subtidal areas during summer (usually from late June to August). Before being transplanted to grow-out areas, spat usually undergo an intermediate rearing period known as ''hardening/stunting'' (Ventilla 1984, Arakawa 1990, Bae & Han 1998). Spat ''hardening'' is a common practice in Korea and Japan. During the hardening period, spat attached to empty oyster shells are suspended on a rack installed in the lower intertidal zone, where they are exposed daily to air for several hours for a period of 6–11 mo. These spat are dubbed ''hardened'' or ''stunted'' spat or oysters. These stunted oysters showed higher survival rates than nonstunted oysters after they were transplanted to grow-out areas (Ventilla 1984, Arakawa 1990).

Gamakman Bay (34°40′N, 127°42′E) is one of the largest oyster farming areas in Korea, with a total surface area of 112 km^2 and a mean depth of 6.3 m. Oyster culture facilities occupy 13% of the surface area of the bay (Lee 2001). In Gamakman Bay, the oyster industry uses naturally collected oyster spat as seeds. Spat are collected from early July to late August using oyster shells attached to a rope. As in other oyster farms on the southern coast, spat in the Bay are stunted for 10–11 mo before grow-out. After hardening, the stunted seed oysters are transplanted to grow-out areas located in the middle of the bay, where they are suspended on submerged (1–4 m deep) longlines until they reach more than 70 mm in shell length (SL) (Bae & Han 1998, Kang et al. 2000, Hyun et al. 2001). However, despite the high production of oysters from this bay, limited information is available on the growth and reproduction of oysters in this area (Ngo et al. 2002, Ngo et al. 2006). Hardening of spat prior to transferring to grow-out areas has been practiced widely in Korean oyster industries for several decades, but only a few studies have investigated the growth and reproduction of stunted spat during the first year of grow-out (Park et al. 1999a, Park et al., 1999b, Kang et al. 2000), and there is no information on the growth and reproductive condition of stunted oysters in Gamakman Bay. Therefore, a study was conducted in Gamakman Bay to monitor the growth, gonad development, and nutritional condition of stunted spat during their first year of grow-out in a suspended longline culture system. In addition, seasonal changes in environmental parameters, such as temperature, salinity, and chlorophyll a level were also monitored. Oyster gonad development and the subsequent spawning are mainly governed by water temperature and food availability (Hilbish & Zimmerman 1988, Ruiz et al. 1992). In particular, food availability determines the seasonal energy storage and subsequent gametogenic cycle of marine bivalves (Bayne, 1976, Newell et al. 1982, Soniat & Ray 1985, Arakawa 1990, Kang et al. 2000).

MATERIALS AND METHODS

Spat Hardening and Grow-out Monitoring

During July to August 1996, oyster spat were collected from Gamakman Bay (34°40′N, 127°42′E) using 9–11-cm oyster

*Corresponding author: E-mail: skchoi@jejunu.ac.kr

shells tied on plastic string and suspended on wooden racks installed in the low intertidal zone for 10 mo for hardening. In May 1997, the stunted oysters were transplanted into a suspended longline culture system in the middle of the bay. From June 1997 to July 1998, 130–190 oysters from this suspended longline culture system were sampled monthly for measurements of SL, tissue weight, and histological analysis of gonad development and digestive tubule atrophy (DTA). Also, water temperature, salinity, and the chlorophyll a level in the water column at a depth of 0–3 m from the surface were recorded monthly at the times oysters were sampled.

Growth and Reproductive Condition

Sampled oysters were measured for (the longest axis of the shell) and tissue wet weight (TWT) to assess growth. After recording SL and TWT, at each sampling time 20–40 of the sampled oysters were fixed in Bouin's fixative for histology to examine reproductive condition and DTA (a total of 363 oysters for the entire study period). A longitudinal section was made from the center of the fixed tissue following Howard and Smith (1983). The cross-section included gonads, visceral mass, gills, and mantle tissues (Fig. 1). The histological block was sliced to a thickness of 6 μm and then stained with Harris' hematoxylin and eosin Y.

To examine the reproductive condition, 4 microscopic fields of gonad of each oyster were randomly selected, and gonad development was categorized into 1 of 8 gametogenic stages: 1, sexually undifferentiated; 2, early development; 3, mid development; 4, late development; 5, fully mature; 6, partially spawning; 7, spawning; and 8, spent (Powell et al. 1993). Gonad development was also analyzed quantitatively by measuring the area of gonad occupying the area of total cross-section. To assess the gonad area, we scanned the histological slide of each oyster and measured the areas using an image analyzer. A total of 363 oysters were analyzed for percent of gonad area (PGA), the ratio of gonad area to total areas (Kang et al. 2003a).

DTA is a condition characterized by thinning of the lumen and tubule epithelium of the digestive tubules in marine bivalves, induced by environmental stresses including poor food supplies, hypo- or hypersalinity, and water contamination (Morton 1983, Couch 1984, Lowe 1988, Gauthier et al. 1990, Winstead 1995, Weinstein 1997, Syasina et al. 1997). Winstead (1995) reported that the high degree of DTA observed in oysters in Apalachicola Bay, Florida, was associated with poor nutrition during a period of low salinity. Subsequently, DTA, analyzed by histological and histochemical techniques, has been used as a biomarker in numerous environmental studies of marine molluscs (Ellis et al. 1998, Najle et al. 2000, Tay et al. 2003, Kim & Powell 2004, Zorita et al. 2006, Kim & Powell 2009).

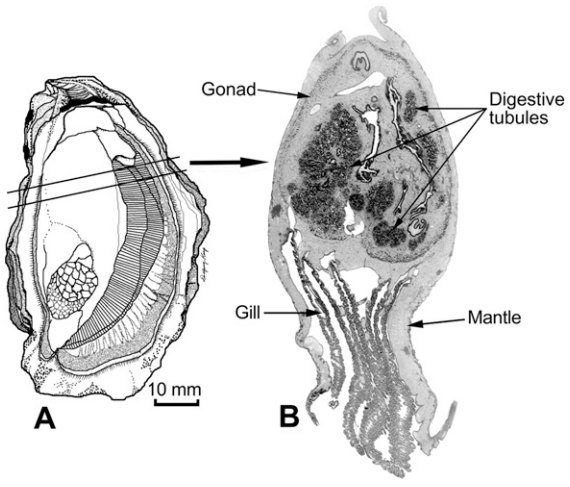

Figure 1. Preparation of histology to assess gonad development and digestive tubule atrophy. (A) Location of the longitudinal section taken. (B) Image cross-section.

The histological preparations used for the reproductive condition analysis was used to determine the level of DTA ($n = 363$ oysters), using a compound microscope (Olympus BX 50, Tokyo, Japan). To evaluate DTA level, 4 random microscopic fields exhibiting digestive tubules were selected from each slide (i.e., each oyster) and graded from 0–4 based upon the level of thinning of the lumen and digestive tubule (Ellis et al. 1998): (0) absorptive stage I, (1) holding stage, (2) absorptive stage II, (3) disintegrating stage, and (4) reconstituting stage (Fig. 2). The DTA level of each oyster was then calculated by averaging the numerical grade of the 4 randomly selected microscopic fields (Ellis et al. 1998). Accordingly, higher scores indicate a greater tubule atrophy.

The seasonality of DTA and PGA was tested using analysis of variance (ANOVA) and Duncan's multiple range test using SAS statistical software. In all cases, $P < 0.05$ was the accepted level of significance.

RESULTS

Water Temperature, Salinity, and Chlorophyll a *Levels*

During the grow-out period, seawater temperatures ranged from 7.5 (January 1998)–26.1°C (August 1997; Fig. 3) and salinity ranged from 28.2 (August 1997)–34.0 psu (February 1998). The relatively lower salinity observed in the bay in August was the result of a summer monsoon that brought vast amounts of freshwater into the bay. The chlorophyll a level in the bay showed a clear seasonality, ranging from 0.1–3.5 μg/L (Fig. 4). There were 2 peaks of chlorophyll a in the water column: one in spring (March to May 1998) and the other in fall (September and October 1997). During the spring phytoplankton bloom, levels of chlorophyll a in the water column ranged from 3.3 (March)—3.5 μg/L (April 1998). The magnitude of the fall bloom was somewhat smaller, with a concentration of chlorophyll a 1.5–2.4 μg/L (September and October 1997). The seasonal chlorophyll a minimum of 0.1 μg/L was observed in November 1997.

Digestive Tubule Atrophy

Levels of DTA fluctuated over time: increasing from July to September, decreasing from September to October, increased from October to November, remaining at a relatively similar level from November 1997 to February 1998, then decreasing from February to March 1998 (Fig. 4). The lowest DTA level (1.5 ± 0.40) was observed in July 1997 and April and March 1998. The highest DTA level (2.6 ± 0.8) was recorded in September 1997. DTA indices recorded in the fall and winter were significantly higher than spring ($P < 0.05$, Fig. 4). The increase in DTA in September 1997 and from November 1997

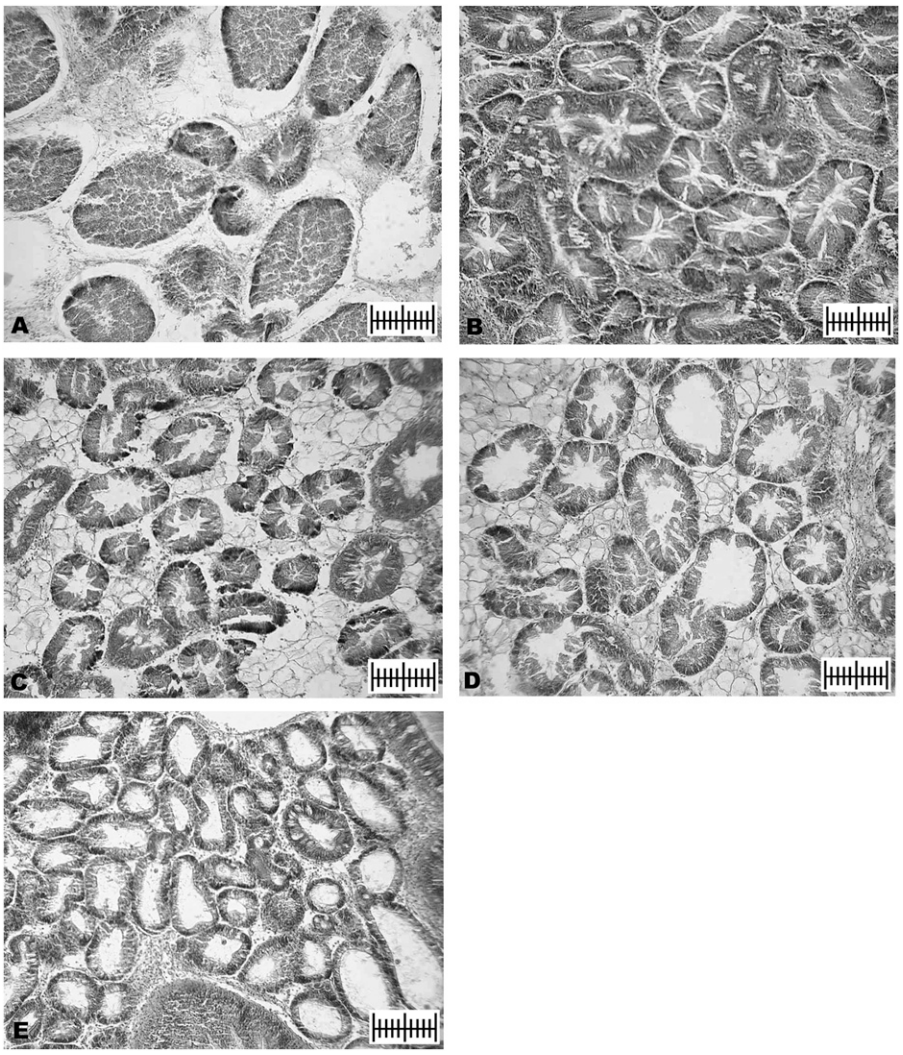

Figure 2. Photomicrographs showing digestive tubule atrophy conditions. A, absorptive stage I (0); B, holding stage (1); C, absorptive stage II (2); D, disintegrative stage (3); E, reconstituting stage (4). Scale bar: 100 μm.

to February 1998 appears to couple with low levels of chlorophyll a in the bay. The decline of DTA levels from February to May 1998 coincided with increases in the level of chlorophyll a in the bay. This suggests that food availability and subsequent DTA is closely linked and DTA may be a good biomarker to assess nutritional condition of oyster.

Growth Patterns of Stunted Oysters During the First Year of Grow-out

During the 12-mo period of cultivation, TWT increased from 0.49 ± 0.17 g in June 1997–12.85 ± 3.62 g in May 1998 (Fig. 5). The tissue growth was exponential during June and October 1997; during this period, the oysters increased in tissue weight from 0.49 ± 0.17–4.65 ± 2.50 g. Tissue growth then remained stable between late fall and early spring. In the spring of 1998, another rapid increase in somatic tissue was observed, from 4.61 ± 1.98 g (March 1998)–12.85 ± 3.62 g (May 1998).

Monthly mean SL increased from 15.36 ± 5.58 mm in June 1997–74.20 ± 11.30 mm in May 1998 (Fig. 5). As with tissue growth, shell growth also showed a fast increase during the first 4 mo of grow-out (15.36 ± 5.58 mm in June–55.62 ± 12.11 mm in October 1997). Shell growth then remained stable between late fall and early spring (November to March). Again, as with tissue growth, shell growth entered a second exponential phase between March and May 1998; an SL of 74.20 ± 11.30 mm was recorded in May 1998.

Reproductive Condition

In June 1997, oysters with an SL of 10–27 mm exhibited fully mature gonads, and some of them appeared ready to spawn. Spawning occurred between July and September, with a peak in August. In October 1997, all oysters became sexually inactive and the sexes could no longer be differentiated using histological preparation. Gametogenesis initiated again in January 1998, when the water temperature reached 7.5°C. Most oysters collected in February were in the early development stage (87%) when the water temperature reached 10°C. In May 1998, most females (90%) were fully mature and ready to spawn. Male oysters showed a similar pattern of annual gametogenesis, although initiation of spermatogenesis occurred a month earlier than oogenesis initiation (Fig. 6).

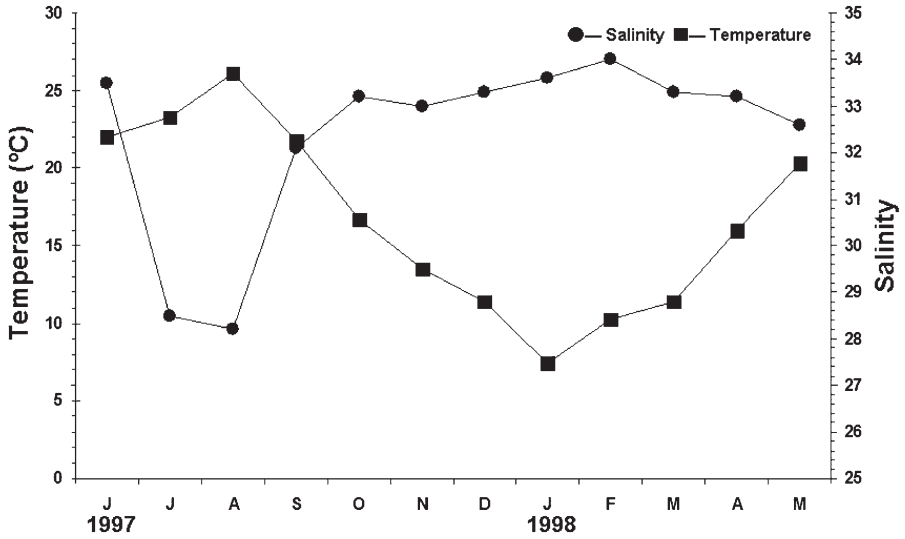

Figure 3. Monthly changes in the sea surface temperature and salinity in Gamakman Bay.

PGA ranged from 19.7 ± 6.3% (September 1997)–50.0 ± 11.7% (April 1997, Fig. 7). PGA decreased markedly from August to September 1997 (ANOVA, $P < 0.05$), indicating that most oysters had completely spawned by September, and the level stayed low until January 1997. PGA then increased gradually starting in February 1998, and it reached a level similar to that observed in June and July 1997 (ANOVA, $P < 0.05$). As with annual gametogenesis, the seasonal fluctuations in PGA followed the seasonal changes in water temperature in the bay.

DISCUSSION

For decades, hardening/stunting spat for grow-out in a long-line suspended culture system has been a general practice in the Korean oyster farming industry. Only a few studies have examined first year shell and tissue growth in several grow-out areas in Korea. However, none of these previous studies on stunted spat followed their reproductive cycle during their grow-out period, and no study has been conducted in Gamakman Bay, which is one of the major oyster production grounds. Our study not only measure stunted spat growth, but also their reproductive development during their first year grow-out.

Growth Patterns of Stunted Oysters During the First Year of Grow-out

Shell growth (i.e., increase in SL) of stunted Pacific oysters appears to vary with geographical locations. The SL of stunted oysters raised in Gamakman Bay was lower compared with results reported in previous studies conducted in other regions. Oh et al. (2002) reported that the mean SL of stunted oysters in Goseong Bay, Korea, after 10 mo of grow-out was 85 mm. Stunted oysters raised for 8 mo in the Seto Inland Sea in Japan reached 93.1 mm in SL (Kobayashi et al. 1997). Relatively slow growth observed in the current study may be associated with lower levels of food in the water column, although the influence of other parameters such as water temperature and salinity cannot be ruled out. Along with water temperature, food availability is one of the most crucial factors governing oyster

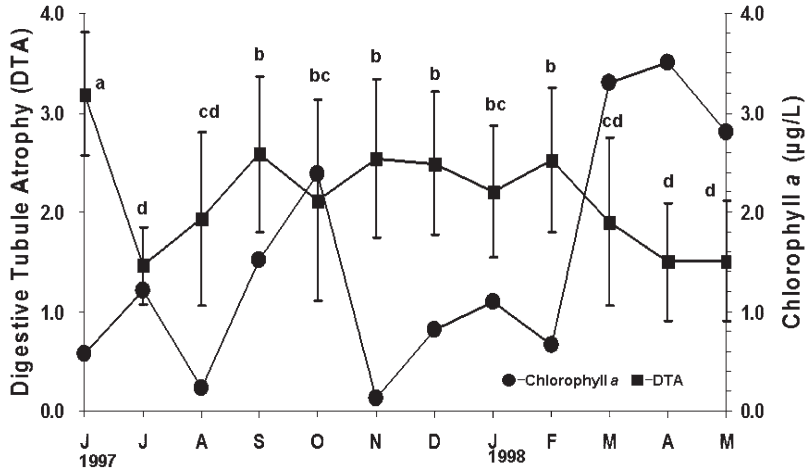

Figure 4. Monthly changes in chlorophyll a concentration and digestive tubule atrophy (DTA, mean with SD) of oysters. Letters on DTA indicate statistically significant differences among different sampling periods (ANOVA, F = 15.81 $P < 0.0001$; Duncan's multiple range test, $P < 0.05$).

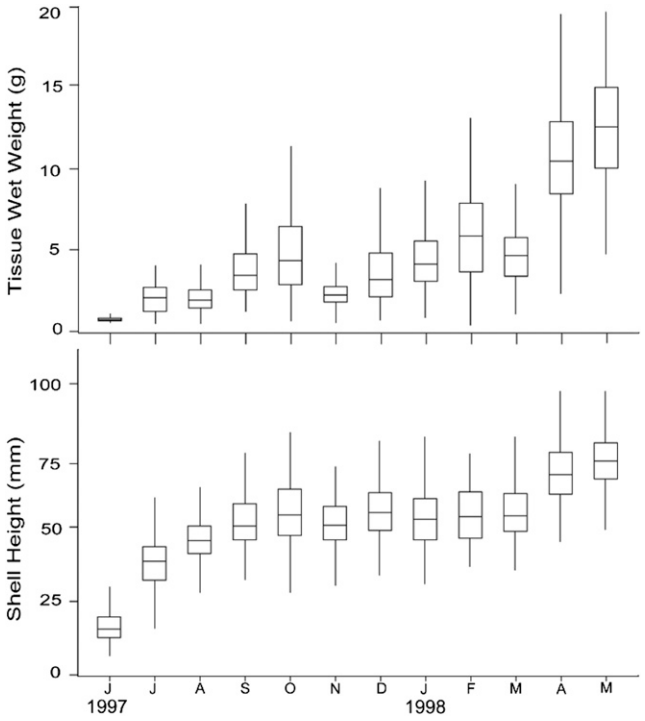

Figure 5. Monthly mean shell length and wet tissue weight of oysters transplanted in Gamakman Bay for a year after hardening.

growth (Hofmann et al. 1992, Powell et al. 1995, Kang et al. 2000, Ngo et al. 2006). Oh et al. (2002) reported that chlorophyll a in Goseong Bay, Korea, ranged from 0.2–6.2 μg/L annually. The concentration of chlorophyll a in the Seto Inland Sea ranged from 0.33–7.27 μg/L (Kobayashi et al. 1997). The chlorophyll a levels recorded in the current study were 0.1–3.5 μg/L, which is about 2-fold lower than those recorded in the previous 2 studies, and comparatively lower than the levels reported from other oyster-farming areas. Thus, lower food supply in the grow-out area may have resulted in slower growth of the "stunted" oysters in the current study (see the review of oyster growth rates and chlorophyll a levels by Gangnery et al. (2003)).

The observed slower growth of SL in the current study than the 2 previous studies could be also be the result of the size of stunted spat transferred to grow-out. SL of the transplanted stunted oysters in June 1997 was smaller than those reported in the studies by Oh et al. (2002) and Kobayashi et al. (1997). The stunted spat sizes used for grow-out were 18 mm and 27 mm in Oh et al. (2002) and Kobayashi et al. (1997), respectively. The size of stunted oysters sampled 1 mo after transplanting in June 1997 was 15.36 ± 5.58 mm in the current study.

Annual Reproductive Cycle of Stunted Oysters

Results of histological analysis suggest that stunted spat spawned as early as June 1997, a month after their translocation from the intertidal hardening ground to the center of the bay, and spawning continued until September (Fig. 7). The vast decrease of PGA from August to September (from 40% to 19%) indicates that spawning was at its most intense between late August and September. The transplanted stunted oysters exhibited only a single spawning peak during August and September in 1997. This spawning pattern is somewhat different from previously reported spawning patterns on adult oysters. Kang et al. (2003b) monitored monthly changes in the egg masses of 2- to 3-y-old Pacific oysters in Goseong Bay off the southern coast of Korea using an immunological method. They observed 2 major spawning peaks in oysters: one in late June and another one in late July to mid August. Park et al. (1999a, 1999b) also observed 2 spawning peaks in oysters in small bays near Goseong Bay: one in late June to early July and the other in August. Pacific oysters raised in Hiroshima, Japan, which is

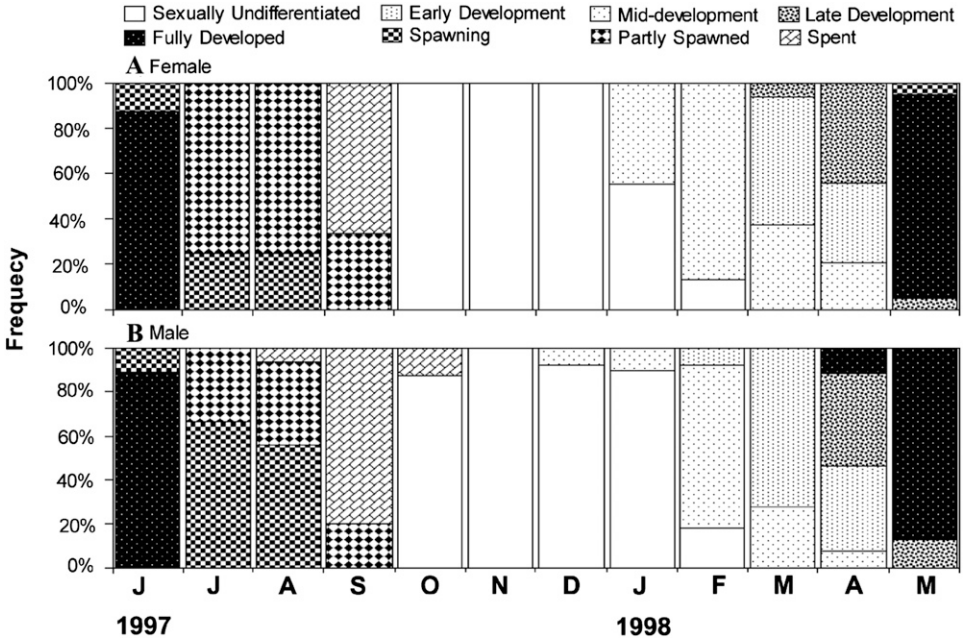

Figure 6. (A, B) Frequency distributions of the gonad development of oysters in Gamakman Bay for females (A) and males (B).

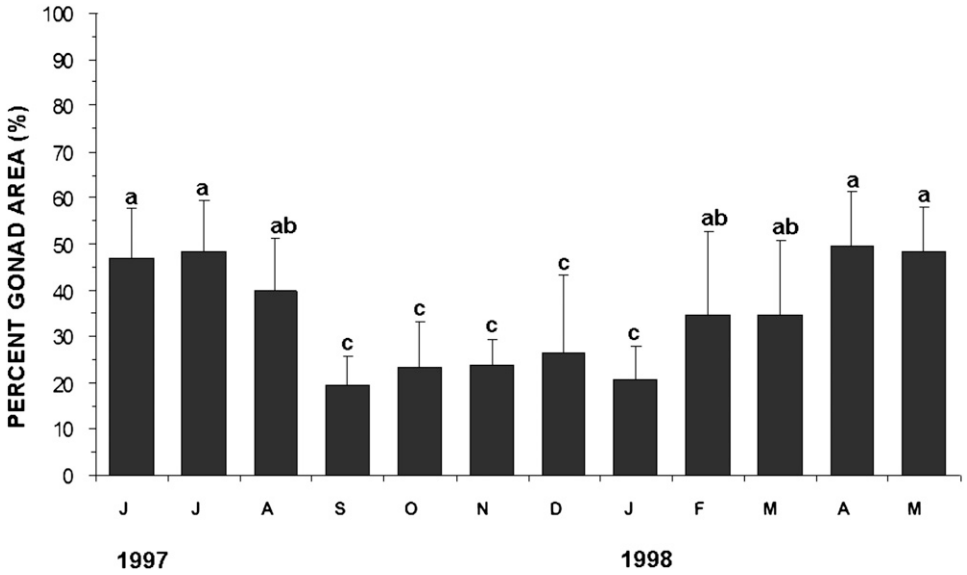

Figure 7. Monthly changes in the mean and SD of percent gonad area of oysters in Gamakman Bay. Letters on percent gonad area indicate statistically significant differences among different sampling periods (ANOVA, F = 14.25 $P < 0.0001$; Duncan's multiple range test, $P < 0.05$).

geographically parallel to the southern coast of Korea, showed a similar spawning pattern to our study (Arakawa 1990). In Hiroshima, Japan, oysters spawn from May to September, with a single spawning peak (July to August).

In the current study, the PGA of stunted oysters measured during the first spawning period (40–47% in 1997) was comparatively higher than the PGA reported in stunted oysters in Japan. According to Ogasawara et al. (1962) (quoted from Ventilla (1984)), the PGA of stunted oyster was approximately 35%, whereas the PGA of raft culture oysters was 69%. They also reported that stunted oysters contained more glycogen and less water during spawning, because they spend less energy on reproduction.

This suggests that similar to the stunted oysters in Japan, the stunted oysters transplanted in Gamakman Bay produced fewer gametes than did raft or longline cultured (i.e., suspended culture) oysters. The less gamete production in stunted oysters in Gamakman Bay could be associated with differing energy allocations of net energy to growth and reproduction; stunted oysters may use more of their net energy on shell and somatic growth than on reproduction during their first spawning season after a hardening period.

Annual Digestive Tubule Atrophy Levels

The current study is the first attempt to investigate the correlation between annual variation in DTA in stunted oysters and seasonal variation of food supply. It appears that seasonal DTA variations in stunted oysters correlated negatively with the seasonal fluctuations in chlorophyll a in Gamakman Bay. The highest DTA recorded in September 1997 suggests that oysters were in poor nutritional condition and/or were under spawning stress, as previous studies have suggested. This suggestion is supported by the recorded low chlorophyll a level in August 1997 and the abrupt decrease of PGA from August to September in 1997. From February to May 1998, the chlorophyll a level increased rapidly as a result of the spring phytoplankton bloom in the bay. The low DTA level observed at this time coincided with a period of rapid increase in tissue weight and SL (Fig. 6). Oysters in small bays off the southern coast of Korea, active accumulation of glycogen and proteins in the mantle as energy reserves for the upcoming spawning in summer generally happens during this early spring period (Kang et al. 2000, Ngo et al. 2006). Thus, a synchrony in the timing of phytoplankton bloom probably dominated by diatoms (a better nutritional condition), low DTA level, and active accumulation of energy reserves (glycogen and proteins) in oyster tissues suggests that DTA may be a good indicator to be used for monitoring the nutritional condition in oysters.

ACKNOWLEDGMENTS

This work was supported by the International Research Cooperation Program of the Ministry of Land, Transportation and Maritime Affairs, Republic of Korea (PM55800). We also thank the staff of the Shellfish Aquaculture and Research Laboratory at Jeju National University for their help in data collection and analysis.

LITERATURE CITED

Arakawa, K. Y. 1990. Natural spat collecting in the pacific oyster *Crassostrea gigas* (Thunberg). *Mar. Behav. Physiol.* 17:95–128.

Bae, P.- A. & C.- H. Han. 1998. Effects of nursery environmental factors on the growth of Pacific oyster, *Crassostrea gigas*. *Korean J. Aquacult.* 11:391–400. [in Korean with English abstract].

Bayne, B. L. 1976. Aspects of reproduction in bivalve mollusks. In M. L. Wiley, editor. Estuarine processes. New York: Academic Press. pp. 432–448.

Couch, J. 1984. Atrophy of diverticular epithelium as an indicator of environmental irritants in the oyster, *Crassostrea virginica*. *Mar. Environ. Res.* 14:525–526.

Ellis, M. S., R. D. Barber, R. E. Hilman, Y. Kim & E. N. Powell. 1998. Histopathology analysis. In: G. G. Lauenstein & A. Y. Cantillo, editors. NOAA technical memorandum NOS ORCA 130 (Sampling and analytical methods of the national status and trends program). Silver Spring, MD: NOAA. pp. 198–215.

Gangnery, A., J.- M. Chabirand, F. Lagarde, P. Le Gall, J. Oheix, C. Bacher & D. Buestel. 2003. Growth mode of the Pacific oyster, *Crassostrea gigas*, cultured in Thau Lagoon (Méditerranée, France). *Aquaculture* 215:267–290.

Gauthier, J. D., T. M. Soniat & J. S. Rogers. 1990. A parasitological survey of oysters along salinity gradients in coastal Louisiana. *J. World Aquacult. Soc.* 21:105–115.

Hilbish, T. J. & K. M. Zimmerman. 1988. Genetic and nutritional control of the gametogenic cycle in *Mytilus edulis*. *Mar. Biol.* 98:223–228.

Hofmann, E. E., E. N. Powell, J. M. Klinck & E. A. Wilson. 1992. Modeling oyster population: III. Critical feeding periods, growth and reproduction. *J. Shellfish Res.* 11:399–416.

Howard, D. & C. Smith. 1983. Histological techniques for marine bivalve mollusks. NOAA technical memorandum NMFS-F/NEC-25. Woods Hole, MA: NOAA. 97 pp.

Hyun, K.- H., I.- C. Pang, J. M. Klinck, K.- S. Choi, J.- B. Lee, E. N. Powell, E. E. Hofmann & E. A. Bochenek. 2001. The effect of food composition on Pacific oyster *Crassostrea gigas* Thunberg growth in Korea: a modeling study. *Aquaculture* 199:41–62.

Kang, C.- K., M.- S. Park, P.- Y. Lee, W.- J. Choi & W.- C. Lee. 2000. Seasonal variation in condition, reproductive activity, and biochemical composition of the Pacific oyster, *Crassostrea gigas* (Thunberg) in suspended culture in two coastal bays of Korea. *J. Shellfish Res.* 19:771–778.

Kang, D.- H., I.- Y. Ahn & K.- S. Choi. 2003a. Quantitative assessment of reproductive condition of the Antarctic clam, *Laternula elliptica* (King and Broderip), using image analysis. *Invertebr. Reprod. Dev.* 44:71–78.

Kang, S.- G., K.- S. Choi, A. A. Bulgakov, Y. Kim & S.- Y. Kim. 2003b. Enzyme-linked immunosorbent assay (ELISA) used in quantification of reproductive output in the Pacific oyster, *Crassostrea gigas*, in Korea. *J. Exp. Mar. Biol. Ecol.* 282:1–21.

Kim, Y. & E. N. Powell. 2004. Surf clam histopathology survey along the Delmarva mortality line. *J. Shellfish Res.* 23:429–441.

Kim, Y. & E. N. Powell. 2009. Effects of climate variability on interannual variation in parasites, pathologies, and physiological attributes of bivalves from the U.S. East, Gulf, and West Coasts. *Environ. Bioindicat.* 4:67–96.

Kobayashi, M., E. E. Hofmann, E. N. Powell, J. M. Klinck & K. Kusaka. 1997. A population dynamics model for the Japanese oyster, *Crassostrea gigas*. *Aquaculture* 149:285–321.

Lee, W. C. 2001. Modification and application of an ecosystem model for carrying capacity in oyster culturing ground. PhD diss. Department Of Environmental Engineering, Pukyong National University. 132 pp.

Lowe, D. M. 1988. Alterations in cellular structure of *Mytilus edulis* resulting from exposures to environmental contaminants under field and experimental conditions. *Mar. Ecol. Prog. Ser.* 46:91–100.

Morton, B. 1983. Feeding and digestion in Bivalvia. In: A. S. M. Saleuddin & K. M. Wilbur, editors. The Mollusca, 5. New York: Academic Press. pp. 65–131.

Najle, R., M. Elissondo, S. Gentile, M. Gentile, G. Vacarezza & H. Solana. 2000. Histopathology of the digestive gland of an Antarctic limpet exposed to cadmium. *Sci. Total Environ.* 247:263–268.

Newell, R. I. E., T. J. Hilbish, R. K. Koehn & C. J. Newell. 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* (Bivalvia, Mytilidae) from locates on the East Coast of the United States. *Biol. Bull.* 162:299–310.

Ngo, T. T. T., S.- G. Kang & K.- S. Choi. 2002. Seasonal changes in reproductive condition of the Pacific oysters, *Crassostrea gigas* (Thunberg) from suspended culture in Goseong Bay, Korea. *Korean J. Environ. Biol.* 20:268–275.

Ngo, T. T. T., S.- G. Kang, D.- H. Kang, P. Sorgeloos & K.- S. Choi. 2006. Effect of culture depth on the proximate composition and reproduction of the Pacific oyster, *Crassostrea gigas* from Gosung Bay, Korea. *Aquaculture* 253:712–720.

Ogasawara, Y., U. Kobayashi, R. Okamoto, A. Furukawa, M. Kisaoka & K. Nogami. 1962. The use of the hardened seed oyster in the culture of the food oyster and its significance to the oyster culture industry. *Bull. Naikai Reg. Fish. Res. Lab.* 19:1–13.

Oh, K.- H., I.- C. Pang, E. E. Hofmann, Y. Kim, S.- Y. Kim, Y.- J. Park & K.- S. Choi. 2002. Modeling oyster population dynamics I. Effects of available food on growth of the Pacific oyster *Crassostrea gigas* in Goseong Bay, Korea. *J. Korean Fish. Soc.* 35:327–335. [in Korean with English abstract].

Park, M.- S., H.- J. Lim, Q. Jo, J.- S. Yoo & M.- J. Jeon. 1999a. Assessment of reproductive health in the wild seed oysters, *Crassostrea gigas*, from two locations in Korea. *J. Shellfish Res.* 18:445–450.

Park, M.- S., H.- Y. Lyu & T.- S. Lee. 1999b. Investigation on the cause of bad natural seed collection of the Pacific oyster, *Crassostrea gigas*: relationships between the conditions of mother shell and viability of the released eggs and larvae based on the pathological and embryological survey. *J. Korean Fish. Soc.* 32:62–67. [in Korean with English abstract].

Powell, E. N., E. A. Wilson-Ormond & K.- S. Choi. 1993. Gonadal analysis: *Crassostrea virginica* In: G. G. Lauenstein & A. Y. Cantillo, editors. Comprehensive descriptions of complementary measurements: sampling and analytical methods of the national status and trends program national benthic surveillance and mussel watch projects. Vol. II. NOAA technical memorandum NOS ORCA 71. Silver Spring, MD: NOAA. pp. II.85–II.102.

Powell, E. N., J. M. Klinck, E. E. Hofmann, E. A. Wilson-Ormond & M. S. Ellis. 1995. Modeling oyster population V: declining phytoplankton stocks and the population dynamics of the American oyster (*Crassostrea virginica*) population. *Fish. Res.* 24:199–222.

Ruiz, C., M. F. Abad, L. O. Sedano, L. O. Garcia-Martin & J. L. Sánchez-López. 1992. Influence of seasonal environmental changes on the gamete production and biochemical composition of *Crassostrea gigas* (Thunberg) in suspended culture in El Grove, Galicia, Spain. *J. Exp. Mar. Biol. Ecol.* 155:249–262.

Soniat, T. M. & S. M. Ray. 1985. Relationships between possible available food and the composition, condition and reproductive state of oysters from Galveston Bay, Texas. *Contrib. Mar. Sci.* 28: 109–121.

Syasina, I. G., M. A. Vaschenko & P. M. Zhadan. 1997. Morphological alterations in the digestive diverticula of *Mizhhopecten yessoensis* (Bivalvia: Pectinidae) from polluted areas of Peter the Great Bay, Sea of Japan. *Mar. Environ. Res.* 44:85–98.

Tay, K.- L., S.- J. Teh, K. Doe, K. Lee, & P. Jackman. 2003. Histopathologic and histochemical biomarker responses of Baltic clam, *Macoma balthica*, to contaminated Sydney Harbor sediment, Nova Scotia, Canada. *Environ. Health Perspect.* 111:273–280.

Ventilla, R. F. 1984. Recent developments in the Japanese oyster culture industry. *Adv. Mar. Biol.* 21:1–57.

Weinstein, J. E. 1997. Fluoranthene-induced histological alterations in oysters, *Crassostrea virginica*: seasonal field and laboratory studies. *Mar. Environ. Res.* 43:201–218.

Winstead, J. T. 1995. Digestive tubule atrophy in eastern oyster, *Crassostrea virginica* (Gmelin 1791), exposed to salinity and starvation stress. *J. Shellfish Res.* 14:105–111.

Zorita, I., M. Ortiz-Zarragoitia, M. Soto & M. P. Cajaraville. 2006. Biomarkers in mussels from a copper site gradient (Visnes, Norway): an integrated biochemical, histopathological and histological study. *Aquat. Toxicol.* 78S:S109–S116.