Effect of culture depth on the proximate composition and reproduction of the Pacific oyster, *Crassostrea gigas* from Gosung Bay, Korea

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Abstract

We investigated seasonal variation in the reproductive output and proximate composition of tissues (protein, lipid, and carbohydrate) of the Pacific oyster, *Crassostrea gigas* Thunberg, at the top (0–2 m) and bottom (3–5 m) of a long-line suspended culture in Gosung Bay, Korea. The water temperature was 2–3 °C higher at the surface than at the bottom from early spring to mid-summer. The chlorophyll *a* level was also higher at the surface during March and April, when a spring phytoplankton bloom occurred in the bay. The seasonal variation in the proximate composition of oyster tissues differed between the surface and the bottom as well. Carbohydrate levels in oysters at the surface were somewhat higher in fall and winter, when the oysters were actively accumulating carbohydrates in their tissues for future growth and reproduction. Oysters at the surface tended to produce more eggs during the spring to early summer spawning period; the gonadosomatic index (GSI) was significantly higher in surface oysters than in bottom oysters (*p*<0.05). The overall growth and reproduction rates of the surface oysters were higher, even though the bottom oysters were located only 1–3 m below them. Accordingly, our data suggest that culture depth in the traditional long-line suspended culture needs to be re-evaluated to maximize oyster production.

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1. Introduction

Interannual or local differences in the energy storage cycle and reproduction of marine bivalves are often associated with changes in environmental conditions, particularly the amount of food in the water column (Newell et al., 1982; Rodhouse et al., 1984; Bricelj et al., 1987; Navarro et al., 1989; Kang et al., 2000). In coastal areas, available food for bivalves and other suspension feeders decreases with increased depth. The poor feeding
conditions associated with greater water depths result in reduced growth and reproductive output. For example, Barber et al. (1988) demonstrated that the gonad weight and gonad index of *Pecten magellanicus* was significantly greater in shallow water (13–20 m) than in deep water (170–180 m).

The Pacific oyster, *Crassostrea gigas*, is widely cultured along the southern coast of Korea, where a number of small, shallow bays (mostly <10 m in depth) are protected by numerous islands. Oysters are intensively cultured in these bays using a traditional long-line suspended culture system. Oyster spat is collected for culturing from mid-summer to early fall using 5- to 6-m long oyster-culture strings tied with numerous oyster shells. Oyster spat that settle on the strings undergo 7–9 months of hardening in intertidal areas. After the hardening period, the oysters are relocated to a grow-out field in the middle of the bay. The strings containing the hardened oysters are suspended on a 100- to 200-m horizontal long-line and submerged 1–2 m from the surface to prevent fouling. Oysters are then harvested during late winter and mid-spring, 9–11 months after grow-out.

Kang et al. (2000) suggested that food availability is the major environmental parameter determining the growth and gonad proliferation of *C. gigas* in Jaran and Hansan–Koje Bays on the southern coast of Korea. They reported that differences in gonad development and the biochemical composition of tissues among oysters in these bays were strongly correlated to a site-dependent variation in the storage-utilization cycle of energy reserves, particularly glycogen. Several studies have also reported that the fecundity of oysters is highly variable within and among locations. This variation has been attributed to differences in oyster size, asynchrony and variation in time since a previous spawning, the prevalence of parasites, and different temperature and salinity regimes (Cox and Mann, 1992; Choi et al., 1993; Mann et al., 1994; Kang et al., 2003). These studies suggest that changes in environmental parameters with increasing depth, particularly temperature, salinity, and food abundance, could significantly impact oyster growth and reproduction. The actual influence of these parameters has yet to be determined for major oyster grow-out grounds on the southern coast of Korea (Hyun et al., 2001; MOMAF, 2001).

We previously reported the development of an immunological technique for quantitatively evaluating the reproductive efforts of oysters and described seasonal changes in the reproductive effort (Kang et al. 2003). This study is part of a continuing effort to improve quantitative descriptions of the relationships between culture depth, energy storage, and reproductive effort of oysters in Gosung Bay. In this study, we (1) compared the biochemical composition of oysters from different depths, i.e., from the surface (0–2 m) and bottom (3–5 m) of suspended long-line cultures, and (2) determined the depth-dependent variation in the role of energy storage relative to the reproductive effort of oysters reported by Kang et al. (2003).

2. Materials and methods

2.1. Oyster sampling

This study was carried out at Gosung Bay on the southern coast of Korea (Fig. 1). For the analysis, market-sized oysters (shell length >7 cm) were collected from three sampling locations. At each sampling site, an oyster string was randomly selected, and oysters were collected from the surface (0–2 m) and bottom (3–5 m) layers. Sampling continued monthly from January to December 2000. From June to August, oysters were sampled biweekly to follow spawning activity. After the shell length (mm) was recorded using Vernier calipers, the soft tissues of the oysters were removed and weighed after blotting excess water with absorbent paper. After freeze-drying for 24 h, the dry tissue weight (g) of each oyster was determined using a microbalance. The water temperature and salinity were recorded in situ when the oysters were collected. Chlorophyll a concentrations in the bay, was assessed from Ministry of Maritime Affairs and Fisheries of Korea reports (MOMAF, 2001).

2.2. Biochemical measurements of oyster tissues

The dry tissue of each oyster was homogenized with a mortar. To determine the biochemical composition, a known quantity of dry tissue was homogenized in phosphate buffer solution (PBS) using an ultrasonifier. The total protein in the tissue was measured using a BCA Protein Assay with BSA as a standard. Total carbohydrates were quantified photometrically by the phenol-sulfuric acid method, with dextrose used as a standard (Taylor, 1995). Lipids were extracted using a mixture of chloroform and methanol (Bligh and Dyer, 1959) and charred following the method of Marsh and Weinstein (1966). The quantity of extracted lipid was determined photometrically with tripalmitin as a standard. The calculation of total protein, lipid, and carbohydrate was based on the dry tissue weight of each individual and determined as a percentage (%).
Fig. 1. The location of Gosung Bay on the southern coast of Korea.

2.3. Reproductive effort of oysters

Kang et al. (2003) reported the reproductive effort of the oysters used in our biochemical analysis. To investigate the effect of depth on reproductive effort, the oysters were grouped into surface (0–2 m) and bottom (3–5 m) categories. In brief, the reproductive effort of the oysters was measured using an egg-specific antibody in an enzyme-linked immunosorbent assay (ELISA). The total weight of the eggs in each assayed oyster was expressed as the gonadosomatic index (GSI), which is the ratio of egg dry weight to total tissue dry weight. The

Fig. 2. Seasonal variation in water temperature (A) and salinity (B) of the surface (open circles) and bottom (solid circles) layers from January to December 2000 in Gosung Bay.

Fig. 3. Seasonal variation in chlorophyll a concentration of the surface (open circles) and bottom (solid circles) layers during the sampling period.
fecundity of the oysters during the spawning season (May–August) was also estimated by dividing the egg mass estimated from the ELISA by the weight of a single egg (i.e., 13 ng; Kang et al., 2003).

2.4. Statistical analyses

Reproductive effort and proximate composition of oysters at the two culture depths were compared by month using Student's t-test (Sokal and Rohlf, 1981).

3. Results

3.1. Seasonal variation in environmental conditions

Fig. 2 shows the seasonal variation in temperature and salinity of surface and bottom waters. The temperature of the surface water varied from 4.3 °C (February) to 30.4 °C (July), while that of the bottom water ranged from 4.2 °C (February) to 26.6 °C (August). In general, temperature at the surface was higher (by 1–2 °C) than at the

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Fig. 4. Seasonal variation in protein (A), carbohydrate (B), and total lipid (C) in oysters from the surface (open bars) and bottom (solid bars) layers during the sampling period. Vertical lines indicate the standard deviations. Significant difference levels for the t-test: *, p<0.05; **, p<0.01; ***, p<0.001.
bottom from April to August, indicating that the water column was stratified during this period. In May, a maximum salinity of 34.3 psu was recorded at the surface, with 34.2 psu at the bottom. An annual minimum salinity was recorded at the surface in July (27.6 psu) due to massive freshwater input to the bay during the summer monsoon period (Fig. 2B).

Fig. 3 illustrates the monthly changes in chlorophyll a concentration in the bay. Chlorophyll a peaked at the surface in April (4.9 µg/L), July (3.6 µg/L), and September (1.9 µg/L) and at the bottom in April (4.2 µg/L), June (3.3 µg/L), and September (3.9 µg/L). The chlorophyll a level was higher at the surface during a spring plankton bloom and higher at the bottom in fall.

3.2. Proximate composition of oyster tissue

Fig. 4A shows the seasonal variation in total protein in the oyster tissues. At the surface, total protein varied from 11.4% (January) to 40.4% (October), with three peaks, in mid-June (39.4%), mid-August (37.9%), and late October (40.4%). Total protein in bottom oyster tissues ranged from 12.5% (February) to 42.8% (September) with peaks in late June (40.0%), mid-August (36.3%), and September (42.8%). The summer protein maximum coincided with the OSI peaks, indicating that seasonal changes in total protein are closely related to the annual reproductive cycle.

The seasonal variation in total carbohydrates in the oyster tissues is plotted in Fig. 4B. In contrast to the proteins, carbohydrate levels were higher in winter and spring: carbohydrates peaked in February in both surface (48.2%) and bottom (44.7%) oysters, decreasing in late June to a minimum of 12.4% for surface and 9.2% for bottom oysters. After the spawning period, oysters from both depths tended to recover the carbohydrate level during the fall, although the process seemed more rapid at the surface (Fig. 4B).

Unlike the proteins and carbohydrates, lipids showed only one peak in the oyster tissues, in early summer just before the first spawning. The maximum lipid level was recorded in late May (24.7%) for bottom oysters and in mid-June (31.9%) for surface oysters (Fig. 4C). During August and September, individual surface oysters had higher total lipids while bottom oysters registered higher lipids in April (p<0.05).

3.3. Gonadosomatic index and fecundity

Fig. 5 displays seasonal changes in GSI of oysters from both depths during the sampling period. For surface oysters, the monthly mean GSI increased rapidly from March to April (0–16.4%), reaching a maximum of 49.5% in mid-June during gametogenesis and the first spawning period. The mean GSI decreased dramatically in late June (10.4%), and then increased again before the second spawning in late July (26.7%). The mean GSI decreased from mid- to late August (22–18.8%). For bottom oysters, the mean GSI also increased continuously from March to April (0–15.6%), with a maximum in mid-June (41.1%); however, the magnitude of change was somewhat lower than that of surface oysters (p<0.05). However, the GSI of bottom oysters recorded in late August (12.6%) was higher than that of surface oysters (2.2%, p<0.05). Our data indicated that the spawning process of surface oysters was somewhat faster than that of bottom oysters, and the surface oysters tended to produce more eggs in the annual reproductive cycle.

Table 1 summarizes the fecundity of oysters from each depth during the spawning period. In May, surface oysters produced 39.4 million eggs/oyster and bottom oysters averaged 28.2 million each. Fecundity peaked in
Table 1

Mean shell length (mm), dried tissue weight (g) and fecundity (million of eggs/female) of oysters from different depths during spawning period in Gosung bay, Korea

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Surface oysters</th>
<th></th>
<th>Bottom oysters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SL Mean DTW</td>
<td>Fecundity Range</td>
<td>SL Mean DWT</td>
<td>Fecundity Range</td>
</tr>
<tr>
<td>May</td>
<td>90.3 (10.5)</td>
<td>1.8 (0.5)</td>
<td>74.4 (10.3)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>14-Jun</td>
<td>81.9 (6.4)</td>
<td>2.4 (0.6)</td>
<td>86.2 (12.7)</td>
<td>3.1 (1.0)</td>
</tr>
<tr>
<td>28-Jun</td>
<td>74.5 (12.8)</td>
<td>1.4 (0.2)</td>
<td>88.2 (15.9)</td>
<td>5.4 (4.3)</td>
</tr>
<tr>
<td>14-Jul</td>
<td>89.4 (9.1)</td>
<td>1.4 (0.4)</td>
<td>72.2 (3.4)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>28-Jul</td>
<td>82.3 (15.2)</td>
<td>1.2 (0.2)</td>
<td>74.6 (8.7)</td>
<td>0.9 (0.4)</td>
</tr>
<tr>
<td>14-Aug</td>
<td>95.2 (13.4)</td>
<td>1.2 (0.7)</td>
<td>84.2 (6.5)</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>28-Aug</td>
<td>82.3 (15.7)</td>
<td>0.9 (0.1)</td>
<td>79.8 (4.4)</td>
<td>0.6 (0.1)</td>
</tr>
</tbody>
</table>

SL, shell length of oysters (mm); DTW, dried tissue weight; data in parentheses indicate standard deviation.

mid-June, with 107 million eggs for surface oysters and 99 million for bottom oysters. After the first spawning, the fecundity of both oyster groups decreased, and then increased slightly to reach a second peak in late July. The number of eggs decreased rapidly during the second spawning period, with 26 million eggs/oyster at the surface and 20 million eggs/oyster at the bottom.

4. Discussion

4.1. Effects of temperature and food on oyster reproduction

Water temperature was several degrees higher at the surface than at the bottom from April to August (Fig. 2). Such a temperature variation could result in different rates of gametogenesis for oysters in the two layers. Numerous studies have reported that temperature and food availability in the water column are the two main environmental parameters controlling the rate of gametogenesis in oysters (Hofmann et al., 1992; Choi et al., 1994). Kang et al. (2000) reported on the spatio-temporal variation in oyster gametogenesis of two oyster populations collected from Osu and Koje-Hansan Bays adjacent to Gosung Bay. The variation in annual gametogenesis was explained in part by the different levels of food availability and the water temperature at the two locations. In Gosung Bay, Ngo et al. (2003) also investigated the annual gametogenic cycle of oysters cultivated at different depths on a long-line culture system. They found that gonad maturation was faster for oysters at a depth of 1–2 m than for oysters at 4–6 m, particularly during the early developmental stage. Despite the asynchronous development of the surface and bottom oysters in the spring, spawning was more or less synchronous during the summer, indicating that the effect of water temperature was somewhat limited in the early developmental stage.

4.2. Seasonal variation in the proximate composition of oyster tissue

The increase in the total protein level in oyster tissues during June and early August occurs at a time of intense, energetic demand for gamete development. In Gosung Bay, oysters from different depths also exhibited elevated protein levels during and at the end of the spawning period. These data are in accord with previous suggestions that protein could serve as an energy reserve during and at the end of gametogenesis, while carbohydrate is the main storage component during the resting and early development stages (Barber and Blake, 1981; Ruiz et al., 1992). From mid-June onward, especially during the second spawning period, protein levels in the bottom oysters were greater than in surface oysters, indicating the important role of protein when carbohydrate and lipid reserves were limited. Whyte et al. (1990) pointed out that during extended periods of food depletion, proteins contribute more to energy maintenance in oysters than carbohydrates do, even when sufficient carbohydrates are apparently available. Furthermore, Beninger and Lucas (1984) demonstrated the important role of protein when bivalves were in an imbalanced energy condition. On the other hand, protein levels of oysters from both depths were high in September and October, a period when chlorophyll a levels were also high. Oysters are opportunistic, and the food ingested during phytoplankton blooms could be used to accumulate energy reserves for the second spawning period.

4.3. Nutrient storage cycle and annual gametogenesis of oysters

During late fall to winter (November to January), when the oysters were in the recovery and accumulation phase of the annual gametogenic cycle, the surface
oysters had higher levels of carbohydrates than those from the bottom. In mid-July after the first spawning, the carbohydrate level in surface oysters was also significantly higher than that of the bottom oysters (Fig. 4B). Our findings concurred with those of Lodeiro et al. (2001), who showed that carbohydrate content decreased markedly with depth, especially during the phase of limited reproductive investment. In addition, the accumulation and utilization of carbohydrates in the surface oysters were highly correlated with protein and lipid levels. The natural gametogenic cycle in bivalves is closely linked with cycles of glycogen storage and subsequent de novo synthesis of lipids during vitellogenesis at the expense of the stored glycogen (Gabbott, 1975). Gallagher and Mann (1986) suggested that impediments to this process might result in the production of fewer eggs or eggs of suboptimal quality. In our study, surface oysters had a higher reproductive output than the oysters from greater depths during the first spawning (Fig. 5). The limited accumulation of carbohydrates presumably relates to insufficient food availability, possibly affecting the necessary energy allocation to gametogenesis in the bottom oysters.

Protein–carbohydrate and lipid–carbohydrate bioconversions were clearly demonstrated in surface oysters during gametogenesis. Oysters in the surface layer possibly possessed a better ability to bioconvert energy reserves for gonad development. Ruiz et al. (1992) observed that C. gigas used stored glycogen during gametogenesis and utilized proteins and lipids during winter when food was scarce. In Gossung Bay, seasonal changes in the biochemical composition of the oysters were similar. Robledo et al. (1995) reported that levels of protein were lower in the hemolymph of mussels kept at 5 m than at 2 m. The authors suggested a possible rhythmic mobilization of the reserves, i.e., in response to differing environmental conditions, such that carbohydrate accumulation may occur at one time and protein accumulation at another.

4.4. Effect of culture depth on oyster reproduction

In this study, the bottom oysters grew slower and produced relatively fewer eggs during the first spawning peak (Fig. 5). The low level of food at the bottom during the spring likely resulted in the lower reproductive output. The effect of water depth on the reproduction of marine bivalves has been well documented. Barber et al. (1988) suggested that insufficient food in deep water would best account for the slower gonad maturation, greater decrease of resorption, and lower fecundity of deep-water scallops. MacDonald and Bourne (1987) also reported that the reproductive output of the giant scallop Placopecten magellanicus is greater for individuals inhabiting shallow water (10 m) than for those in deeper water (30 m). Loosanoff (1965) compared the reproductive output of oysters at different depths in Long Island Sound. The gonads of Crassostrea virginica from shallow (3 m) water were thicker than those of oysters in deeper (10 m) water, indicating that the shallow-water oysters produce more eggs during the spawning period. These studies agree that the differences in gonad production are mostly attributable to differences in food resources between shallow and deeper water; in most cases, food is more plentiful in shallower water. The effect of food on marine bivalve reproduction has also been experimentally demonstrated. PIPE (1985) experimentally starved mussels (Mytilus edulis), which resulted in a decline in reproductive output and retarded gonad development. Consequently, the main effect of culture depth on oyster reproduction in the long-line culture system would be the depth-dependent food supply for the oysters.

In late August, the GSI of the bottom oysters was higher than that of the surface oysters. The chlorophyll a level at the bottom was approximately three-fold higher than at the surface, and the water temperature reached its annual maximum. The high GSI observed in bottom oysters at that time could be due to the ample food supply and the rapid energy conversion for gametogenesis resulting from the elevated water temperature. Several studies have shown that marine bivalves rapidly transfer assimilated food from the digestive gland to the gonadal tissue when sufficient food is supplied during gametogenesis (Gabbott, 1976; Urrutia et al., 2001). For M. edulis, this rapid transfer may occur over a period of about 7 days (Thompson, 1972). Using a computer model, Hofmann et al. (1994) demonstrated that variations in water temperature and food supply, not adult size, are the two main factors that control the reproductive effort of the American oyster (C. virginica).

Table 1 summarizes the fecundity of the surface and bottom oysters, which was estimated from the GSI during the spawning period from May to late August. The number of eggs released per oyster at the first spawning peak can be estimated by calculating the difference between fecundity measured on June 14 (before spawning) and July 18 (after spawning). We estimate that each oyster on the surface released up to 96 million eggs, while the bottom oysters each released 93 million eggs during the June spawning period. The data also indicate that oysters in the bay spawned again between late July and mid-August, although fewer eggs were released during the second
spawning (approximately 10 million eggs per surface oyster and 12 million eggs per bottom oyster). In late August, bottom oysters exhibited a higher GSI than the surface oysters, suggesting that spawning at the bottom is more extended. However, whether the eggs produced in late August contribute significantly to larval recruitment is uncertain. Several studies have shown that eggs produced at the end of an annual reproductive cycle are often resorbed when the water temperature drops, rather than being discharged into the water column (Thompson et al., 1996). However, the eggs released during the first spawning, regardless of depth, would contribute greatly to larval recruitment in the bay.

This study is the first attempt to correlate the proximate composition and reproductive effort of C. gigas raised at different depths in a long-line suspension culture. Our data suggest that the depth of the suspended culture affects seasonal changes in the biochemical composition of tissues as well as reproduction. The faster gonad development and faster recovery of the oysters in the surface water layer indicate that they are in better nutritional condition with significantly greater gonadal reserves. This study also suggests that the culture depth of oysters in the suspended long-line system in Gosung Bay needs to be re-evaluated in order to establish the best conditions for growth and reproduction.

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