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Growth and reproduction of early grow-out hardened juvenile Pacific oysters, *Crassostrea gigas* in Gamakman Bay, off the south coast of Korea

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A B S T R A C T

The southern coast of Korea comprises many small bays where the Pacific oyster, *Crassostrea gigas*, has been cultivated intensively employing a long-line hanging culture system. Naturally harvested oyster spats used in the long-line culture system are hardened in mid-intertidal area and transplanted to the center of the bay for grow-out usually in May. The May transferred oyster produced considerably smaller quantity of eggs during spawning in September. To enhance the reproductive effort and growth during grow-out, we transferred the hardened oyster juveniles to grow-out sites in January rather than May to allow them to accumulate energy reserves at the time of spring phytoplankton bloom that normally occurs during April and early May. The January transferred oyster juvenile (JTO) showed fast somatic and shell growth during grow-out, reaching a marketable size in August, while the juvenile oyster transplanted in May (MTO) did not reach a market size until November. JTO spawned from June to September, with a single spawning pulse in July, while most of the MTO spawned in September. Quantity of eggs produced by the JTO was 5.7 times higher than MTO, indicating that early grow-out enhanced egg production. The meat weight and levels of tissue carbohydrate and protein of JTO harvested in November were significantly higher than MTO harvested at the same time, suggesting that the meat quality of JTO is superior to the MTO. Overall, our results suggest that transplanted hardened juveniles to grow-out in January increase natural oyster seed harvest in the bay as well as improve quality of the oyster for marketing.

Statement of relevance: Oyster farming is one of the leading aquaculture industries in the world, producing over 4 million tons of oysters annually world-wide. In the oyster industry, *Crassostrea gigas*, the Pacific oyster, has been cultured extensively in Asia, and recently in Europe using different modes of culture. In Asia, the Pacific oysters are raised using subtidal hanging long-line system, while they are cultured in intertidal areas using net bags and rack system in Europe.

In Korea and Japan, the oyster seeds are supplied mostly from wild, by harvesting oyster larvae during post-spawning season. The newly harvested oyster spats are stunted in intertidal before they are transplanted to the subtidal hanging long-lines, to maximize survival of the seed oysters. This stunting process is called hardening and the hardening of oyster has been practiced for several decades in Korea and Japan. In Korea, the hardening seed oysters are transplanted in May, and the May transplanted oysters are harvested in December as they grow up to 8 cm in shell length. The May transplanted oysters produce relatively smaller quantity of eggs during their first spawning, although the small reproductive effort may increase their survival during post-spawning season. The natural oyster spats harvested in late summer or early fall for the oyster culture in Korea are mostly originated from the May transplanted oysters. Currently, the natural oyster spat harvest in Korea suffers from insufficient quantity. In part, this insufficiency is associated to the poor reproductive activity of the May transplanted oyster. Low reproductive effort of May transplanted oysters during their first spawning is, in part, associated to too late transplantation to the bay, and earlier transplantation of the stunted oysters from the hardening ground to the grow-out ground in the bay may enhance the reproductive effort, resulting in subsequent successful larval recruitment and settlement of the larvae. Accordingly, we experimentally transplanted the hardened oysters 4 months earlier to the traditional transplanting period (January) and monitored their reproduction and growth. Although the hardening process has been practiced over 50 years, no studies have compared growth and reproduction of differentially transplanted oysters in Korea. As the data demonstrated, January transplanted oyster demonstrated earlier gonad maturation and produced 5× more eggs during spawning, compared to

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May transplanted oysters. No severe mortality was observed from the early transplanted oysters during the course of study, although reproductive activity of January transplanted oysters was relatively intensive, compared to May transplanted oysters.
The present study first attempted to evaluate effectiveness of transplantation timing of the hardened oysters, in terms of growth and reproduction. Also, quantity of the eggs produced from differentially transplanted female oysters was first measured and compared in this study using ELISA. As the data indicated, January transplanted oyster produced 5× more eggs, which may enhance the natural oyster spat harvest in the bay, as well as to evaluate the transplantation period and management of oyster farming on the south coast of Korea.

1. Introduction

The Pacific oyster, *Crassostrea gigas* has been widely cultured in numerous semi-enclosed bays on the south coast of Korea, where the high level of primary production provides sufficient food for their growth and reproduction. The Korean oyster farming industry mostly uses natural seeds harvested from the bay during the post-spawning season (Choi, 2008). On the south, oyster spats are collected twice a year, one in July to early August and the other one in September and early October (Kang et al., 2000; Jeong et al., 2001). Currently 70% of the seeds used in the oyster aquaculture on the south coast are collected during September and early October and the larvae are believed to be derived from the May transplanted oyster (Jeong et al., 2001). The naturally harvested spats during late summer are placed in upper intertidal area for 8–10 months before they are transplanted in the bay for grow-out. This process is called "hardening" and it has been practiced commonly in Korea and Japan oyster aquaculture for the past decades. During hardening, growth and reproduction of the spats are suppressed due to periodic exposure to atmosphere and subsequent reduced feeding time (Fujiya, 1970; Ventilla, 1984; Arakawa, 1990; Choi, 2008). Eight to 10 months after hardening, the stunted oyster spats are transplanted to the long-line hanging culture facility in May and harvested in November. According to Fujiya (1970) and Ventilla (1984), the survival rate of the hardened oysters in Hiroshima waters was 70–90%, while the rate was only 20% with no hardening. After 6–8 months of grow-out, the hardened oysters reach over 80 mm in shell length and 5–6 g in tissue wet weight (Kang et al., 2000, 2010; Hyun et al., 2001). However, quantity of the harvested spats fluctuates year to year in an unpredictable manner (Park et al., 1999a; Jeong et al., 2001; Min et al., 2004). The decline in wild spat supply on the south is believed to be associated to the poor reproductive condition of the oysters during grow-out (Park et al., 1999b). Unlike normal adult oysters, the hardened young females transplanted in May produce relatively small quantity of egg due to the hardening (Choi, 2008; Kang et al., 2010).

Despite the long history of Korean oyster farming, studies of effectiveness of hardening duration and physiology of the juvenile oysters during grow-out are limited. Kang et al. (2010) reported that the May transplanted hardened oysters spawned in late August and September, one to two months delay, compared to the subsequent 2–3 years old adults, which spawned in June and July (Kang et al., 2003; Ngo et al., 2006). Also, the quantity of the eggs produced by the May transplanted juvenile is remarkably smaller than that of the adults. Kang et al. (2003) and Ngo et al. (2006) determined the gonad-somatic index (GSI, a ratio of weight of the egg to the total tissue weight) of 2–3 years old adult raised in the suspended long-line on the south coast prior to the spawning in June and July, and found it ranged from 40 to 60%. On the other hand, Mondol et al. (2012) reported the GSI of 7-month old hardened females transplanted in May and raised in the suspended long-line until August and September, prior to spawning, was low, ranging only 8–10%. This may result in poor larval recruitment and insufficient natural seed supplies to the oyster aquaculture industry year after. Therefore, the lower gonad-somatic index and egg production from the young oysters derived from May transferred juveniles, is a concern.

The objective of the present study was to test whether giving more times to the hardened juveniles to settle and grow in the grow-out site and accumulate energy reserves during the April to early May phytoplankton bloom would improve their growth and reproductive performance, by transferring them to grow-out facilities 4 months earlier, in January rather than in May.

2. Materials and methods

2.1. Study area and sampling effort

Gamakman Bay (34°40′ N, 127°42′ E) is one of the major oyster farming areas located off the southwest coast of Korea, encompassing a 112 km^2 surface area with a mean depth of 9 m (Lee et al., 1995, Fig. 1). Traditionally, Pacific oysters are cultured using a suspended long-line system in the bay (Choi, 2008). For the present study, oyster spats were collected from the sub-tidal area of the bay during the spawning season in July 2008 using dead oyster shells tied in a rope (i.e. oyster spat collector). The oyster spat collectors which contained the newly settled spats were suspended on wooden racks located in mid- to upper intertidal zone for hardening until they were moved to the suspended long-lines in May (Kang et al., 2010; Kim et al., 2009). During the hardening period, the oyster spats were exposed to the atmosphere during low tide.

To test effects of early transplantation on growth and reproductive performance, the first batch of the hardened juveniles with a mean shell length (SL) of 12.0 $\pm$ 0.5 mm was transplanted from the hardening ground to the long-line grow-out system in January 2009, after 6 months hardening. As reference, the second batch of the hardened juveniles was transplanted from the hardening ground to the grow-out facility in May 2009 (i.e., 10 months of hardening at the intertidal hardening ground), as they became 19.4 $\pm$ 0.4 mm in SL.

To monitor the growth and reproduction, 5 oyster strings of the January transferred oysters (JTO) containing over 100 growing oysters were collected monthly from February 2009 to January 2010, whereas 5 oyster strings of the May transferred oysters (MTO) were collected from the grow-out site from June 2009 to January 2010. For each sampled month, 30–40 of the oysters were randomly selected from the oyster strings and measured for SL, tissue wet weight (TWWT), gonad-somatic index (GSI, quantified egg production), levels of total protein and carbohydrate in the tissue, and determined gametogenic condition. The surface water temperature and salinity were recorded monthly using a CTD meter (YSI 556 MPS, USA). Chlorophyll a level in the water column was determined monthly using the spectrophotometric method.

2.2. Biometric measurements and histology

Thirty to forty oysters were removed from the oyster strings, cleaned of adherent epibionts, and the SL was recorded to the nearest 0.1 mm. The soft tissues were separated from the shells and weighed to mg. For histology, 1–2 mm-thick dorso-ventral section was made in the middle of the body and preserved in Davidson's fixative. The remaining tissue was weighed, freeze dried, and homogenized for biochemical