Effects of artificial upwelling on the environment and reared oyster *Crassostrea gigas* in Omura Bay, Japan

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Artificial upwelling was tested at Seihi, Omura Bay, Nagasaki Prefecture, as a way to improve environmental conditions for Pacific oyster farming. Aeration was performed from the sea bottom during two summer seasons in 2011 and 2012. Oceanographic parameters (temperature, salinity, dissolved oxygen concentration, chlorophyll *a* concentration, and suspended solids) and oyster performance (growth, survival, condition index, and glycogen levels) were monitored monthly. Aeration was shown to be efficient in improving water conditions for oyster farming, especially in the beginning of summer, by locally lowering water temperature by approximately 1°C, redistributing nutrients, and increasing diatom biomass. Dissolved oxygen concentration increased from October, at the beginning of autumn. The condition index of oysters was negatively related to distance from the aeration point. Furthermore, a reproductive season occurring when the aeration could not overcome high temperatures and formation of hypoxic water resulted in poor oyster health (condition index and glycogen levels decreased in September). Our results indicate that aeration can improve bivalve cultures if it is performed at a rate that overcomes hypoxia formation and high water temperatures throughout the summer period.

Key words: hypoxia, enclosed bay, artificial upwelling, Pacific oyster culture, condition index

Introduction

Bivalves are key organisms for developing sustainable aquaculture because they are usually farmed in coastal areas and do not require artificial feeding, instead depending mostly on primary production in their environment. Thus, bivalve production is extremely susceptible to characteristics of the natural habitat and requires areas with good year-round water quality (Mizuta et al., 2012).

Bivalve cultures are usually produced in enclosed bays characterised by calm waters suitable for aquaculture logistics. However, the water column in enclosed coastal areas is usually stratified in summer, resulting in a decrease in water quality for mariculture farms, which thus tend to experience oyster mortality events (e.g., Malham et al., 2009). Summer mortality events are a global problem (Kanno et al., 1965; Goulletquer et al., 1998), and many bivalve mortality precursors have been described. These include oxygen depletion (Akagi and Hirayama, 1991), high-temperature eutrophic conditions (Malham et al., 2009), virus infections (Burge et al., 2007), fouling organisms (Alagarswami and Chellam, 1976), and carbohydrate anabolism linked to reproduction (e.g., Cotter et al., 2010), including even shell asymmetry (Fréchette et al., 2003). The most accepted theory, however, is that mass mortalities are generally caused by a complex interplay of synergetic events (Chávez-Villalba et al., 2007; Malham et al., 2009).

The influences of low oxygen and high temperature have often been highlighted as causes of oyster summer mortalities. Although most coastal populations can tolerate exposures to low dissolved oxygen (DO) concentrations, prolonged exposures to conditions of less than 60% oxygen saturation may result in altered behaviour, reduced growth, adverse reproductive effects, and mortality (Goulletquer et al., 1998; Karim et al., 2002). Oxygen levels below the limit of 3 mg L^{-1} are considered hypoxic and deleterious for marine biota. For *Crassostrea gigas*, the optimum temperature range is, approximately 18–23°C, and temperatures higher than 33°C are thought to cause thermal shock and stress (Le Gall and Raillard, 1988; Bourlés et al., 2009). Temperature is also known to control gametogenesis in oysters.

Bivalve mortalities have been attributed to excessive gonad development and low glycogen levels in post-spawning periods. Glycogen is one of the main energy reserves in bivalves. The reserves accumulate when food is abundant but are subsequently utilised when metabolic demand is high, such as during periods of reproduction (Li et al.,

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2000). A low-energy condition could increase the sensitivity of oysters to various stresses (e.g., thermal stress, hypoxia) and thus be directly linked to oyster health (Berthelin et al., 2000). Glycogen reserves are often studied together with the condition index, which is a measure for recognising the nutritive state of bivalves that is also related to gametogenesis activity (Chávez-Villalba et al., 2007; Liu et al., 2010).

Periods of deteriorating water quality (e.g., eutrophication) producing anoxia have also been correlated with *C. gigas* mass mortality (Soletchnik et al., 2007). Hypoxia stress has been recognised to induce molecular responses, mainly in genes involved with cell communication, protein regulation, and most importantly, the immune system (David et al., 2005).

In this context, artificial upwelling has been used recently to enhance water quality and increase production in mariculture established areas (Berntsen et al., 2002). Positive effects of the induced upwelling flux include reduced hydrographical stratification promoted by turbulence, enrichment of superficial layers due to nutrients brought up with the deep water, and lower water temperatures (e.g., Strand, 1996). In recent years, experiments and models have been developed to improve water quality and favour phytoplankton blooms in bivalve farming locations (Williamson et al., 2009). In a Norwegian fjord, for example, artificial aeration was designed to evaluate the growth stimulation by upwelled nutrient-rich water of non-toxic algae used as food by mussels (Handa et al., 2013). Water brought up from lower layers successfully increased the biomass of the non-toxic algae by increasing the silicate input to euphotic layers. These results were considered promising for improving non-toxic algae biomass as food for cultured mussels.

However, the artificial upwelling method is not free of problems. The efficiency of upwelling depends strongly on the aeration design, such as the air flow rate, and on characteristics of the study area, such as the nutrient concentration and initial phytoplankton biomass. Particularly, with respect to increasing the oxygen concentration, the initial aeration bubble size and water depth are key factors determining the efficiency with which bubbles dissolve in the water column (Wüest et al., 1992). Trials of artificial upwelling have resulted in fairly localised break down of stratification, dilution of nutrient concentrations of upwelled water, and cases of both decreases and slight increases in oxygen concentration (Strand, 1996; Willianson et al., 2009; Handa et al., 2013). Despite these problems, artificial upwelling has been reported widely to be an important management tool for bivalve mariculture. However, no tests have been carried out to verify the extent to which an artificially improved environmental condition positively affects the performance of bivalves. Nevertheless, the response of cultured bivalves to artificial upwelling is a critical point in deciding whether to install artificial upwelling in bivalve mariculture areas.

Located in the north-western part of Omura Bay, Nagasaki Prefecture, Japan, the Seihi area has been a site of Pacific oyster farming for two decades (Fig. 1). In summer, however, more than half of the oyster production is lost. Such high mortality is a major concern in many bays all over Japan (e.g., Hirano et al., 2005), and it has a huge impact on the income of many families that depend on this farming activity. In the past, several anoxic-water upwelling events were reported to have been directly linked to fish mass mortalities and damage to other marine resources in Omura Bay (Akagi and Hirayama, 1991). For this reason, oyster mortality and low final production in Omura Bay have been speculated to be caused by hypoxic waters linked to high temperatures in summer. To reduce the mortality of cultured oysters, a pilot study involving closed systems, called 'mesocosms', in which water was aerated from the bottom has already been completed in a small bay within Omura Bay (Yamaguchi et al., 2007). Tests with and without aeration and with and without oysters were conducted. The results seemed promising for improvement of the water condition and oyster growth in these closed aerated systems.

As a next step, an open aeration system was installed on the bottom of the bay at the site of a Pacific oyster farm. The main purposes of the study were to assess the efficiency of the aeration on overall water quality improvement and investigate the effects of the aeration on oyster health and culture. Our hypothesis was that the aeration system would induce an artificial upwelling of cold, nutrient-rich water and locally increase the dissolved oxygen concentration in the water column. Nutrients would increase phytoplankton biomass, and the aeration flux would result in longer suspension of particulate organic matter in the water column, thus increasing food availability for filter-feeding oysters. As a result, oysters near the aeration were expected to be healthier in proportion to the distance from the aeration point.

Materials and Methods

The Seihi oyster culture area has a mean depth of 6.0 m and open contact with the larger Omura Bay on its western side (Fig. 1). Freshwater flows into the bay from a small river, the Daimioji River, located on the eastern side of the bay. Oysters are farmed in the southern part of the Seihi area.

Positioned on the bottom of the farming area, the aeration system consisted of two concentric circles with radii of 10 m and 15 m, respectively. Air was supplied by 40 air

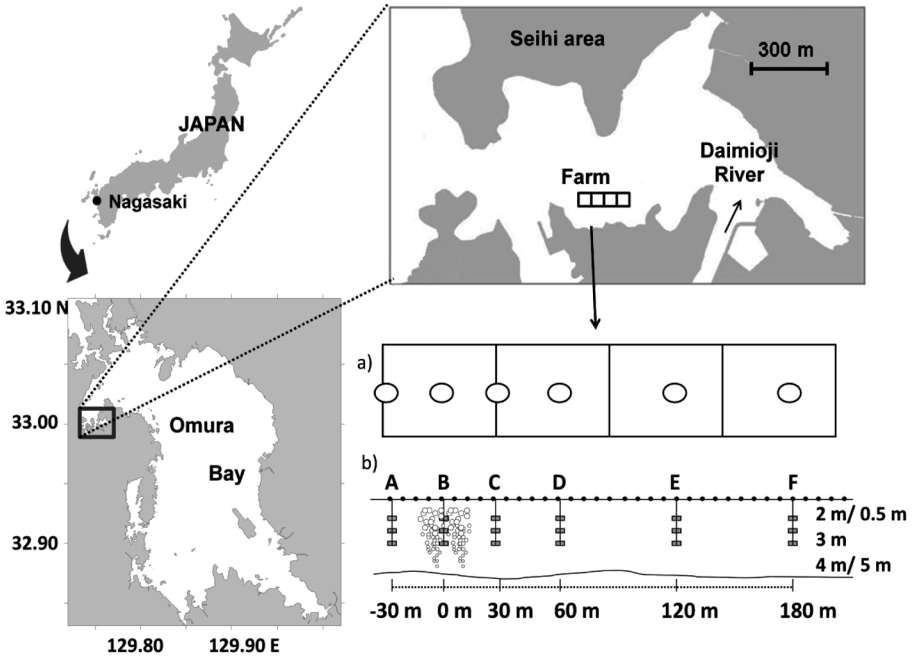

Figure 1. Location of Seihi in the northwestern part of Omura Bay, Nagasaki, Japan and the design of an aeration system: a) aerial view, and b) vertical view, showing the aeration point at St. B, location of other stations, as well as oyster cage depths in 2011 and 2012.

cocks connected to two air compressors (Hitachi oil-free screw compressors) installed on land at a rate of 0.15 m^3 min^{-1}. Aeration periods were two summer seasons: 6 September to 15 November 2011 and 26 June to 15 November 2012.

Cohorts of *Crassostrea gigas* spat, which consisted of three oysters between 50 and 60 mm in height, four oysters between 60 and 70 mm, six oysters between 70 and 80 mm, five oysters between 80 and 90 mm, and two oysters between 90 and 100 mm in 2011, and 56 oysters with shell height of 21.9±2.8 mm (mean±standard deviation) in 2012, were used to fill each of 18 cages (top diameter of 40 cm, bottom diameter of 45 cm, and height of 15 cm) with a mesh size of 5 mm. Oysters were smaller in 2012 because adult oysters were not available for purchase as a consequence of extreme decreases in oyster seed production after the Tohoku tsunami of the previous year. To assess the aeration effect, the cages were placed at different distances from the aeration point (St. B=0 m, Sts. A and C=30 m, St. D=60 m, St. E=120 m, and St. F=180 m) near the surface (2-m and 0.5-m depth in 2011 and 2012, respectively), middle (3 m in both years), and bottom layers (4-m and 5-m depth in 2011 and 2012, respectively) (Fig. 1). Cage depths differed between the two years because in the first year of the experiment, no differences were found in oyster condition in relation to cage depth, a fact that was attributed to the proximity of the cages. In 2012, cages were hung vertically with at least a 2-m depth difference separating them in a further attempt to investigate differences between depths.

Samplings and observations were performed at intervals of approximately 30 days from 28 August to 15 December in 2011 and from 30 June to 6 November in 2012 (Table 1). The sampling on 28 August 2011, which was conducted before the aeration had been turned on, constitutes some control data for the environmental characteristics in summer. The sampling dates for both years are specified in Table 2.

Environmental data

Temperature, salinity, fluorescence, and dissolved oxygen (DO) concentrations were measured using a CTD profiler (Rinko AAQ125, JFE Advantech). Water samples for chlorophyll analysis were collected at intervals of approximately 30 days at Sts. B, D (included only in 2012), and F. Station D was included in the water sampling in 2012 because, unlike St. F, it is not directly influenced by the Daimioji River. In 2012, DO recorders were installed at St. B at 0.5-, 3-, and 5-m depths and at St. F at 3- and 5-m depths for the duration of the experiments.

Simple stratification indices based on temperature (ΔT) and salinity (ΔS) were calculated for the St. B water column before (August 2011) and after (September 2011) the first-year aeration by the following formula:

Table 1. Summary of 2011 and 2012 sampling years main materials and methods.

Sampling year	Sampling period	Aeration period	Oyster culture period	Cage depths (m)	Number of oysters	Oyster height (mm)	Studied variables
2011	28 August to 15 December	6 September to 15 November	14 September to 15 December	2 3 4	3 4 5 5 2	50–60 60–70 70–80 80–90 90–100	Environmental variables Oyster growth & survival Oyster CI
2012	30 June to 6 November	26 June to 15 November	31 July to 6 November	0.5 3 5	56	21.9±2.8 (average±S.D.)	Environmental variables Oyster growth & survival Isotopes POM & oyster muscle Oyster CI Glycogen

$$\Delta T \text{ or } \Delta S_{\text{Month 2011}} = \text{Xs} - \text{Xb} \quad (1)$$

where Xs is the surface temperature or salinity at 0.2-m depth, and Xb is the temperature or salinity at 6-m depth.

To compare temperature and dissolved oxygen concentrations close to and far from the aeration system, differences in these parameters between St. B (centre of aeration) and St. D (just outside the 30-m region affected by aeration; see results in Fig. 3) were calculated from CTD profiler data for the whole water column and are expressed as vertically averaged values and standard deviations.

Water samples were collected with a Van Dorn bottle sampler at the respective cage depths for each year. The water was filtered immediately after collection. Chlorophyll *a* (Chl *a*) samples were extracted from particulate organic matter on a GF/F filter in the dark for 12 h by 90% acetone, and concentration was measured using a calibrated fluorometer (Trilogy Laboratory Fluorometer). Extracted Chl *a* concentrations and in situ fluorescence showed good correlation (R^2>0.80); thus, fluorescence was calibrated to Chl *a* concentrations (μg L^{-1}). Water samples of 500 mL collected at the same depths as the oyster cages were preserved with formalin (10%) for diatom biomass determination (cells L^{-1}). Suspended solid concentrations, expressed as annual means per station, were calculated as the difference between dried filter weights before and after filtration and the known amount of filtered water. Dissolved inorganic nitrogen (NO_2^- and NO_3^-), phosphate (PO_4^-), and silicate (SiO_2^-) concentrations were measured using an AutoAnalyzer (Bran-Luebbe, TRACSS 2000).

Oyster performance

Oyster samplings were started in the second month of each sampling season, as oyster cages were placed in the field during the first sampling (Table 1).

Growth and survival

Growth was recorded at intervals of approximately 30 days or annually as mean shell height (from umbo to valve extremity, in mm) of live oysters in the cage. Percentage survival was recorded based on the numbers of live and dead oysters and is expressed in relation to the oysters initially stocked in the previous sampling or at the beginning of the experiment to obtain approximate monthly or annual estimates, respectively. Both parameters were calculated for each station and are expressed as mean values with standard deviations.

Oyster condition

In each sampling, up to three oysters were collected from each cage, and both dry shell weight and dry meat weight were measured to calculate the condition index (CI) of the oysters, which is expressed by

$$\text{CI} = (\text{dry soft tissue weight}/\text{dry shell weight}) \times 1000. \quad (2)$$

Oysters were shucked and weighed after drying in an oven at 60°C to a constant weight (24–72 h). Although it has been argued that condition index analysis alone should not be used in ecophysiological studies, CI is regarded as a good index of oyster health if used together with collaborating growth and environmental data (Brown and Hartwick, 1988). Additionally, CI is a trustworthy tool for analysing growth of oysters because volume and consistency of body tissues may not conform to the increase in the shell (Yonge, 1960).

Oyster mantle glycogen

Glycogen is one of the main energy reserves in bivalves and is regarded as a quantitative measure of physiological changes. Glycogen storage activity usually occurs under favourable environmental conditions, and mobilisation and conversion of reserves usually occur during activities with high metabolic demand, such as reproduction (Li et al., 2010).

In each sampling during 2012, up to three oysters from each cage were dissected, and mantle tissues were identified, extracted, and weighed. Homogenised mantle tissue samples (0.02–0.1 g) were suspended in 30% KOH and saponified by heating to 100°C for 2 h. Precipitates were

Table 2. Nutrient concentrations at Sts. B, D and F in 2011 and 2012. Values under the detection limit are indicated by "<DL". "*" means samplings in which the aeration was off.

Sampling	Station	Depth (m)	$[NO_3^-]$ (μM)	$[NO_2^-]$ (μM)	$[PO_4^-]$ (μM)	$[SiO_2^-]$ (μM)
28 August 2011*	B	0	46.52	<DL	1.08	59.44
28 August 2011*	B	5	14.24	0.14	1.50	26.15
28 August 2011*	F	0	40.53	<DL	1.22	63.31
28 August 2011*	F	5	16.25	<DL	1.25	19.90
14 September 2011	B	0	14.57	0.14	1.27	24.84
14 September 2011	B	5	14.69	0.38	1.55	22.06
14 September 2011	F	0	14.81	<DL	1.15	24.14
14 September 2011	F	5	13.70	<DL	1.49	21.53
13 October 2011	B	0	15.69	<DL	1.27	32.69
13 October 2011	B	5	15.03	<DL	1.28	23.47
13 October 2011	F	0	16.20	<DL	1.23	44.81
13 October 2011	F	5	15.08	<DL	1.29	21.38
23 November 2011	B	0	0.79	<DL	0.23	19.49
23 November 2011	B	5	0.07	<DL	0.25	10.87
23 November 2011	F	0	1.23	<DL	0.20	32.67
23 November 2012	F	5	0.07	<DL	0.25	8.92
14 December 2011*	B	0	0.00	0.03	0.19	18.49
14 December 2011*	B	5	0.00	0.09	0.35	27.67
14 December 2011*	F	0	16.56	0.26	0.37	50.10
14 December 2011*	F	5	1.15	0.64	0.20	9.93
30 June 2012	B	0	20.11	0.32	0.02	58.92
30 June 2012	B	3	<DL	0.16	0.28	27.74
30 June 2012	B	5	1.15	0.26	0.48	32.62
30 June 2012	D	0	27.10	0.35	0.00	70.77
30 June 2012	D	3	<DL	0.16	0.18	24.05
30 June 2012	D	5	2.06	0.28	0.41	38.47
30 June 2012	F	0	48.25	0.39	0.02	95.78
30 June 2012	F	3	<DL	0.19	0.31	27.05
30 June 2012	F	5	1.60	0.27	0.40	31.51
31 July 2012	B	0	21.93	0.30	<DL	80.50
31 July 2012	B	3	<DL	0.03	<DL	4.55
31 July 2012	B	5	<DL	0.13	0.02	11.97
31 July 2012	D	0	5.88	0.17	<DL	86.64
31 July 2012	D	3	<DL	0.03	<DL	3.66
31 July 2012	D	5	<DL	0.06	0.16	9.52
31 July 2012	F	0	10.39	0.14	<DL	101.95
31 July 2012	F	3	<DL	0.02	<DL	3.51
31 July 2012	F	5	<DL	0.04	<DL	5.48
4 September 2012	B	0	0.40	0.08	0.16	5.86
4 September 2012	B	3	0.01	0.12	0.25	1.46
4 September 2012	B	5	0.10	0.35	0.41	3.44
4 September 2012	D	0	0.09	0.02	0.00	15.51
4 September 2012	D	3	<DL	0.05	0.18	0.00
4 September 2012	D	5	0.04	0.23	0.28	2.26
4 September 2012	F	0	<DL	0.02	0.08	16.78
4 September 2012	F	3	0.08	0.02	0.07	0.00
4 September 2012	F	5	0.15	0.26	0.65	5.46
2 October 2012	B	0	3.57	0.14	0.35	11.39
2 October 2012	B	3	0.81	0.08	0.31	2.80
2 October 2012	B	5	1.58	0.06	0.29	2.18
2 October 2012	D	0	21.60	0.24	0.65	84.14
2 October 2012	D	3	0.46	0.05	0.29	3.89
2 October 2012	D	5	0.75	0.10	0.36	4.14
2 October 2012	F	0	8.66	0.12	0.20	45.61
2 October 2012	F	3	0.59	0.09	0.25	2.58
2 October 2012	F	5	0.33	0.06	0.43	5.30
6 November 2012	B	0	12.62	0.29	0.78	30.48
6 November 2012	B	3	1.34	0.49	0.29	8.54
6 November 2012	B	5	1.08	0.45	0.22	7.56
6 November 2012	D	0	6.05	0.19	0.43	16.99
6 November 2012	D	3	1.55	0.36	0.24	8.54
6 November 2012	D	5	1.09	0.59	0.31	7.85
6 November 2012	F	0	16.29	0.21	0.81	38.79
6 November 2012	F	3	0.43	0.35	0.21	6.35
6 November 2012	F	5	1.56	0.58	0.31	9.29

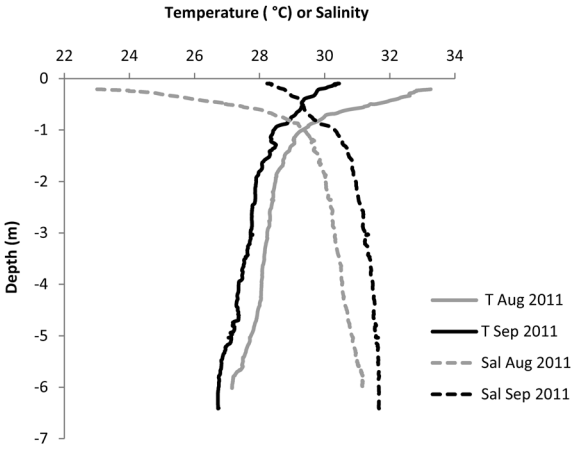

Figure 2. Stratification comparison before (31 August 2011) and after artificial upwelling (14 September 2011) at St. B. Full lines represent temperature profiles and dashed lines represent salinity profiles.

obtained after the addition of EtOH and overnight cold-temperature storage. To the centrifuged precipitates, 2% sodium sulphate and 95% EtOH were added, and the mixture was left to cool overnight. Samples were again centrifuged, and 1 N sulphuric acid was added to the precipitates to obtain the final supernatant. Samples were then treated with cold anthrone solution, boiled for 15 min, and cooled. Absorbance of the resulting coloured complex, which indicates the concentration of glycogen in the samples, was measured at a wavelength of 620 nm.

Aeration was expected to locally increase available food and change the water temperature. Therefore, mantle tissues were selected for the glycogen analyses due to reported sensitivity of these tissues to chlorophyll levels and reproduction, which is believed to be triggered by high temperature (Li et al., 2009).

Statistical analysis

Data analyses were performed with PASW Statistics software. Variance homogeneity was tested with Levene's test, and the differences in means between stations were tested by one-way ANOVA. The significance limit was set to 0.05. Relationships between parameters were evaluated by regression models.

Results

Hydrology and phytoplankton

Before the aeration period, in a control sampling in August 2011, water column stratification was strong, especially within the top 1 m of the surface water ($\Delta T_{Aug2011}$=6.1°C and $\Delta S_{Aug2011}$=−8.15, Fig. 2). Sampled stations showed similar characteristics, including well-defined thermoclines and haloclines. Dissolved oxygen concentration decreased to as low as 2 mg L^{-1} in the bottom layer during this control month. The first sampling after aeration was begun, in September 2011, showed a relatively less stratified water column ($\Delta T_{Sep2011}$=3.1°C and $\Delta S_{Sep2011}$=−2.9). With the aeration system turned on, the vertical profiles at Sts. A, B, and C changed dramatically, and both years showed similar results. Stratification in the water column was weaker within 30 m of the aeration point. Isotherms and DO isopleths were inclined toward the surface near the aeration, indicating upwelling of water from lower layers to the surface (Fig. 3).

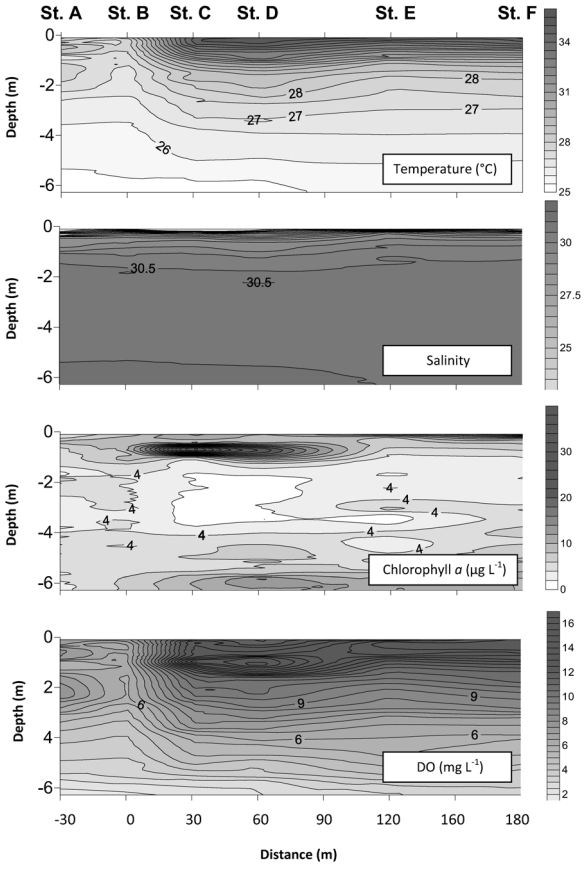

Figure 3. Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen on 31 July 2012, after 35 days of aeration.

Throughout the aeration period, especially in September and October 2011 and July and September 2012, the water column at St. B was less thermally stratified and more homogeneous in DO than those at Sts. D and F, indicating mixing induced by the aeration (Figs. 4 and 5). The temperature was 0.9±1.3°C lower at the aeration spot compared to the water column more than 30 m from it. However, DO was 1.7±1.8 mg L^{-1} lower near the centre of aeration due to upwelling of low-oxygen bottom water.

Hypoxic water formed near the bottom layers in August 2011 and July 2012, but the oxygen concentrations remained

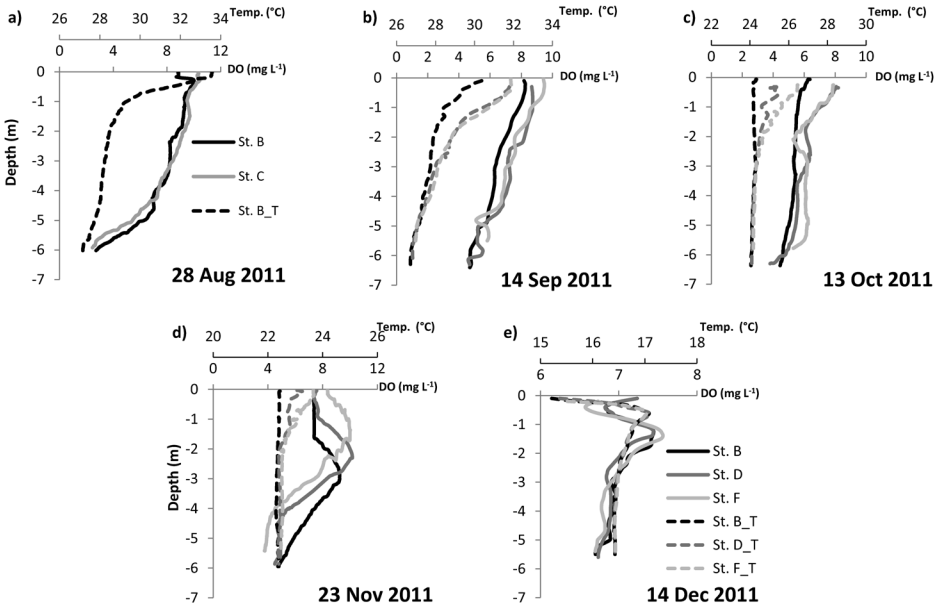

Figure 4. Vertical profiles of dissolved oxygen (mg L^{-1}, solid lines) and temperature (°C, dashed lines) for the year 2011 at: a) Sts. B, C in August, b) Sts. B, D and F in September, c) Sts. B, D and F in October, d) Sts. B, D and F in November, and e) Sts. B, D and F in December. The legend for a) is beside its own graph, while other months share the same legend located beside e) graph.

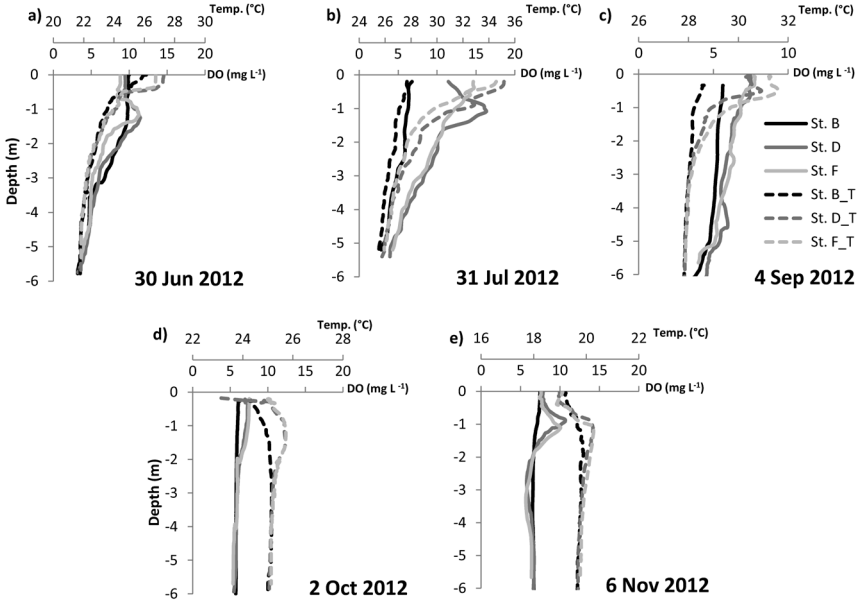

Figure 5. Vertical profiles of dissolved oxygen (mg L^{-1}, solid lines) and temperature (°C, dashed lines) for the year 2012 at Sts. B, D and F in: a) June, b) July, c) September, d) October, and e) November.

higher than the defined hypoxic limit of 3 mg L^{-1} in successive months in both years. From the end of September (when the temperature started to decrease), data from DO recorders indicated an increase in DO concentration in the middle and bottom layers (saturation values close to 100%) at St. B (Fig. 6). Moreover, temperatures in the middle and bottom layers were similar during this period, and ΔDO reached values close to zero at St. B, indicating mixing. Compared with St. B, St. F did not show the same water mixing trend between the middle and bottom layers, instead showing more oxygen in the middle than in the bottom layer (Fig. 6).

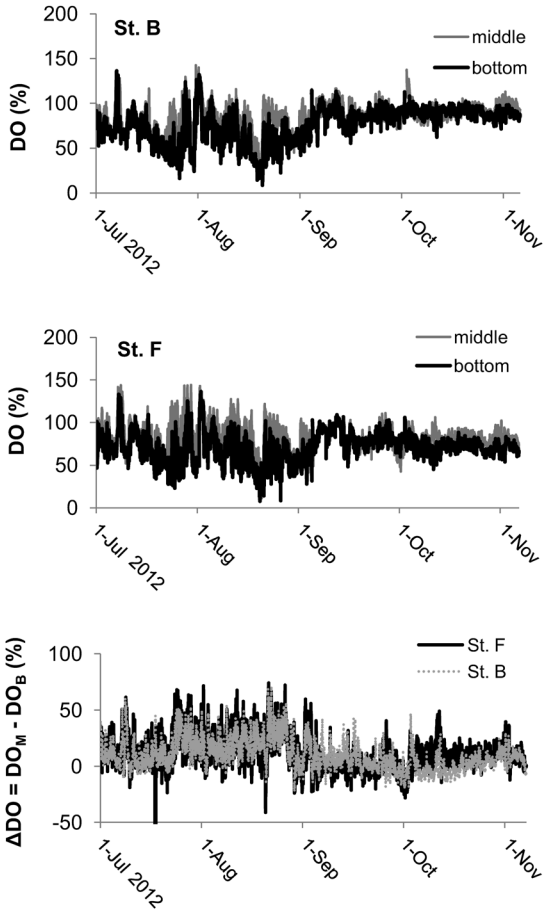

Figure 6. Time changes in dissolved oxygen (%) measured by installed sensors at Sts. B and F.

The first sampling of 2012 (31 June) showed an increased chlorophyll *a* level between 2- and 4-m depth at St. B compared with the other sampled stations (Fig. 7). In September 2012, the surface layer at the same station showed chlorophyll *a* concentration values of about 10 μg L^{-1}, whereas the Chl *a* concentrations at Sts. D and F were lower, at least within the 3-m depth. On the other hand, after September, chlorophyll *a* vertical profiles showed lower values in the water column at St. B compared with the other two stations. Furthermore, the values at St. B were entirely homogeneous, while Sts. D and F had chlorophyll peaks at different depths. Vertical profiles of chlorophyll *a* in 2011, a year in which aeration was started in September, showed exactly the same trends as for 2012 during the same months. In September 2012, diatom biomass at St. B was increased by as much as 5 times at the surface and 3 times at the bottom layer compared with Sts. D and F (Fig. 8). The most abundant diatom species were *Chaetoceros* spp., *Skeletonema* spp., *Thalassiosira* spp., and species of Pennate diatoms.

Suspended solids were slightly different among stations, with mean values of 0.018 mg mL^{-1} and 0.016 mg mL^{-1} at Sts. B and F, respectively, during the aeration period in 2011. In 2012, mean values were 0.048 mg mL^{-1}, 0.047 mg mL^{-1}, and 0.044 mg mL^{-1} at Sts. B, D, and F, respectively.

Nutrients

In the first sampling year, no differences in nutrient concentration that could have been induced by aeration were found between Sts. B and F. In the second sampling year, in July and September 2012, the nitrate concentration in the

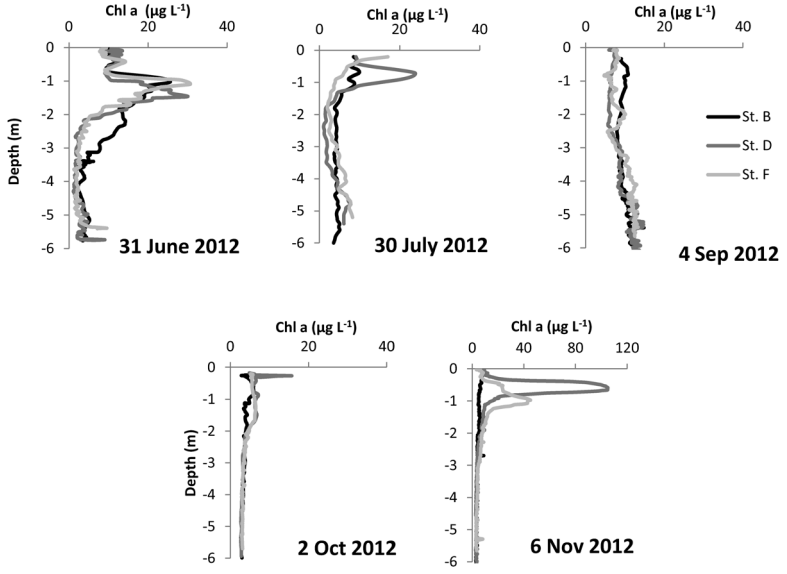

Figure 7. Vertical profiles of chlorophyll *a* (μg L^{-1}) at Sts. B, D and F in 2012.

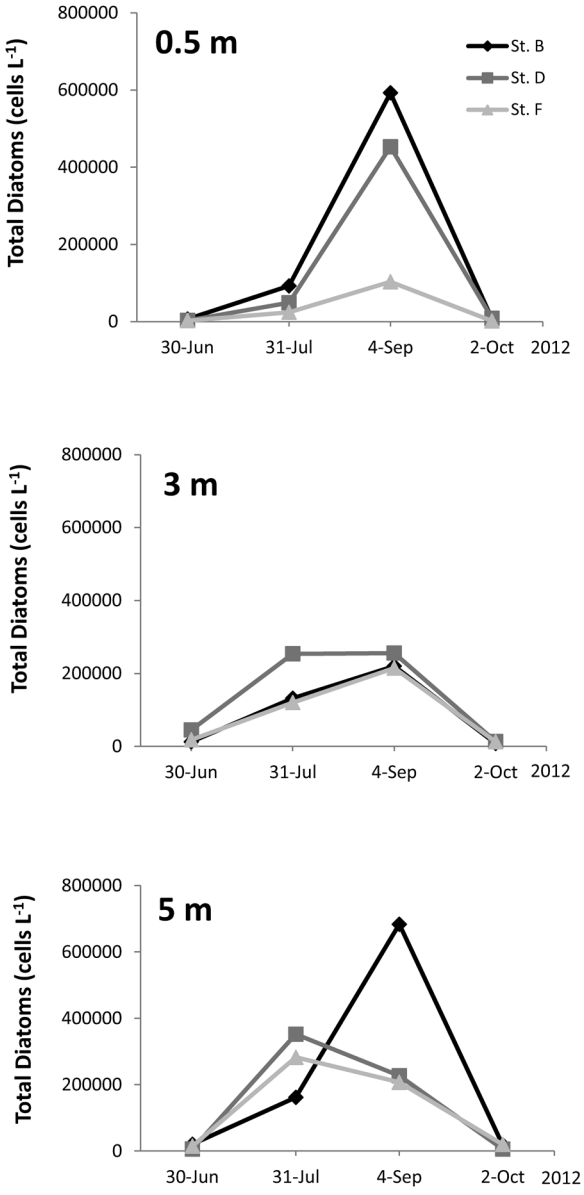

Figure 8. Diatom biomass (cells L^{-1}) in 3 different layers at Sts. B, D and F in four samplings in 2012.

surface layer at St. B was markedly higher than at the other two stations (Table 2). Nitrite and phosphate concentrations were low during the observation period at all stations. For those nutrients, St. B showed markedly low values in the July and September samplings, with maximum values occurring in the bottom layer (maximum values at 5-m depth of NO_2^-=0.35 μM and PO_4^-=0.41 μM). Dissolved silicate concentrations were similar among Sts. B, D, and F at the beginning of the experiment. However, silicate concentrations subsequently decreased in the surface layer at St. B in September and October, whereas St D. and St. F maintained relatively higher values in the same layer

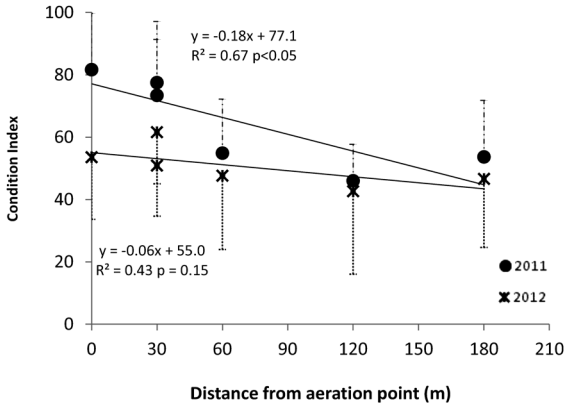

Figure 9. Condition index of oysters expressed as annual means per station, in relation to the distance from the aeration point in 2011 and 2012.

(Table 2).

Oyster crop performance

With a final mean of 90.0±1.2 mm, oyster growth in 2011 was similar among all stations and showed no pattern of differences among depths. In 2012, oysters at St. F (71.1±13.8 mm) had the best final growth, followed by those at St. E (68.4±13.0 mm), and St. B (65.3±12.7 mm), although the best growth fluctuated across stations and months. Survival rates in 2011 did not differ among stations or among depths in any month. Final survival was lower (mean=80.3%) in September compared to the other months. In 2012, final survivals were higher at Sts. D, E, and F (mean=38.9%) than at Sts. A, B, and C (mean=16.7%). A distinctively high survival rate in this year was achieved by oysters at the St. F surface layer cage (mean=91.7%).

Significant negative correlations were found in 2011 between the distance from aeration and the CI oysters per station (R^2=0.67, p<0.05; Fig. 9) and per cage (R^2=0.61, p<0.01). In 2012, the CI was generally higher at stations closer to the aeration point, but the difference was not significant per station (R^2=0.43, p=0.15; Fig. 9) or per cage (R^2=0.16, p=0.92). Relationships between CI and environmental parameters were not significant for the entire experimental period. However, in the first month of sampling in 2012, CI was negatively related to temperature and DO (R^2=0.58, p<0.01 and R^2=0.56, p<0.01, respectively) and positively related to chlorophyll *a* (R^2=0.37, p<0.05) in the middle and bottom layers.

Glycogen values were significantly higher in oysters grown in the surface cages in 2012 (ANOVA p<0.05). However, glycogen levels in oysters did not differ significantly among stations (ANOVA p=0.63) when all depths were considered. At all stations, the glycogen contents of all oysters decreased sharply in the second sampling (September 2012) but recovered by November, the time of the

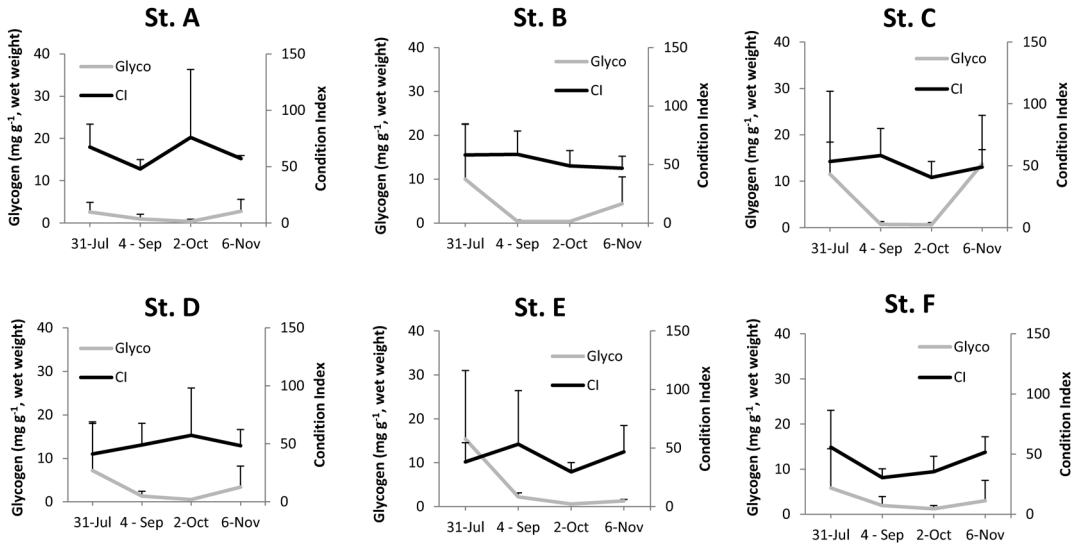

Figure 10. Variations in glycogen contents (mg g^{-1}, wet weight) in oyster mantle tissue and condition index, through the aeration period in 2012.

last sampling. Glycogen trends were followed by CI at all stations (Fig. 10). Glycogen showed a negative logarithmic relationship with mortality ($y=-7.64 \ln x+26.0$; $R^2=0.29$, $p<0.01$; Fig. 11a). In contrast, glycogen concentration in the mantle was positively related to DO concentration ($R^2=0.35$, $p<0.001$) in 2012 (Fig. 11b).

Discussion

Artificial aeration was employed on the bottom of a semi-enclosed bay in an attempt to boost production in an oyster farm by improving water quality and thereby producing healthier oysters in summer. The collected data consisted of hydrological parameters and biological measurements of oysters at different distances from the aeration point. As will be discussed, the aeration was shown to change the environmental conditions in the water column, especially at the beginning and end of the summer season. Because the experiment was started in the middle of summer during the first year (August 2011), it was considered that our data could not show the effects that may have resulted during that year had the experiment started earlier. As a result, 2012 data provide a more accurate demonstration of the potential outcomes of aeration during the summer season.

The general effects of artificial upwelling were investigated by comparing the periods of aeration with a control sampling when the aeration was off (August 2011), as well as with comparisons among stations located at progressive distances from the aeration point. Most of the data showed no differences among depths; therefore, these comparisons will only be discussed when relevant. Specific effects of the aeration are discussed based on the results of samplings prior to oyster spawning season.

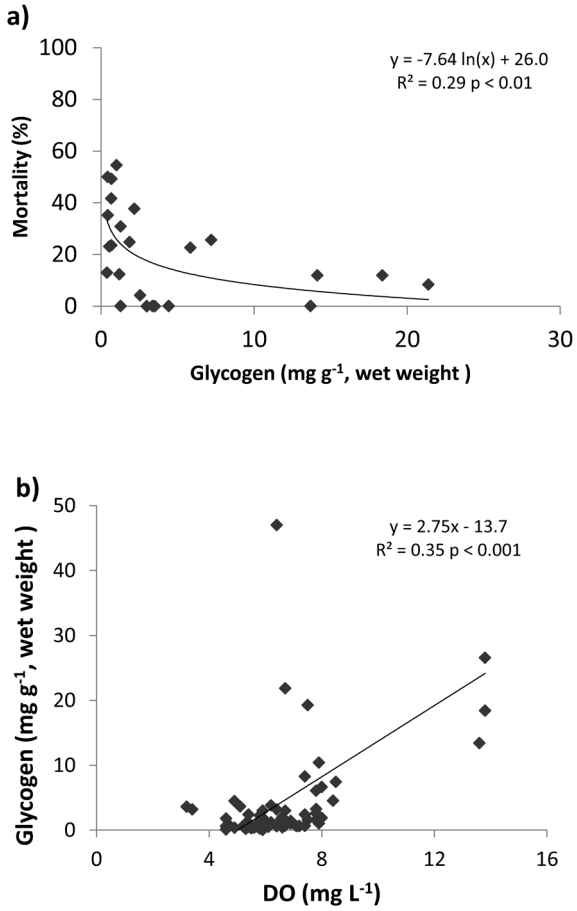

Figure 11. Relation between glycogen concentration (mg g^{-1}, wet weight) and: a) mortality (%); b) dissolved oxygen (mg L^{-1}).

From the CTD data, it was possible to establish two distinct areas: an area within 30 m of the aeration, where the effects of bubbling could be detected and the water column was mixed, and an area farther from the aeration, where the water column was stratified and patterns of temperature, salinity, and oxygen stratification were similar (Fig. 3). The temperature in the former area decreased because of the constant upwelling of cold bottom water towards the surface. Surprisingly, the aeration did not increase DO concentration in the water near the aeration point. Instead, cold, nutrient-rich but low-oxygen water reached the surface. This is at odds with our expectation of improved DO concentration, and it shows that factors such as gas dissolving rate and instantaneous water characteristics have to be considered in order to set the appropriate aeration rate to increase the DO concentration in the water (Wüest et al., 1992; Strand, 1996).

In our study area, hypoxic water formed in the summer of both years of the experiment. Water with low DO concentration was detected in early summer samplings during August 2011 and July 2012 (Figs. 4a and 5b). The former sampling occurred prior to the beginning of aeration, but aeration was being performed during the latter and was not sufficient to counter the formation of hypoxia. Rather than being affected by the aeration system, the formation of oxygen-deficient water in the bottom layer from July to September was a result of seasonal variation. Above the 4-m depth and within 30 m of the aeration point, the aeration reduced DO concentration due to upwelling of low-oxygen waters previously concentrated at the bottom. Fortunately, levels of oxygen in the water column were sufficiently high (>3 mg L^{-1}) to accommodate this change. Thus, the negative impact of lower oxygen on the oysters is believed to have been secondary to the effects of high temperature. The aeration increased DO concentration only at the end of summer when temperature was already mild, maintaining mixed middle and bottom layers (Fig. 6).

No effect of aeration on suspended solids was detectable in our data. Nevertheless, the concentration of suspended solids at the aeration point (St. B) was similar to that at St. F, which was close to the river.

The pattern of nutrients differed between the water column near the aeration and that at more distant stations (Table 2), and this was related to primary production. Phytoplankton, whose production is often limited by nitrogen and/or phosphate, almost immediately take in nutrients that were supplied at higher than normal rates from upwelled water (Sakshaug and Olsen, 1986). In June 2012, the water column at St. B between 2- and 4-m depths had increased Chl *a* concentration, and in July, nutrients showed higher values at the surface at St. B than at the other stations. With abundant nitrogen, the quick utilisation of phosphate in the food web might explain why it was not possible to detect higher concentrations of phosphate close to the artificial upwelling in our data. In September, nutrient-rich water reached the surface, enabling a phytoplankton bloom. This bloom seems to have consumed the nutrients and, together with the on-going mixing of the water column that caused nutrient dilution, prevented the support of late-summer primary production in October and November at St. B (Figs. 7 and 8). On the other hand, Sts. D and F showed higher primary productivity in late summer, as nutrients from the bottom layers were redistributed to upper layers due to decreased water column stratification.

At St. B, it is possible that nutrient dilution was one of the outcomes of artificial upwelling, as already described in models by Williamson et al. (2009). These researchers called attention to the importance of determining the minimum nutrient concentration required to sustain a viable phytoplankton population in the area intended to host an artificial upwelling. Nonetheless, St. B maintained higher nutrient concentrations during the beginning and end of each sampling year, suggesting that the aeration promoted increased nutrient concentrations. Phytoplankton abundance at St. B was also higher than at the other control stations, possibly due to the upwelling of nutrients and cysts of microphytobenthos in the sediment (e.g., Fuji and Matsuoka, 2006).

Dominance of diatoms over dinoflagellates in the phytoplankton community represents a desired situation in relation to food quality for bivalves because diatoms are higher quality food than are dinoflagellates for bivalves (Strand, 1996). Previous work indicated that both dinoflagellates and diatoms are present in Omura Bay (Fuji and Matsuoka, 2006; Ishii et al., 2011). Species of the former dominate in oligotrophic stratified water columns, whereas the latter often dominate in weakly stratified and homogeneous waters (Cushing, 1989). The three most abundant diatom species found in Seihi, *Chaetoceros* spp., *Skeletonema* spp., and *Thalassiosira* spp., are well known for forming resting spores (Ishii et al., 2011; Wang et al., 2013). Increases in diatom biomass at Seihi are believed to be a result of nutrient availability and resting-stage cells suspended in the water column due to the aeration.

Although the final growth of oysters at St. B was relatively good, the shell height at St. F, especially at the surface layer, surpassed that at the other locations. It is usual for oysters grown in surface layers to display higher growth due to abundant food, but at our site, the difference also seemed to be related to the proximity of freshwater. Many bivalve-fouling organisms are suspension feeders and might compete with oysters for food (Gosling, 2004). Freshwater runoff usually kills fouling organisms, favouring oyster growth (Oczkowski et al., 2011; Pollack et al.,

2011).

It is known that care is required when measuring growth by shell height because the volume and consistency of soft body tissues may not conform to the changes in shell height (Yonge, 1960). Therefore, in addition to shell growth, condition index measurements were obtained because they also take body tissues into account.

Some research has related CI with temperature, salinity, chlorophyll *a* (Rheault and Rice, 1996; Yildiz et al., 2011), dissolved oxygen concentration, and sexual maturation (Maldonado-Amparo, 1998; Li et al., 2010; Liu et al., 2010). However, other studies found no direct relationships between bivalve CI and these environmental parameters (Li et al., 2009) or reproduction (Chávez-Villalba et al., 2007). At Seihi, negative relationships of CI with temperature and DO could be detected in the first sampling in the middle and bottom layers, as well as a positive relationship between CI and Chl *a* level. Because DO concentration near the aeration system was lower until the end of the summer season and the results of the present study showed a positive relationship between proximity to the aeration point and oyster CI, oxygen does not seem to have directly affected CI. Despite the DO concentration, by lowering temperature and increasing plankton quality (favouring diatoms) and biomass, the aeration improved water quality, and this was reflected in the high CI of oysters in the first samplings. Nevertheless, relationships between CI and environmental parameters were not significant if the entire duration of the aeration was considered, possibly because of the strong negative effects of temperature. When temperature varies in the range of 23–25°C, just above the optimum for this species (Le Gall and Raillard, 1988; Bourlés et al., 2009), any small difference in temperature may affect oyster condition differently. On the other hand, it is likely that at extremely high temperatures of more than 28°C, as were observed in August–September, small variations in temperature do not result in different biological responses, implying that the relationship between CI and temperature is non-linear when a wide range of temperatures is considered.

C. gigas summer mortalities appear to be linked to a sequence of situations. The sequence begins with food availability leading to fast growth, along with stress induced by oxygen depletion and by high temperature, which triggers reproductive activity. In the end, oysters exposed to stressful conditions have low glycogen reserves and are vulnerable to pathogens (Goulletquer et al., 1998). In the 2012 experiment, oysters at Sts. A, B, and C had lower survival rates than those at stations farther from the aeration point. The synergetic effects of lower oxygen concentration and season-induced high temperature may have resulted in higher mortality among these oysters during the period of maximum temperature in summer. Moreover, increased temperature is known to favour pathogen growth at high-salinity sites (Kanno et al., 1965; Powell et al., 1994). Indeed, salinity may be the differentiating factor between the mortality rates at the stations close to the aeration point (Sts. A, B, and C) and those far from it (Sts. D, E, and F). It is well known that freshwater from rivers kills epibionts; thus, oysters that are often bathed by low-salinity waters have fewer predators than those at sites where there is almost no direct freshwater input (Oczkowski et al., 2011; Pollack et al., 2011). Unfortunately, epibiont data were not assessed in this work to reinforce the observations mentioned earlier, but a difference in epibionts between stations closer to the freshwater discharge and stations far from it was clear during the samplings periods.

Mortality has also been linked to periods of low glycogen storage (Goulletquer et al., 1998; Li et al., 2010). Glycogen contents in mantle tissues were measured as a second attempt to check the health of oysters. Although glycogen levels were high in July, they dropped considerably at all stations in September (Fig. 10). The decrease in glycogen content in the mantle tissues was followed by decreased CI in the oysters, but with a time lag. Because the biochemical variations in oysters grown in different locations were similar, it seems likely that decreases in mantle-tissue glycogen occurred during gonadal development. This conclusion is supported by a reproduction study by Li et al. (2010), which described oyster metabolic changes associated with reproduction. Spawning and depletion of glycogen reserves in oysters usually occur after periods of high temperature (>28°C), such as the temperatures in September in our work. When gametogenesis begins, the glycogen stored in tissues is used to support gonad development, a period of high metabolic demand (Liu et al., 2010). This increases the CI index of oysters because energy is allocated from mantle tissues to the development of gonads, which would weigh more during a reproductive stage than the mantle previously did. Chávez-Villalba et al. (2007) reported that high oyster CI during periods of accelerated reproductive activity was associated with mortality events. However, in our data, spawning possibly occurred following a small increase in CI. Then, CI started to decline and recovered only after the spawning season, during increasing energy storage, as indicated by the glycogen content. Although the temperature had already dropped by October, glycogen contents remained low, indicating that the oysters were still in a recovery period. Li et al. (2009) showed that oysters needed a month to recover CI levels after spawning and to concomitantly increase their energy reserve.

As in Li et al. (2009), oyster CI recovery at Seihi seemed to last until November, when oyster glycogen levels rose

again. A spawning season from July to September and subsequent decreases in glycogen and CI index have been identified for Pacific oysters in Japanese waters by Akashige and Fushimi (1992) and were confirmed by our findings.

Glycogen content in the mantle was significantly related to the mortality that occurred between the July and September samplings (Fig. 11a). It is known that oysters after spawning are more vulnerable to environmental stress and diseases (Li et al., 2010). Because glycogen was related to DO concentration in the environment through a highly significant relationship (Fig. 11b), this suggests another possible cause for the differences in mortality between stations close to and those far from the aeration system.

Glycogen synthesis in *Ostrea edulis* is inhibited by anoxia (DO～0 mg L^{-1}), although inhibition does not take place at low oxygen concentrations (L-Fando et al., 1972). As different species of oysters would be expected to react differently to DO levels and glycogen synthesis inhibition, *C. gigas* may store more energy in tissues at increased DO levels. Considering both the oxygen concentration and mortality relationships with glycogen reserves, it is possible to conclude that providing aeration maintains lower water temperature and good levels of dissolved oxygen, oysters can store more energy and thereby increase survival during subsequent spawning periods.

Conclusions

In summary, this study documented hydrological improvements promoted by artificial upwelling that benefitted Pacific oyster health. The upwelling of cold and nutrient-rich bottom waters drastically reduced hydrographical stratification within 30 m of the aeration point. Improvements in water quality included relatively lower temperature, redistribution of nutrients, and re-suspension of dormant phytoplanktonic cells, leading to increased phytoplankton biomass and possible phytoplankton quality improvement. Although the aeration seemed to have a positive effect on oyster condition (CI), which could be explored with respect to aquaculture logistics, this relationship should be investigated further. At the rate performed, aeration could not overcome negative oxygen and temperature effects on oysters from July to September. As a result, reduced stored energy and CI during reproduction season left oysters vulnerable, and mortality followed. However, water quality improved at the beginning of autumn, with optimum temperatures and increased DO levels due to aeration. A further need for an upwelling system is to guarantee the positive benefits on oyster cultures (lower temperatures, improved food, and increased oxygen concentrations) throughout the summer season. It is clear that different aeration rates and designs may lead to better results (Williamson et al., 2009; Fan et al., 2013). Our data also suggest that at Seihi, oyster cultures would have better results if a river-proximal site were used in combination with artificial aeration.

In conclusion, additional research is necessary to explore the effects of aeration rates and experiment designs on oyster production because aeration requirements can differ among target species and localities.

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大村湾における人工的な湧昇が養殖マガキ*Crassostrea gigas*の漁場環境と生育に及ぼす影響

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長崎県大村湾内の西彼地区において，人工的な湧昇によりマガキ養殖場の環境改善を試みた．2011年と2012年の夏季に海底から曝気を行った．水温，塩分，溶存酸素濃度，クロロフィル*a*濃度，懸濁態物質などの海洋環境とマガキの成長，生残，コンディションインデックス，グリコーゲン含有量などの生態学的状態を毎月調べた．曝気により，特に夏季の初めには，曝気点付近で約1℃程の水温低下，上層への栄養塩供給，珪藻の増加などの養殖場の環境改善が見られた．溶存酸素濃度は10月（秋季の初め）に増加した．マガキのコンディションインデックスは，曝気地点からの距離と負の相関があった．しかしながら夏季には高水温と貧酸素状態を解消するほどには至らなかったため，マガキの生育状態の指標となるコンディションインデックスやグリコーゲン量が9月には低下した．曝気によって夏季の貧酸素や高水温状態を解消させることができれば，この方法はマガキ養殖場の環境改善に役立つ有用なツールになると考えられる．

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