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A report on the mass summer mortalities of the farmed Pacific oysters, *Crassostrea gigas* and Bay scallops *Argopecten irradians* in the local waters of Goseong Bay, Korea

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ABSTRACT

Mass mortalities of farmed shellfish, mostly in summer season, thus named mass summer mortalities, have been a global issue in shellfish aquaculture. The 2013 mass summer mortalities in the confined waters of Goseong Bay, Goseong, Korea were quite a unique and intensive for two farmed species, the Pacific oysters, *Crassostrea gigas*, and bay scallops, *Argopecten irradians*. The mortalities were progressive from the bottom of the suspended oysters and caged scallops in the waters, reaching up to 80% for the oyster and 95% for the scallop in about 20 days after the first occurrence, early August, 2013. We monitored a wide range of environmental factors, including water temperature, dissolved oxygen (DO), salinity, turbidity, acidity (pH), organic and inorganic matters, chemical oxygen demand (COD), suspected pathogenic agent, and phytoplankton composition throughout the water column where the two species were suspended or caged. Our survey concluded that the hypoxia or anoxia might be a major cause of the mortalities. Here, we detailed the mortalities and ways to arrive at the conclusion.

Key words: Mass summer mortality, Hypoxia, *Crassostrea gigas*, *Argopecten irradians*.

Introduction

Goseong Bay, Goseong, Korea provides a relatively small but productive water body for oysters and scallops. In August, 2013, farmed oysters *Crassostrea gigas* and scallops *Argopecten irradians* experienced great mortalities in the local waters of the bay. The mortalities occurred over a short period of time and they were significant and quite an abnormal.

Mass summer mortalities of shellfish have been a global happening over a variety of shellfish, including oysters (Cheney *et al*., 2000; Pernet *et al*., 2012) and scallops (Gulka *et al*., 1983; NFRDI, 2005; Xiao *et al*., 2005). A sheer number of studies on the subject have fully unveiled the primary causes for the mortalities: temperature extremes beyond the acclimatization capacities (Gagnaire *et al*., 2006a), pathogenic agents (Chu and La Peyre, 1993), hypoxia or anoxia (Vaquer-Sunyer and Duarte, 2008), reduction in heterozygosity (Watt and Dean, 2000), overall pollutants (Gagnaire *et al*., 2006b), malnutrition (Thompson and Harrison, 1992), bad management (Kaiser *et al*., 1998), recent climate change, and so on (Zillen *et al*., 2008). The underling working mechanism for each cause for the mortality is well documented. But, the mortalities in reality are expected to be much more complicated. The damages, for example, can be exacerbating when more than two causes are co-working (Vaquer-Sunyer and Duarte, 2010; Ivanina *et al*., 2012).

In the cellular consequences of the hypoxia, deficient oxygen elicits the transition to passive tolerance and associated systemic and cellular stress signals like hormonal responses or oxidative stress as well as

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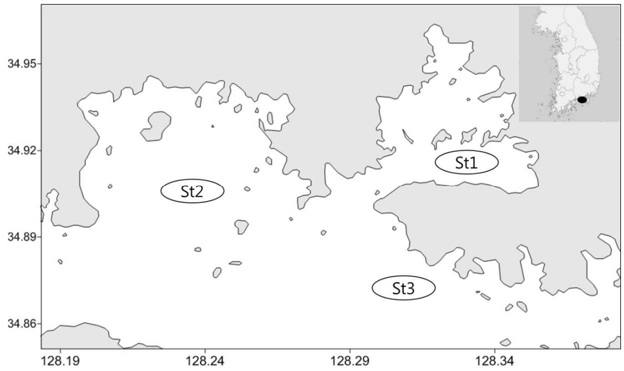

Fig. 1. Location of Goseong Bay, Korea (black spot on the peninsula), showing the study stations: St-1 for oyster farms, St-2 for scallop farms, and St-3 for farms for both species.

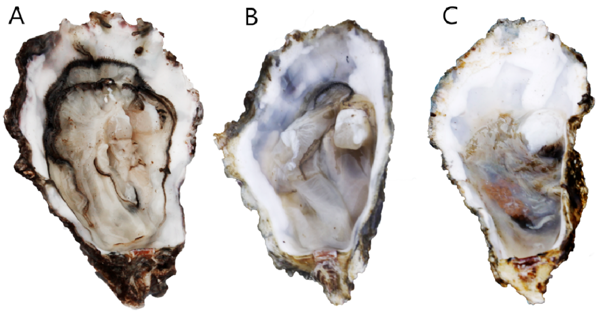

Fig. 2. Representative internal views of *Crassostrea gigas*, normal (**A**), alive but going to mortality (**B**), and dead (**C**).

expression of molecular chaprones and others at thermal extremes (Portner, 2010; Han *et al*., 2013). Therefore, hypoxia drives it in the kernel of discussion in the physiology of mass summer mortality because the likelihood that deficiency of oxygen can exacerbate the damage is high. In this regard, we monitored the 2013 summer mortalities of oysters and scallops in terms of the animal mortalities and their ambient water qualities that can be potential causes for the mortalities with a special focus on hypoxia together with temperature.

Materials and Methods

1. Study area

Three study sites named stations 1-3 were places in the highly sheltered and relatively small bay, Goseong Bay, Goseong, Korea where both of oysters suspended and scallops lantern-caged exhibited significant mortalities (Fig. 1). Each station differed by the species farmed: station 1 (St-1) for oysters, station 2 (St-2) for scallops, and station 3 (St-3) for both.

2. Mortality count

Mortalities of the oysters and scallops were directly counted on the research vessel. Individuals demonstrating an apparent tendency or on the verge of mortality by appearance were considered as a mortality count (Fig. 2).

3. Environmental measurement

Temperature, dissolved oxygen (DO), acidity (pH), and salinity were directly determined from bottom to surface waters on every meter basis with an aid of a water quality monitoring instrument (YSI-6000, YSI, USA). For the measurements of chemical oxygen demand (COD) and nitrogenous compounds such as NH_4-N, NO_2-N, and NO_3-N, surface and bottom waters were sampled using a water sampler (Niskin water sampler-1010, General Oceanics, USA) and immediately bottled and transferred into the laboratory for the analyses. Water transparency was measured by measuring Secchi depth. All the analytical details, otherwise mentioned, followed Standard Methods for the Examination of Environment Pollution (2010).

4. Pathological monitoring

Five samples from each species representing animal status normal and tendency of mortality for oysters and live for scallops were kept alive prior to laboratory analysis. Analyzed were the adduct muscles and heart tissues for bacterial agents and gill and heart tissues for parasites under microscope (Carlzeiss, IMAGE A2, Germany). For the analysis of *Perkinsus olseni*, DNA samples of mantle and adductor muscles were analyzed in PCR (Biorad 170-8710, USA) with an aid of PolsITS primer (5'-GAC CGC CTT AAC GGG CCG TGT T-3') and PolsITS-600R primer (5'GGR CTT AGC ATC CAA AG-3') (Lee *et al*., 2010).

5. Phytoplankton composition

Waters of study area were collected (1L) using water sampler. The collected samples were moderately concentrated and then analyzed for phytoplankton identification under a light microscope.

Results and Discussion

1. Mortalities

Table 1 summarizes aquaculture and mortalities of the two species in study. The ways of aquaculture for the two species were normal except for way for scallop in which lantern cage deployment or suspension interval was relatively narrow. The lantern cage suspension intervals of our study site were 40-50 cm, about half a normal interval for other scallops (Parsons, 1992). This, however, is more related to growth performance than to mortality (Zhang *et al.*, 1991; Parsons, 1992). The mortalities initiated from early August ranged 80-95% for oyster and 50-98% for scallop in two to 3 weeks. They were exceptional, compared with those observed from our routine mortality monitoring, at most 10-20% (data, not shown). Interestingly, there was a narrow vertical range carrying 2-3 collectors in the suspended culture line. The most of the oysters in the collectors were characterized by two types: dead or alive but lacking viability. The oysters upper the range were normal or alive but lacking viability. Strikingly, total or almost total mortalities were found down the range, reminiscent of a sign explaining the progressive mortalities "upward".

2. Water qualities

Water qualities representing study stations were graded on Table 2. The waters of the study stations were highly turbid by appearance (less than 2m in Secchi disk). However, the measurements exhibited standard level or slightly lowered the standard, except for the measurements of COD (see Korean Standard Methods for the Examination of Environment Pollution, 1998).

3. Phytoplankton composition

All the stations were dominated by protozoan flagellate sized smaller than 1 μm followed by diatom *Chaetoceros* spp. For example, total cell counts for St-1 were 20,025 cell/ml for flagellates and 12,475 cell/ml for diatoms. The trends were similar for the waters of ST-2 and St-3. A flagellate Akashiwo sanguineum remained as a minor composition, 5-20 cell/ml. A few of *Ceratium fusus* and *Dinophysis* sp. were also noticed in our phytoplankton survey. However, none of

Table 1. Aquaculture operation and mortality for the two species *Crassostrea gigas* and *Argopecten irradians* in the survey

Species cultured	Culture type	Suspension length (m)	Suspension* interval (cm)	Culture** density	Shell length size (cm)	Mortality (%)
Scallop	Lantern cage	5-6	40-60	15-50	2.5-6.0	50-100
Oyster	Longline	5-8	40-50	5-8	8-10	80-95

*Interval of vertical suspension on the longline.
**Ind./collector for *C. gigas* and ind./layer for *A. irradians*.

Table 2. Water quality measurement for the study stations

Location		COD mg L^{-1}	NH_4-N mg/L	NO_2-N mg/L	NO_3-N mg/L	DIN Mg/L	PO_4-P Mg/L	Chl-a μg/L
St-1	SW	3.22 ± 0.017	0.019 ± 0.001	0.001 ± 0.0007	0.010 ± 0.0002	0.031 ± 0.0002	0.003 ± 0.0002	6.6 ± 1.2
	BW	2.49 ± 0.125	0.029 ± 0.002	0.003 ± 0.0012	0.012 ± 0.0002	0.045 ± 0.0002	0.015 ± 0.0002	
St-2	SW	2.20 ± 0.047	0.011 ± 0.002	0.003 ± 0.0008	0.018 ± 0.0002	0.032 ± 0.0002	0.004 ± 0.0002	2.8 ± 0.5
	BW	2.34 ± 0.255	0.016 ± 0.003	0.004 ± 0.0013	0.020 ± 0.0002	0.041 ± 0.0002	0.014 ± 0.0002	
St-3	SW	2.69 ± 0.851	0.007 ± 0.001	0.002 ± 0.0004	0.012 ± 0.0002	0.021 ± 0.0002	0.003 ± 0.0002	4.2 ± 1.5
	BW	0.14 ± 0.002	0.002 ± 0.000	0.004 ± 0.0022	0.005 ± 0.0002	0.006 ± 0.0002	0.007 ± 0.0002	

Location abbreviations: BW, bottom water; SW, surface water.

Table 3. Depth dependent DO (ppm) and mortality (%) of *C. gigas* and/or *A. irradians* at the study stations

Location	Depth (m)					
	1	2	3	4	5	6
St-1	6.71 ± 0.45 (23.3 ± 7.7)*	6.17 ± 0.25 (81.7 ± 10.4)	4.49 ± 1.11 (95.33 ± 3.1)	2.61 ± 0.15 (99.7 ± 0.6)	1.65 ± 0.08 (100 ± 0.0)	1.47 ± 0.08 (100 ± 0.0)
St-2	6.65 ± 0.06 (51.8 ± 8.6)	6.25 ± 0.21 (69.0 ± 9.4)	6.06 ± 0.05 89.7 ± 4.1	2.38 ± 0.35 96.6 ± 5.8	1.85 ± 0.12 (100 ± 0.0)	1.55 ± 0.01 (100 ± 0.0)
St-3	6.87 ± 0.28 (37.5 ± 10.0)	6.47 ± 0.06 (75.3 ± 5.8)	5.15 ± 0.54 92.5 ± 1.0	4.6 ± 0.52 98.2 ± 0.6	4.0 ± 0.32 (100 ± 0.0)	3.87 ± 0.39 (100 ± 0.0)

*Figure in parenthesis implies mortalities: St-1 for *C. gigas*; St-2 for *A. irradians*; St-3 for both species on average.

Cochlodinium spp. was observed. The diatom outgrowth, together with minor appearance of *A. sanguineum*, appeared to be responsible for the turbidity and a sign for a succession in species composition in the study area.

4. Causes of mortalities

There are many causes for shellfish mass summer mortalities. Of them, temperature and dissolved oxygen are worth paying attention because of their bigger damage potentials. Pathogenic agents are also responsible for many mass summer mortalities (Xiao *et al.*, 2005; Thieltges, 2006; Garnier *et al.*, 2007), but they as an opportunistic invader (Lacoste *et al.*, 2001) may remain as a secondary cause.

We monitored a variety of potential causes for the mortalities, including temperature, pathogenic agents, hypoxia or anoxia, salinity, acidity, and overall pollutants. The measurements except for temperature, salinity, and pathogenic agents were absolutely or to some extent unfavorable. Even though the measurements were unfavorable for normal shellfish life, the levels were still within the magnitudes the animal could resist.

One of the most distinctive and conspicuous observations was dissolved oxygen. Fig. 3 detailed the profiles of dissolved oxygen and temperature. Dissolved oxygen levels in the surface waters were overall low. But there was a great fall in dissolved oxygen level with water depth. It was far below the normal level or in hypoxia from depth 4 meter. Compared with each of environment measurements, the mortalities were most closely related with the change of dissolved oxygen (Table 3). This indicates the hypoxia as a key culprit of the shellfish mortalities. The finding that depth of a narrow vertical range in the suspended culture line containing the shellfish dead and alive but lacking viability further support our conclusion.

In the study of hypoxic waters that impact ecosystem, thresholds for hypoxia in coastal ecosystems are higher than previously thought and are not static, but regulated by local and global processes, being particularly sensitive to warming (Steckbauer *et al.*, 2011). The oxygen level, 2ppm, used to be a threshold level for hypoxia (Diaz and Rosenberg, 1995) was argued by many experimental evidences that experienced onsets of hypoxic mortalities at higher concentrations (Gray *et al.*, 2002; Vaquer-Sunyer and Duarte, 2008; Steckbauer *et al.*, 2011). In this regard, the two species placed in the waters 3-4 meters deep appeared to face the hypoxia, resulting in mass mortalities.

The frequency of hypoxic events is increasing due to global eutrophication (Vaquer-Sunyer and Duarte, 2008; Rabalais *et al.*, 2009). This increase in frequency

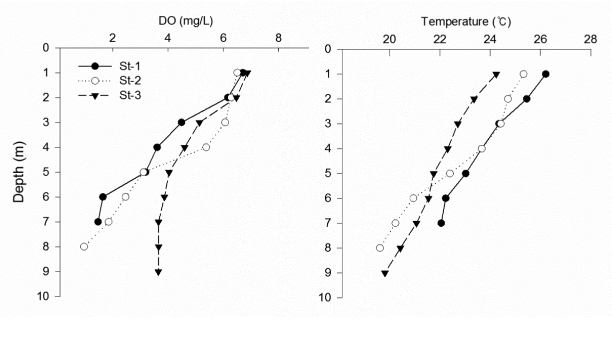

Fig. 3. Vertical profiles of dissolved oxygen (DO) and temperature at three study stations.

is emerging as a common stressor in coastal ecosystem (Vaquer-Sunyer and Duarte, 2008). In general in a given location, hypoxia occurs when respiration by the resident biota, especially photosynthetic microorganisms, outstrips the oxygen input into the water from photosynthesis, diffusion from the nearby oxygenated areas, and atmospheric exchange (Diaz and Rosenberg, 2008; Vaquer-Sunyer and Duarte, 2008; Tilman *et al*., 2001; Ivanina *et al*., 2012). The hypoxia observed in the present study was prominent in the near-bottom water layers, with a gradient increasing upward. Thus, anaerobic metabolism from the near-bottom appeared to be one of the triggers of the hypoxia.

This might be particularly plausible in a highly sheltered and nutritious water body as a spatial extent of hypoxia is highly sensitive to small changes in the bigger water body, with maximum responses at suboxic concentrations where anaerobic metabolisms predominate (Deutsche *et al*., 2011). Even though we could not define the origin of the extremely low dissolved oxygen in the study area, its persistence appeared to be related with the size and geographical structure of the bay. Once generated, the hypoxia appeared to persist due to the highly sheltered structure of the bay, damaging the farmed shellfish.

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