Thesis for Master Degree

Effects of environmental and biological factors on the recruitment and growth of the Manila clam *Ruditapes philippinarum* in the intertidal zone, Taean, west coast of Korea

Advisor: Prof. Ok Hwan Yu

February 2017

Department of Convergence Study on the Ocean Science and Technology

School of Ocean Science and Technology
Korea Maritime and Ocean University

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Effects of environmental and biological factors on the recruitment and growth of the Manila clam *Ruditapes philippinarum* in the intertidal zone, Taean, west coast of Korea

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Abstract

The distribution of the Manila clam, *Ruditapes philippinarum*, which often dominates intertidal zones, is influenced by both environmental and biological conditions. However, few comprehensive studies have examined the interactive effects of these two groups of factors. This study examined the environmental and biological parameters determining the population dynamics of *R. philippinarum* and investigated their influence on macrobenthos communities. We collected *R. philippinarum* and macrozoobenthos (>1 mm long) monthly three times from quadrats (50 cm × 50 cm, 0.25 m^2) in intertidal zones at Euihangri and Padori from August 2013 to January 2015 and seasonally collected using a cancore (0.1 m^2) in intertidal zone of Geunsoman, Taean from April 2011 to December 2014. Physicochemical parameters of the water and sediment were measured at the same times. Water temperature and salinity are high in summer and low in winter. The mean grain size of the sediment was higher at Euihangri than at Padori, while the opposite was true of the total mean density of *R. philippinarum* (325 individuals (ind.)/0.25 m^2 at Padori *vs.* 194 ind./0.25 m^2 at Euihangri). The settled spat (<10mm in length) density was much higher at Euihangri than at Padori (132 *vs.* 12 ind./0.25 m^2, respectively). *R. philippinarum* spats settled on the sediments at Euihangri in October and grew continually until the following May, when they reached adult size. Spats that settled at Padori between March and April were not able to reach the adult stage. As the density of the adult population increased, the condition index of individual clams decreased, while the body condition index increased with the spat population density. The condition index at Euihangri exceeded that at Padori, indicating better growth conditions at Euihangri. The recruitment success of spats was negatively influenced by spat density. At Geunsoman, we found 146 large macrobenthos species, including 60 polychaetes, 53 crustaceans, and 16 mollusks. The dominant species was *R. philippinarum* (20,020 ind./m^2), followed by *Heteromastus filiformis*, *Ceratonereis erythraeensis*, and *Ampharete arctica*. In this study, the condition index and number of adult clams were influenced by the mean grain size of sediments and the recruitment of clam spats was influenced by the density of adults. Therefore, in order to maintain the optimal environment for clams, it is important to control both the habitat sediment environment and clam populations.

KEYWORDS: Manila clam; Macrobenthos; Tidal flat; Growth rate

서해 태안 조간대 바지락 개체군의 가입과 성장에 영향을 주는 환경 및 생물 요인

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요약

조간대에서 우점하는 바지락의 분포는 환경 요인과 생물 요인들의 영향을 받지만, 두 요인을 포함한 상호관계 연구는 매우 제한적이다. 본 연구에서는 바지락이 우점하는 조간대에서 바지락의 개체군 변동에 영향을 주는 환경 요인과 생물 요인을 분석하였고, 또한 이 요인들이 대형저서동물 군집에 미치는 영향을 알아보았다. 조사지역은 태안 의항리와 파도리 조간대로 2013년 8월부터 2015년 1월까지 매월 방형구 (0.25 m^2) 를 이용하여 1mm 이상 크기의 저서동물을 채집하였고 바지락의 개체군 분석을 위해 채형변수를 측정하였다. 태안 근소만 조간대에서는 2011년 4월부터 2014년 12월까지 계절별로 한 개 정점에서 저서동물을 채집하여 군집구조를 분석하였다. 의항리와 파도리의 수온은 각각 평균 13.9 °C와 14.8 °C로 나타났고, 염분은 평균 29.8, 29.9 로 나타나 차이가 없었다. 퇴적물의 평균입도의 경우 의항리가 4.31 Φ로 3.15 Φ인 파도리보다 높았으며 통계적으로 유의한 차이를 보였다. 클로로필 *a*는 수층과 퇴적물 모두

평균적으로는 파도리가 높았지만 통계적으로 차이를 보이진 않았다. 바지락 전체 서식 밀도는 파도리가 평균 325 개체/0.25m^{2}로 평균 194 개체/0.25m^{2}인 의항리보다 많았다. 그러나 각장 10mm 이하의 치패의 경우 의항리가 평균 132 개체/0.25m^{2}로 평균 12 개체/0.25m^{2}인 파도리보다 많았다. 의항리에서 바지락은 10월부터 퇴적물에 가입되어, 다음해 5월까지 지속적으로 성장하여 성체 가입이 이루어지지만, 파도리에서는 3-4월 사이에 가입된 치패는 성체로 성장하지는 못하였다. 성체의 개체수가 증가할수록 비만도는 감소하였으나, 치패의 개체수가 증가할수록 비만도는 증가하였다. 치패의 가입은 파도리보다 의항리에서 높았다. 근소만에서 대형저서동물 총 출현 종수는 146종이였고, 갯지렁이가 60종으로 가장 많이 나왔고 그 다음으로 갑각류와 연체동물이 각각 53종, 16종으로 많이 나왔다. 우점종으로는 바지락 (*Ruditapes philippinarum*) 이 평균 1335 개체/m^{2}로 가장 많았으며, 그 다음으로 갯지렁인 *Heteromastus filiformis*, *Ceratonereis erythraeensis*, *Ampharete arctica* 순으로 우점하였다. 이 연구에서 바지락의 비만도와 성체는 퇴적물 평균 입도의 영향을 받았고, 바지락 치패의 가입은 성체의 서식 밀도에 많은 영향을 받았다. 그러므로 바지락 최적 서식환경을 유지하기 위해서는 서식지 퇴적물 환경 뿐 만 아니라 바지락 개체수의 조절이 중요할 것으로 여겨진다.

KEYWORDS: Manila clam 바지락; Macrobenthos 대형저서동물; Tidal flat 조간대; Growth rate 성장률

Chapter 1. General introduction

1.1 Macrobenthos community in an intertidal zone

Macrobenthos play a decisive role in the structure and function of marine ecosystems (Brey, 2012; Oug et al., 2012). Benthos are consumed by fish and mammals, providing food for higher trophic levels, and are also important in nutrient and organic matter cycling and serve as a link between the pelagic and benthic divisions of marine ecosystems (Grall & Chauvaud, 2002; Iken et al., 2010; Hill et al., 2011). Benthic communities are susceptible to change over time because of the response of the life cycles of species to environmental variability (Sibly & Calow, 1989; Convey, 1996). They are considered to be good indicators of ecosystem health because of their sedentary nature and longevity, which results in long-term exposure to toxic materials, and are representative taxa that can respond to complex types of stress (Jewett et al., 1999). Studies of the responses of the macrobenthos community structure to environmental gradients are popular in community ecology because macrobenthos communities are affected by environmental gradients, feeding resources, and habit heterogeneity, especially with seasonal changes that may hide the effects of environmental gradients.

Characteristically, the macrobenthos are not very mobile and their spread is influenced by human activities and natural differences. Investigations of the spatial and temporal variability of the macrobenthos community are important for biological assessments (Xie, 2016). Since they are relatively sessile, the wellbeing of marine benthic communities can be used to determine the response to environmental conditions or the effects of human perturbations (Pearson & Rosenberg, 1978; Borja et al., 2000; Guidetti et al., 2000; Hampel et al., 2009). Benthic macroinfaunal communities are central to habitat-assessment programs in near-sea waters worldwide because the macroinfauna are comparatively non-moving residents in sediments, where contaminants accumulate, and they have a variety of life phases and feeding patterns that make them responsive to many types of interruption (Warwick, 1988; Diaz et al., 2004; Borja et al., 2006; Llansó et al., 2009; Gray & Elliott, 2009). While this variety of responses is an asset for capturing ecological community disturbance, it is also challenging when differentiating disturbance-related variation from natural variation in community structure. Benthic communities, comprised of complicated assemblages of organisms living in association with sea-floor sediments, play a central part in the functioning of marine ecosystems, and benthos absorb substantial amounts of organic matter settling on the sea floor, which are relocated to the pelagic and benthic food webs via trophic interactions. Soft-bodied benthic organisms are important sources of food for various demersal fishes (Crisp, 1984; Snelgrove, 1998; Koho et al., 2013). Bioturbation by the benthos enhances the degradation of organic matter and increases oxygen penetration in sediments, where the organic content and occurrence of microalgae are related to particle size, and composition is an important factor influencing the macrobenthic community composition and population densities, which are in turn affected by both particle size and sediment chemistry (Otani et al., 2008; Mermillod-Blondin, 2011; Morais et al., 2016).

1.2 Environmental factors associated with *Ruditapes philippinarum*

Ruditapes philippinarum is native to the Indo-Pacific region, but has been introduced to coastlines in many parts of the world since the 1930s (Flassch & Leborgne, 1992), and is now a dominant species in the intertidal zones of northwestern America, Europe, Korea, China, and Japan. Many species of shellfish occur intertidally on the west coast of Korea (Loosanoff & Davis, 1963; Anderson, 1982), where the tidal range is large and mud flats are well developed. *Crassostrea gigas* and *R. philippinarum* are important, high-yielding fishery species (Kim & Zhang, 1999: Shin & Shin, 1999). When the planktonic larvae of *R. philippinarum* reach ca. 0.3 mm in length in the spring or early fall, they settle on soft bottoms (Coutteau et al., 1994; Drummond et al., 2006). The species grows well in appropriate mixtures of sand and silt, but not when the silt content is high, unless the sediment composition is adjusted by spreading sand or oyster shells on the substrate (Park et al., 2011). Attempts to increase the habitat range of *R. philippinarum* by changing sediment composition should be based on integrated analyses of the environmental and biological conditions that determine the distribution of this species.

Water temperature, salinity, and prey species composition influence the suitability of habitats for *Ruditapes philippinarum*. Water temperature affects the duration of the spawning period and the rate of larval development and increases in water temperature accelerate clearance rates, ingestion, and the respiration rates of bivalves (Walne, 1972; Hoffman, 1983; Griffiths & Griffiths, 1987; Zhang & Yan 2006). *R. philippinarum* is highly resistant to changes in water temperature and salinity; nevertheless, high water temperature and low salinity can greatly affect its growth and reproduction (Claus, 1981; Baud & Bacher, 1990; Shin et al., 2000; Delgado & Pérez-Camacho, 2007; Dang et al., 2010). The condition index of *R. philippinarum*, which reflects both the nutritional status and the stress exposure of the clams, is closely related to spawning and seasonal changes in the phytoplankton component of the water column (Drummond et al., 2006; Toba et al., 2007). Phytoplankton in seawater are the main prey of filter feeders such as bivalves, and chlorophyll, which is associated with bivalve production, including the condition index and morphological patterns, represents the amount of phytoplankton indirectly (Loosanoff, 1942; Moller & Rosenberg, 1983; Kennedy et al., 1996; Gam et al., 2010; Park et al., 2013b; Caill-Milly et al., 2014). The habitat of *R. philippinarum* is limited to the uppermost 10 cm of sediment, where it competes for space with other members of the macrobenthos, although this activity has received little attention. Furthermore, very few studies have examined the environmental and biological conditions that affect the population dynamics of *R. philippinarum*.

1.3 General imformation of study area

The Yellow Sea west of Korea is a semi-enclosed, northwestern Pacific marginal sea into which two of the largest rivers in China flow: the Huanghe (Yellow) and Changjiang (Yangtze) Rivers. The high terrigenous sediment inputs from the surrounding landmasses to the Yellow Sea result in high sediment accumulation in its subaqueous deltas (Alexander et al., 1991, Yang et al., 2003; Yang & Youn, 2007). Along open shores, fine sediments are eroded by frequent storms and the effects of seasonal monsoon winds. By contrast, in the low-energy environment of summer, the fine sediments pile up. Along semi-enclosed shores like the Yellow Sea, sedimentation is primarily under the control of seasonal winds, geological factors (*e.g.*, the form of the back-bay area), and currents in the intertidal zone (Jang & Choi, 1998; Lee et al., 1999; Ryu et al., 1999; Ryu et al., 2001). Along the western shore of Korea, the changes in the sea surface are great, due to the large differences in ebb and flow in the morning and evening and the complex actions of astronomical tides and storm surges. Along the western shore, where the water is shallow and there are wide beaches at low tide, the mean sea level has been changed by seashore development, such as the reclamation of land from the sea (Jung, 2014). In other areas, the sea level has risen continuously due to climate change and other factors (Kang et al., 2005a).

1.4 Aims of this study

Various kinds of shellfish inhabit the western shore of Korea, and their habitats are affected by the tides and wide mud flats. The shellfish include *Ruditapes philippinarum* and oysters, which are very important fishery species. Through an environmental and biological analysis of different intertidal zones of the sedimentary facies of the Yellow Sea, this study analyzed changes in *R. philippinarum* populations and density of the macrobenthos, including biomass and species diversity, to understand the intra- and interspecies competition of the intertidal macrofauna. Ultimately, the study aim is to identify suitable environments for *R. philippinarum* and suggest ways to increase productivity.

Chapter 2. Effects of environmental and biological factors on the recruitment and growth of *Ruditapes philippinarum*

2.1 Introduction

Macrobenthos parameters have been used to assess the health of the coastal ecosystem because the component species often have long life cycles, limited habitat ranges, and high sensitivity to environmental change (Pearson & Rosenberg, 1978; Bamber & Spencer, 1984). The distribution of intertidal macrobenthic taxa is strongly influenced by prevailing environmental and biological conditions. The environmental conditions of importance are temperature, including the duration of exposure to extreme heat or cold (Myer, 1977; Beukema, 1989), particle composition (Gerard & Saskia, 2005), the organic content of sediments (Isabella et al., 2008), and the slope of the intertidal gradient (Rosarid & Mariano, 2008). Biological determinants of distribution include competition for food resources or habitat space (Wiltse, 1980), predation pressure (Lipcius & Hines, 1986), and the disturbance effects of dominant species (Levinton et al., 1985). Sediment composition is an important factor influencing macrobenthic community composition and population densities, which are affected by both particle size and sediment chemistry (Otani et al., 2008). Sediment organic content and the occurrence of microalgae are related to particle size (Morais et al., 2016). Few studies have determined whether competition within the same habitat space affects the distribution of macrobenthic species.

Ruditapes philippinarum is endemic to the Indo-Pacific region. It has been introduced to coastlines of many parts of the world since the 1930s (Flassch & Leborgne, 1992), and is now a dominant species in the intertidal zones of northwestern America, Europe, Korea, China, and Japan (Loosanoff & Davis, 1963; Anderson, 1982). Many species of shellfish occur intertidally on the west coast of Korea, where the tidal range is large and mud flats are well developed. *Crassostrea gigas* and *R. philippinarum* are important, high-yielding fishery resources (Kim & Zhang, 1999: Shin & Shin, 1999). When the planktonic larvae of *R. philippinarum* reach ca. 0.3 mm in length in the spring or early fall, they settle on soft bottoms (Coutteau et al., 1994; Drummond et al., 2006). The species grows well in appropriate mixes of sand and mud, and are unstable when the mud content is high. Then the sediment composition is commercially adjusted by spreading sand or oyster shells onto the substratum (Park et al., 2011). Attempts to increase the habitat distribution range of *R. philippinarum* by changing sediment composition should be based on integrated analyses of the environmental and biological conditions that determine the distribution of the species.

Water temperature, salinity, and prey species composition influence the suitability of habitats for this clam. Water temperature affects the duration of the spawning period and the rate of larval development (Hoffman, 1983; Griffiths & Griffiths, 1987; Zhang & Yan, 2006). Increases in water temperature accelerate clearance rates, ingestion, and the respiration rates of bivalves (Walne, 1972; Hoffman, 1983; Griffiths & Griffiths, 1987). *R. philippinarum* is highly resistant to changes in water temperature and salinity (Shin et al., 2000). Nevertheless, high water temperatures and low salinities can greatly impact its growth and reproduction

(Claus, 1981; Baud & Bacher, 1990; Delgado & Pérez-Camacho, 2007; Dang et al., 2010). The condition index of *R. philippinarum*, which reflects both the nutritional status and stress exposure of the clams, is closely related to spawning and seasonal changes in the phytoplankton component of the water column (Drummond et al., 2006; Toba et al., 2007). Phytoplankton in seawater is the main prey of the filter feeder such as bivalvia, chlorophyll *a* represents an indirect amount of phytoplankton (Loosanoff, 1942; Kennedy et al., 1996; Park et al., 2013b). Chlorophyll *a* is associated with bivalve production including condition index and morphological patterns (Caill-Milly et al., 2014; Gam et al., 2010; Moller & Rosenberg, 1983). The habitat for *R. philippinarum* is limited to the uppermost 10 cm of sediment, where it competes for space with the other components of the macrobenthos. These competitive interactions have received little attention. Furthermore, very few studies have examined the environmental and biological conditions that affect the population dynamics of *R. philippinarum*.

We determined the optimal habitat conditions for *Ruditapes philippinarum* by investigating its population dynamics in relation to prevailing environmental and biological conditions.

2.2 Materials and Methods

Ruditapes philippinarum specimens were collected three times from quadrats (50 cm × 50 cm, 0.25 m2) deployed each month in the period from August 2013 through January 2015 at the low tide level of the following sites: Euihangri (N36 49.370, E126 10.514), Sowon-myeon, Taean-gun, Chungcheongnam-do, and Padori (N36 43.920, E126 09.258) (Fig. 1). Macrobenthos that co-occurred with *R. philippinarum* were collected with a quadrat (0.25 m^2 area) by straining the sediments through 1 mm sieves. In a laboratory, we measured the lengths, heights, and

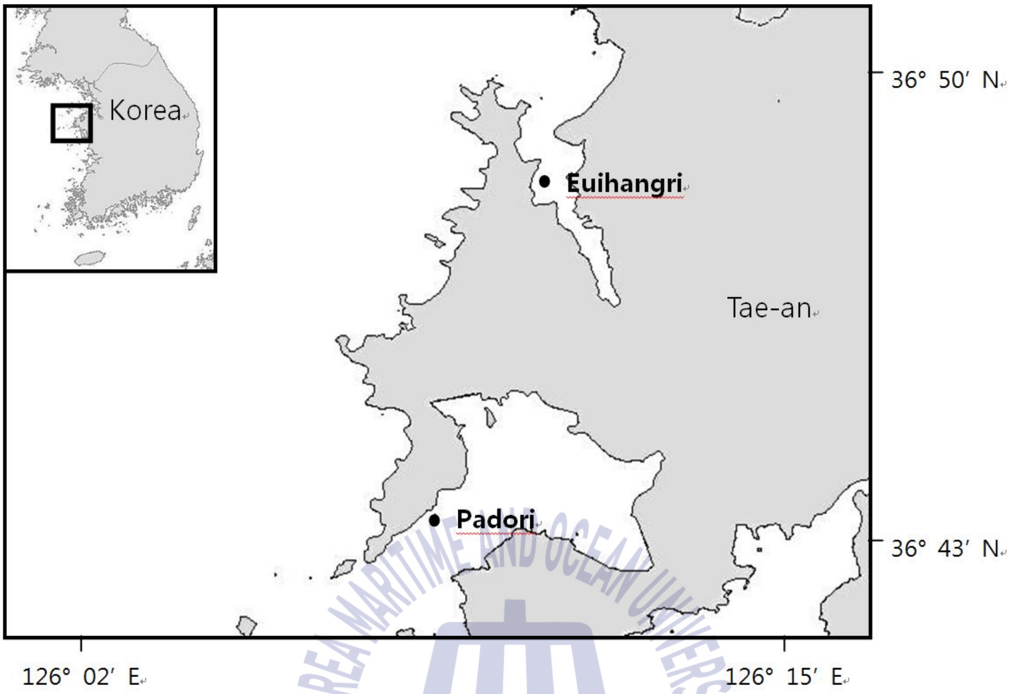

Fig. 1 Locations of sampling stations at Euihangri and Padori on the west coast of Korea

widths of the specimens to the nearest 0.01 mm using a Digimatic Caliper (CD-15CP) and macrobenthos was classified to the order level. We measured the weight of each flesh mass (after separation from the shell) to the nearest 0.001 g, dried it for >24 h at 60°C, and obtained the dry weight to the nearest 0.001 g. We calculated the condition index (CI) of each specimen using the following equation: CI = partial dry weight (g)/dry shell weight (g) × 100. We estimated the total densities and biomasses of whole populations of *R. philippinarum*. The specimens were separated into two size groups (<10 mm long and >10 mm long). Spat <10 mm long were still growing after settlement (Kim, 2005). We measured the salinity and temperature of the seawater in the field using a YSI-30 refractometer. We measured chlorophyll *a* concentrations in samples of bottom water, and collected surface sediments to five times for laboratory measurements of particle size and total organic content. The chlorophyll *a* concentrations were measured using standardized protocols (Ministry of Maritime Affairs & Fisheries, 2010). For the analysis of chlorophyll *a* concentration, 90% acetone was added to the collected samples, and they were extracted in a dark place at about 5°C for 24 hours. The concentration of chlorophyll *a* in the sediments was measured by spectrophotometer and then by UNESCO method. Seawater samples were measured after measuring the absorbance using a fluorometer. Sediment particle sizes were determined after treating collections with a solution of 10% hydrogen peroxide. Sediment samples were heated to >100°C to evaporate the hydrogen peroxide, and then washed >3 times with distilled water to remove organisms and salts. Washed samples were passed through a 63 µm standard sieve (Ingram, 1971). After drying, the sediments trapped by the sieve were weighed and subjected to automatic particle size analysis using a Sedi-graph 5120 apparatus following the addition of a dispersing agent (sodium hexametaphosphate). We calculated the average particle sizes and degrees of sorting. The sediments were categorized according to Folk's classification system.

We used regression analyses to determine the growth of the clams. The relationship between shell length and total wet weight was described by a power function:

$TW = a \times SL^{b}$, (1)

where TW is total wet weight, SL is shell length, *a* is a constant, and *b* is the slope of weight growth based on shell length. Equation (1) was transformed to $\ln(TW) = \ln(a) + (b \times \ln[SL])$. The relationship between total wet weight and flesh dry weight was described:

$FW = a \times TW + b$, (2)

where FW is flesh dry weight, TW is total wet weight, *a* is the slope, and *b* is a constant.

One way anova test or Kruskal test was performed to determine the significant difference between environmental factors and biological factors in two areas. We tested the data for normality before proceeding with statistical analyses. When the assumption of normality was met, we used parametric one-way ANOVA [Analysis Of Variance]. Non-normal data were subjected to non-parametric Kruskal-Wallis tests. We examined the relationships among environmental conditions, clam densities, and body condition using the Spearman rank correlation coefficient (Sigmaplot 12.3).

2.3 Results

2.3.1. Environmental conditions

The average water temperature at Euihangri was 13.9 ± 8.0 °C (mean ± SD), with a low of 3.5°C in December 2014 and a high of 26°C in July 2014. The average water temperature of Padori was 14.8 ± 8.6°C, with a low of 2.6°C in January 2015 and a high of 26.9°C in August 2013 (Fig. 2). Thus, water

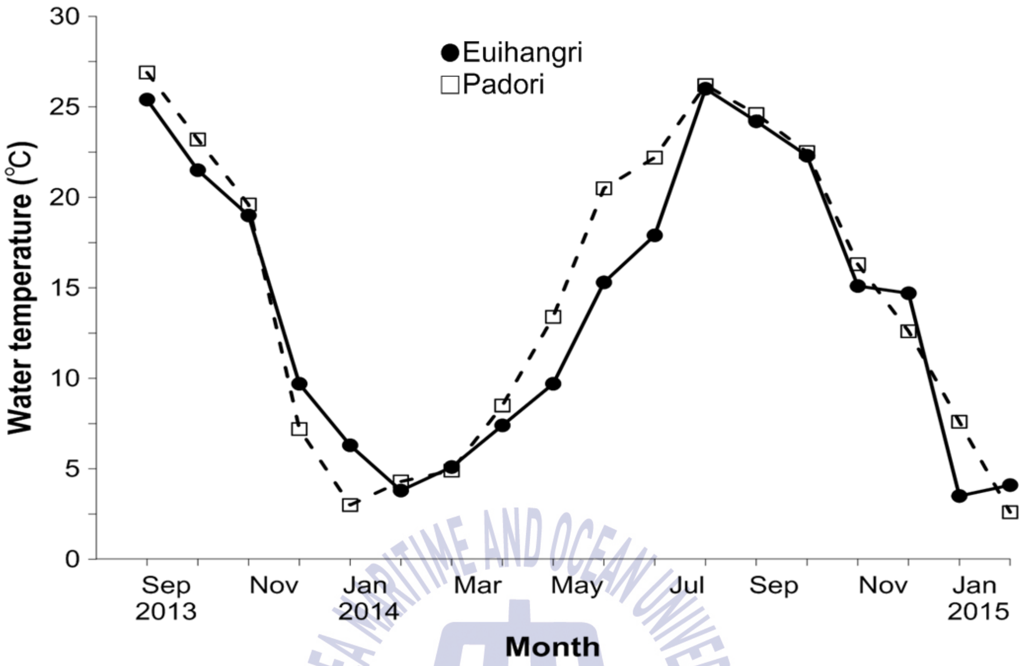

Fig. 2 Monthly variation in water temperature at Euihangri and Padori on the west coast of Korea

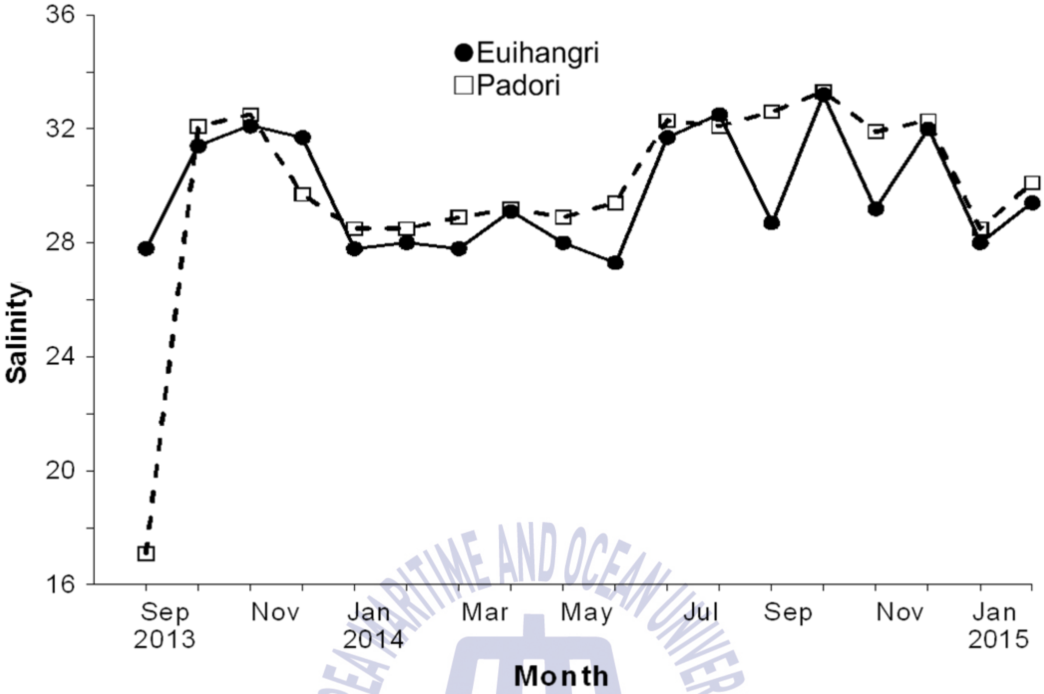

Fig. 3 Monthly variation in salinity at Euihangri and Padori on the west coast of Korea

Table 1 Environmental and biological parameters at two sites on the west coast of Korea. H and F values are summary statistics for Kruskal-Wallis and one-way ANOVA tests, respectively

Contents	Euhang-ri	Pado-ri	H-value or F-value*	P-value
Water temperature (℃)	13.9 ± 8.0	14.8 ± 8.6	0.1	0.76
Salinity (psu)	29.8 ± 2.0	29.9 ± 3.6	2.08	0.15
Mean grain size (Φ)	4.31 ± 1.17	3.15 ± 0.96	10.46*	0.03
Sorting value	2.13 ± 0.44	2.23 ± 0.77	0.22*	0.65
Water chlorophyll *a* (μg/L)	4.32 ± 3.45	5.93 ± 9.66	0.07	0.78
Sediment chlorophyll *a* (μg/mg)	73.07 ± 28.40	81.63 ± 36.37	0.4	0.53
Biomass of macrobenthos (g/m2)	2.32 ± 1.95	2.26 ± 1.28	0.01	0.92
Density of macrobenthos(ind/0.25m^2)	94.83 ± 82.73	80.83 ± 148.06	1.45	0.23
Total density of *Ruditapes philippinarum*	194.4 ± 164.6	325.5 ± 174.4	5.38*	0.03
Adult density of *Ruditapes philippinarum*	62.1 ± 34.1	313.9 ± 172.8	13.87	0.001
Spat density of *Ruditapes philippinarum*	132.3 ± 170.6	11.6 ± 20.9	11.45	0.001
Condition index of *Ruditapes philippinarum*	7.50 ± 0.93	6.15 ± 0.79	22.08*	0.01

temperatures were similar between the two sites (Table 1). The average salinities at Euihangri and Padori were 29.8 ± 2.0 and 29.9 ± 3.6, respectively. Salinities tended to be lower in the winter and spring, and higher in the summer and fall. The lowest salinity at Euihangri was 27.3 in May 2014, and the highest value at this site was 33.2 in September 2014 (Fig. 3). The lowest salinity at Padori was 17.1 in August 2013, and the highest value at this site was 33.3 in 7611 g/m^2 in October.

September 2014. The sediments at Euihangri and Padori were gravelly/muddy sand mixes with sand components exceeding 55% and 69%, respectively. The mean grain sizes at Euihangri and Padori were 4.31 ± 1.17Φ and 3.15 ± 0.96Φ, respectively (Table 1). The mean grain size at Euihangri was most aggregated in January 2015 and was finest in April 2014 (1.35Φ and 5.86Φ, respectively). At Padori, the mean grain size was most aggregated in August 2014 and was finest in October 2014 (1.70Φ and 5.41Φ, respectively) (Fig. 4). The sorting values of sediments were not significantly different between Euihangri and Padori (2.13 ± 0.43 and 2.2 ± 0.76 , respectively) (Table 1).

The average concentrations of chlorophyll *a* in the bottom water samples collected at Euihangri and Padori did not significantly differ and were 4.32 ± 3.45 μg/mg and 5.93 ± 9.66 μg/mg, respectively (Table 1). The concentrations in the water column Euihangri were lowest in January 2014 (1.045 μg/L) and highest in August 2013 (15.55 μg/L) (Fig. 6). At Padori, the water column values were lowest in December 2013 (0.91 μg/L) and highest in August 2013 (42.89 μg/L). The chlorophyll *a* concentrations in the sediment were high at Euihangri prior to June 2014, but were highest in Padori in the following period. Peak values were not significantly different between sites (Table 1). Chlorophyll *a* concentrations in the sediment at Euihangri were lowest in December 2013 (28.76 μg/mg) and highest in January 2015 (154.3 μg/mg). Values at Padori were highest in January 2015 (198.8 μg/mg) and lowest in April 2014 (38.79 μg/mg) (Fig. 7).

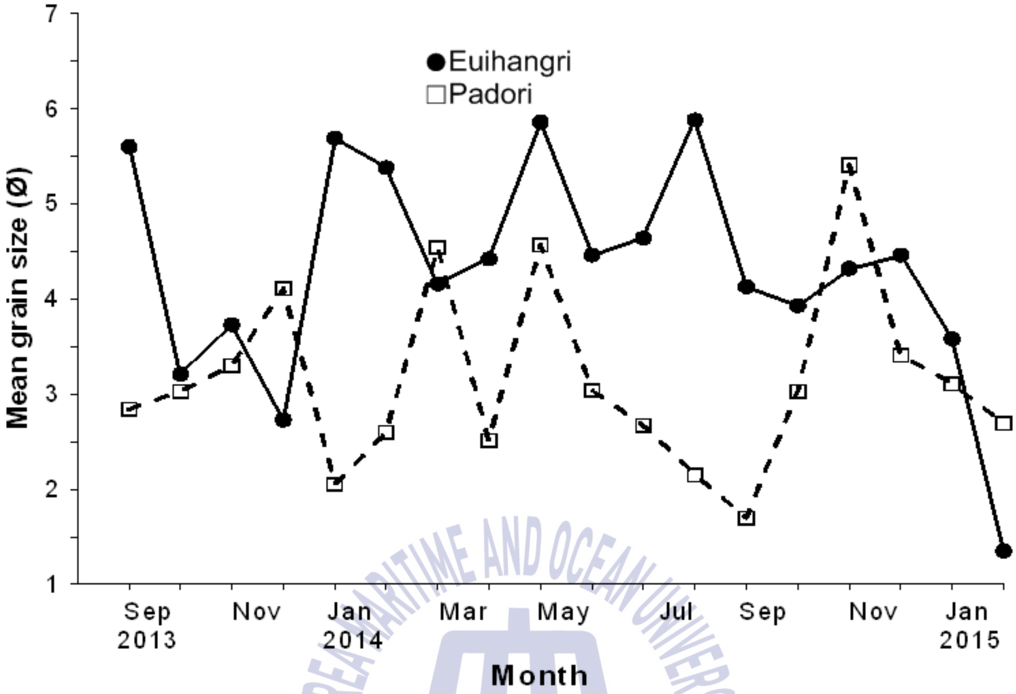

Fig. 4 Monthly variation in mean grain size of sediment at Euihangri and Padori on the west coast of Korea

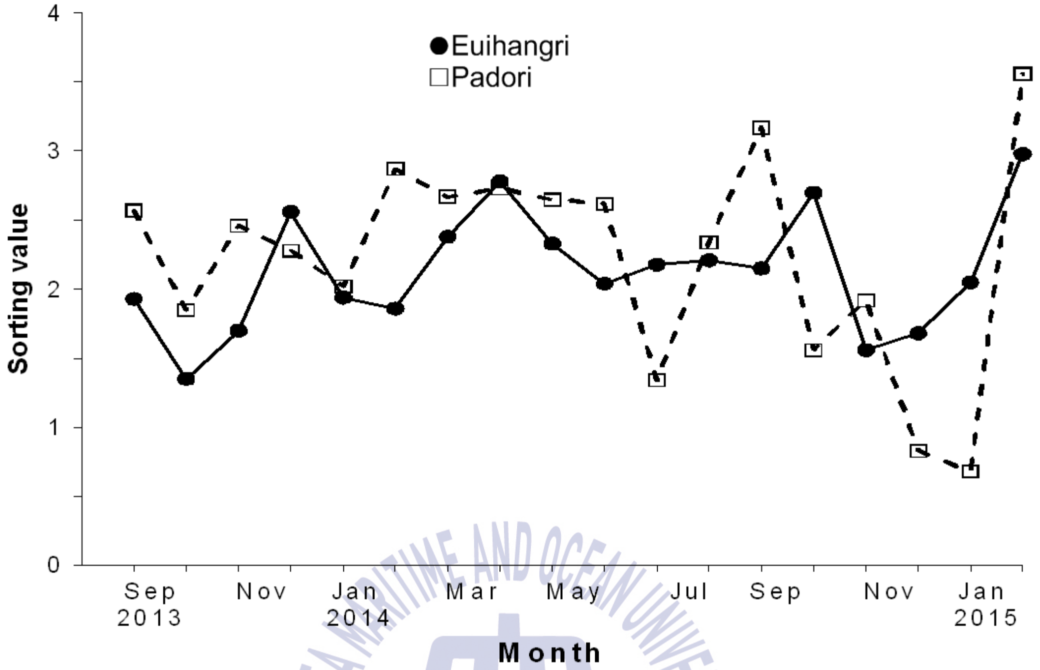

Fig. 5 Monthly variation in sorting value of sediment at Euihangri and Padori on the west coast of Korea

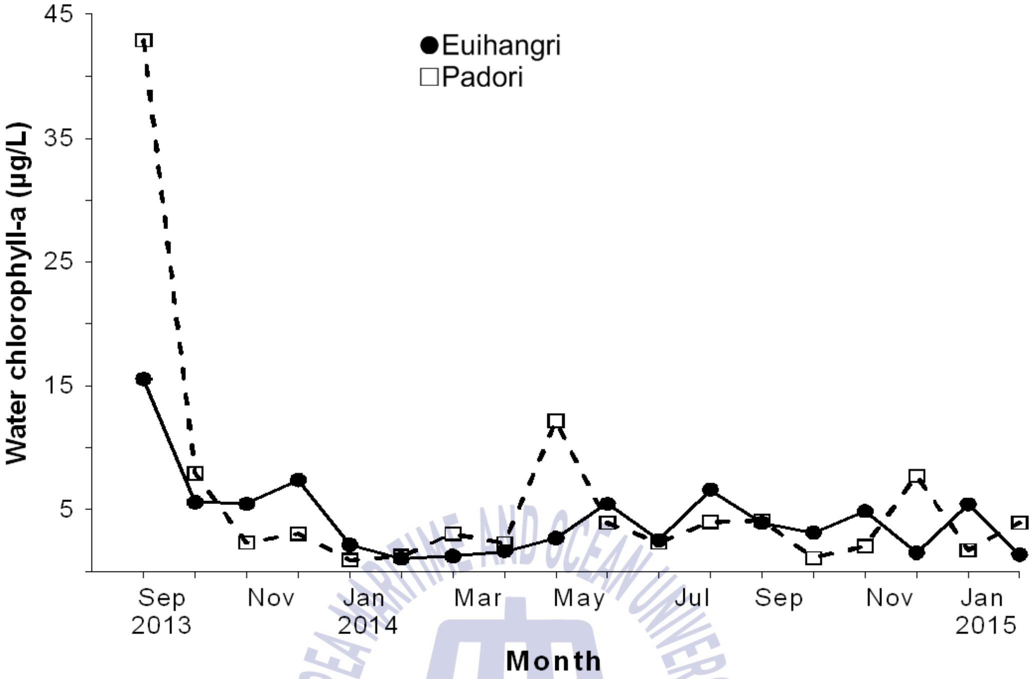

Fig. 6 Monthly variation in water chlorophyll *a* at Euihangri and Padori on the west coast of Korea

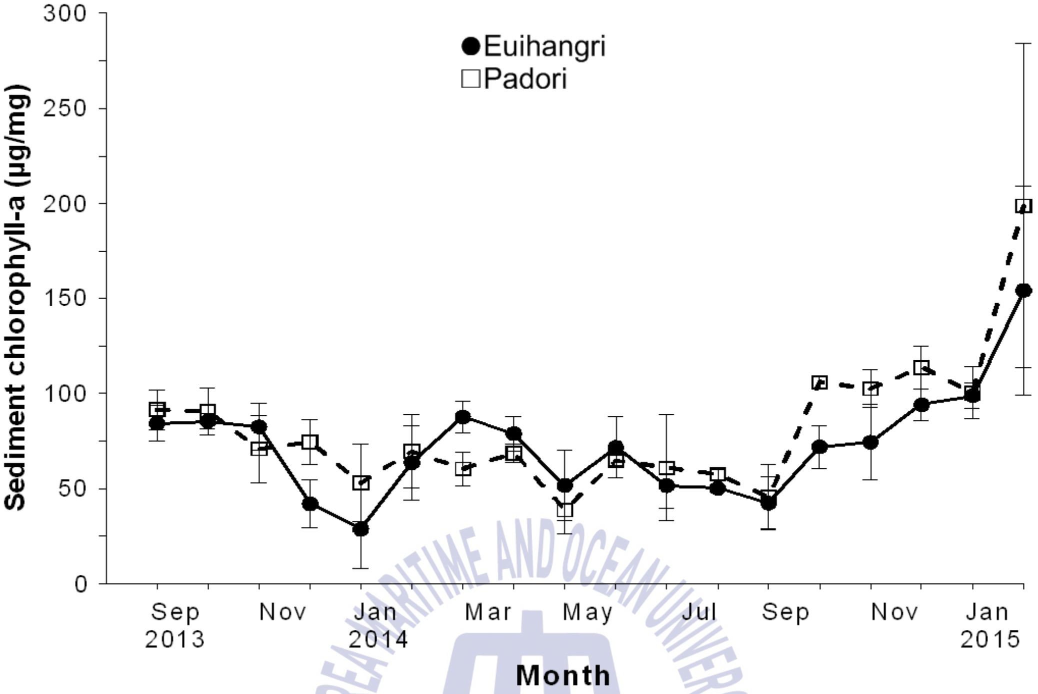

Fig. 7 Monthly variation in sediment chlorophyll *a* at Euihangri and Padori on the west coast of Korea

The average biomass of macrobenthic species other *Ruditapes philippinarum* was higher at Euihangri (2.470 ± 1.875 g/0.25m^2) than at Padori (2.254 ± 1.360 g/0.25m^2), but the differences were not significantly different (Table 1). At Euihangri, the biomass values were lowest in January 2014 (0.159 g/0.25m^2) and highest in July 2014 (6.913 g/0.25m^2). The values at Padori were lowest in October 2014 (0.214 g/0.25m^2) and highest in August 2013 (5.176 g/0.25m^2) (Fig. 8). The average densities of macrobenthic species other than *R. philippinarum* didnot significantly differ and were 94.83 ± 82.73 ind./0.25m^2 and 80.83 ± 148.06 ind./0.25m^2 at Euihangri and Padori, respectively (Table 1). Values at Euihangri were lowest in January 2014 (3 ind./0.25m^2) and highest in April 2014 (244 ind./0.25m^2). Densities at Padori were lowest in November 2014 (11 ind./0.25m^2) and highest in July 2014 (663 ind./0.25m^2) (Fig. 9).

2.3.2 Populations of *Ruditapes philippinarum*

The average densities of *Ruditapes philippinarum* at Euihangri and at Padori were 194 ind./0.25m^2 and 325 ind./0.25m^2, respectively (Table 1). Thus, the densities at Padori were clearly significantly higher than those at Euihangri. The highest densities of *R. philippinarum* at Euihangri and Padori were 663 ind./0.25m^2 (May 2014) and 647 ind./0.25m^2 (August 2013), respectively. When the clams were most dense, the abundances of small individuals (<10 mm) were high at Euihangri, while the abundances of larger individuals (>10 mm) were elevated at Padori (Fig. 10). The lowest densities of *R. philippinarum* at Euihangri and Padori were 20 ind./0.25m^2 (September 2014) and 79 ind./0.25m^2 (January 2015), respectively. The average densities of clams <10 mm were 132 ind./0.25m^2 and 12 ind./0.25m^2 at Euihangri and Padori, respectively. The highest densities of clams <10 mm were 652 ind./0.25m^2 and 86 ind./0.25m^2 at Euihangri (May 2014) and Padori (April 2014), respectively. The average densities of clams >10 mm were 314 ind./0.25m^2 and 62 ind./0.25m^2 at Padori and Euihangri, respectively; the difference between

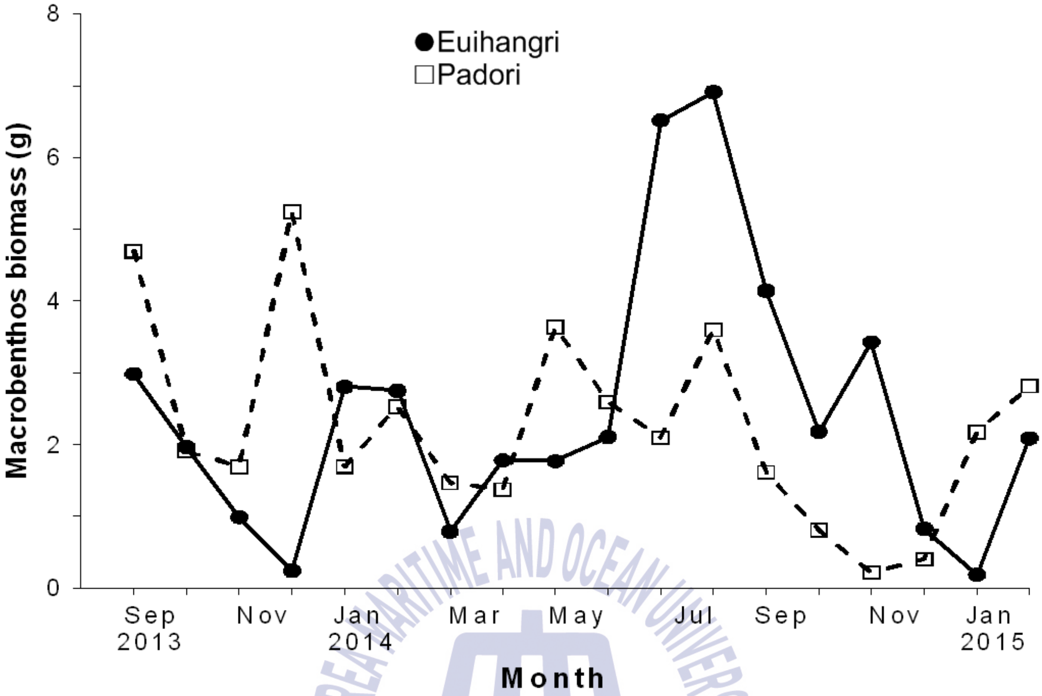

Fig. 8 Monthly variation in biomass of macrobenthos at Euihangri and Padori on the west coast of Korea

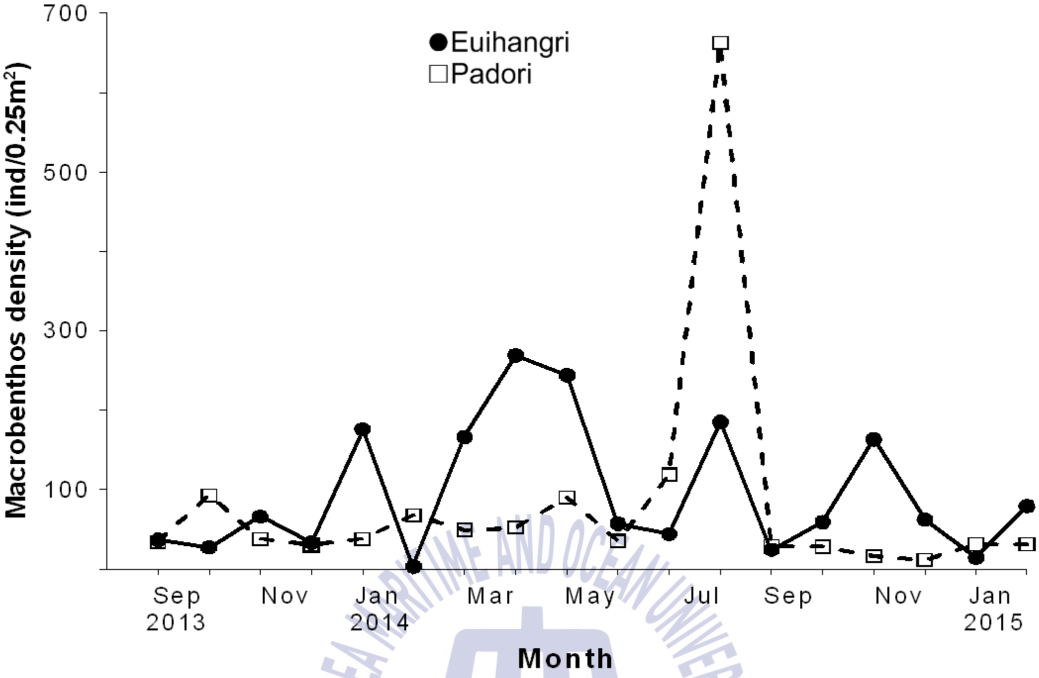

Fig. 9 Monthly variation in density of macrobenthos at Euihangri and Padori on the west coast of Korea

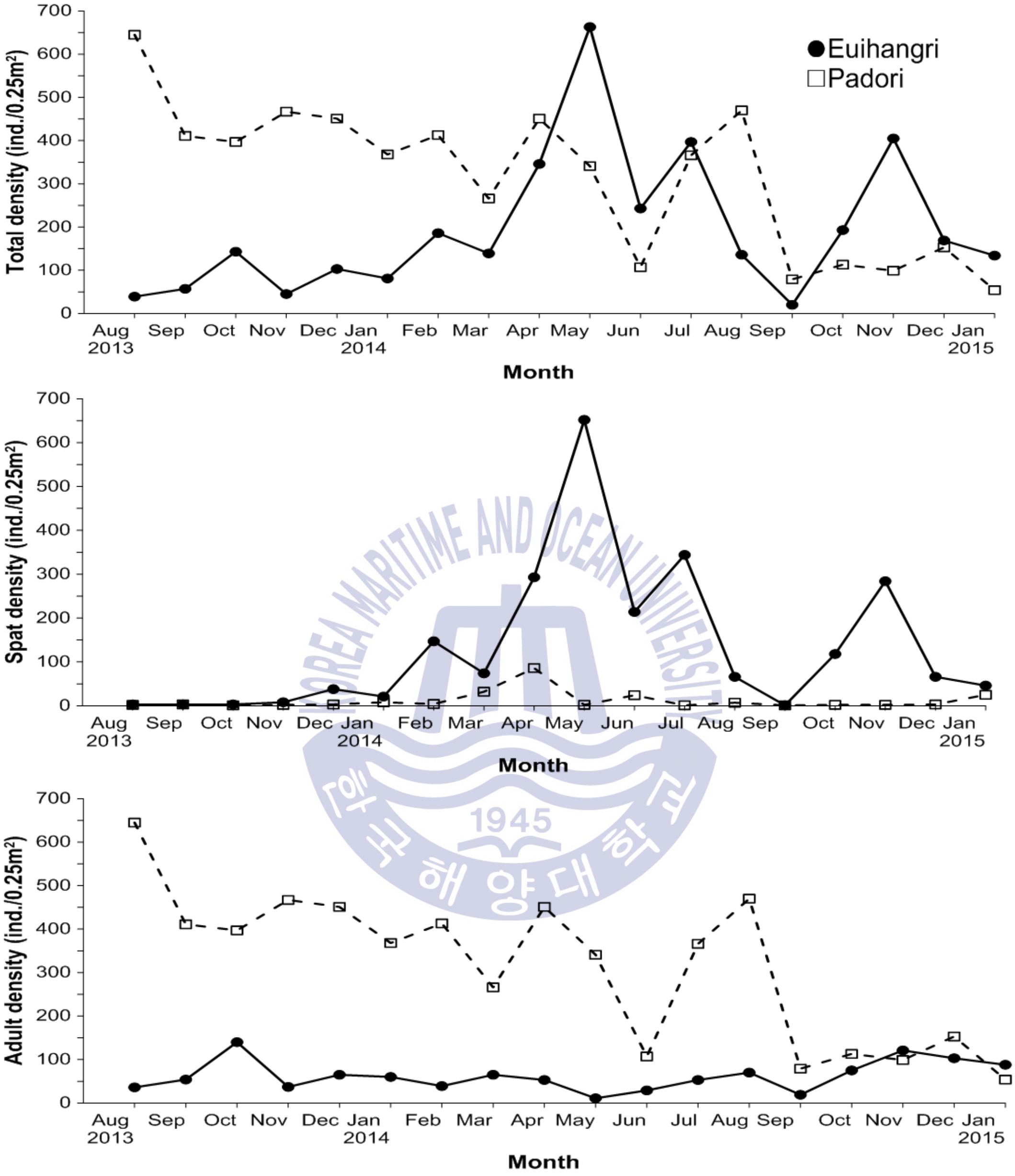

Fig. 10 Monthly variation in density of all of three size groups in *Ruditapes philippinarum* : total individuals, spat(<10 mm long) and adults (>10 mm long)

sites was significant (Table 1). The maximum densities of *R. philippinarum* individuals >10 mm were 140 ind./0.25m^2 and 645 ind./0.25m^2 at Euihangri (October 2013) and Padori (August 2013), respectively.

The monthly frequency distribution plots of clam sizes at Euihangri showed that individuals <10 mm first appeared in October 2013, and continued to appear until the following May (Fig. 11). During this period, the population fell into two size classes. The larger size class rapidly decreased in frequency after June, and individuals in this category rarely appeared after September. At Padori, very few individuals <10 mm appeared in the sediment in August and September 2013, and small clams appeared after February 2014 (Fig. 12). The frequency of clams <10 mm increased between March and June 2014, but no recruits appeared at Padori after July and decreased in September 2014. The frequency of individuals in the 24–30 mm class was constant through the year at this site.

The condition index of *Ruditapes philippinarum* was significantly higher at Euihangri (7.50 ± 0.93) than at Padori (6.15 ± 0.79) (Table 1). At Euihangri, the index was highest in August 2013 (9.85), decreasing thereafter through February 2014 to values in the range of 7.66–6.62 (Fig. 13). The index was 6.68 in March and 9.01 in September, falling to a low value of 6.16 in January 2015. At Padori, the index remained constant at 5.77 ± 0.13 from August 2013 to March 2014, increasing thereafter through June 2015 (8.25). Values decreased from July, reaching a low of 4.32 in January 2015 (Fig. 14, 15). Regression models for the relationship between SL and TW, and between TW and FW were constructed. The SL vs. TW models for Euihangri (Equation 3) and Padori (Equation 4) are:

$TW = 5E\text{-}05 \times SL^{3.337}$ ($R^2 = 0.97$) (3)

$TW = 0.0003 \times SL^{2.769}$ ($R^2 = 0.76$) (4)

The TW vs. FW models for Euihangri (Equation 5) and Padoria (Equation 6) are:
$FW = 0.045 \times TW + 0.002$ ($R^2 = 0.94$) (5)

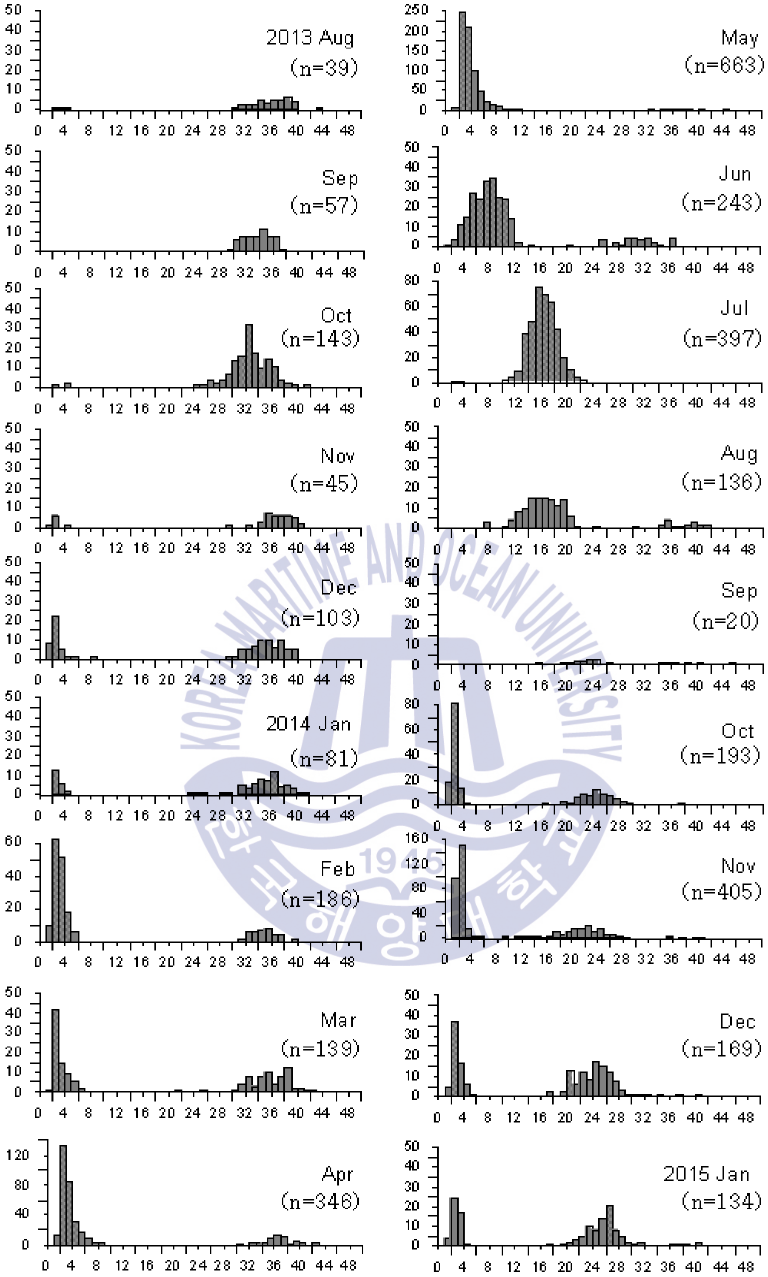

Fig. 11 Size-frequency distribution of *Ruditapes philippinarum* at Euihangri sampled monthy from August 2013 to January 2015

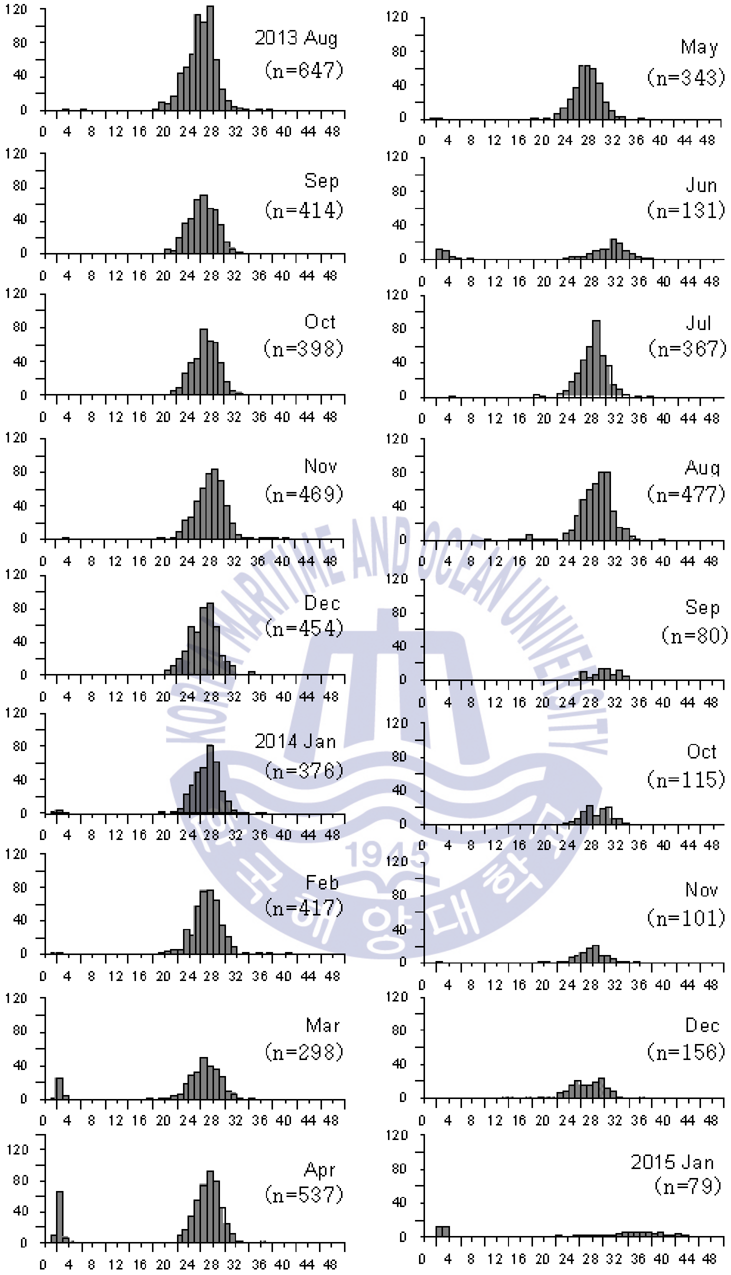

Fig. 12 Size-frequency distribution of *Ruditapes philippinarum* at Padori sampled monthy from August 2013 to January 2015

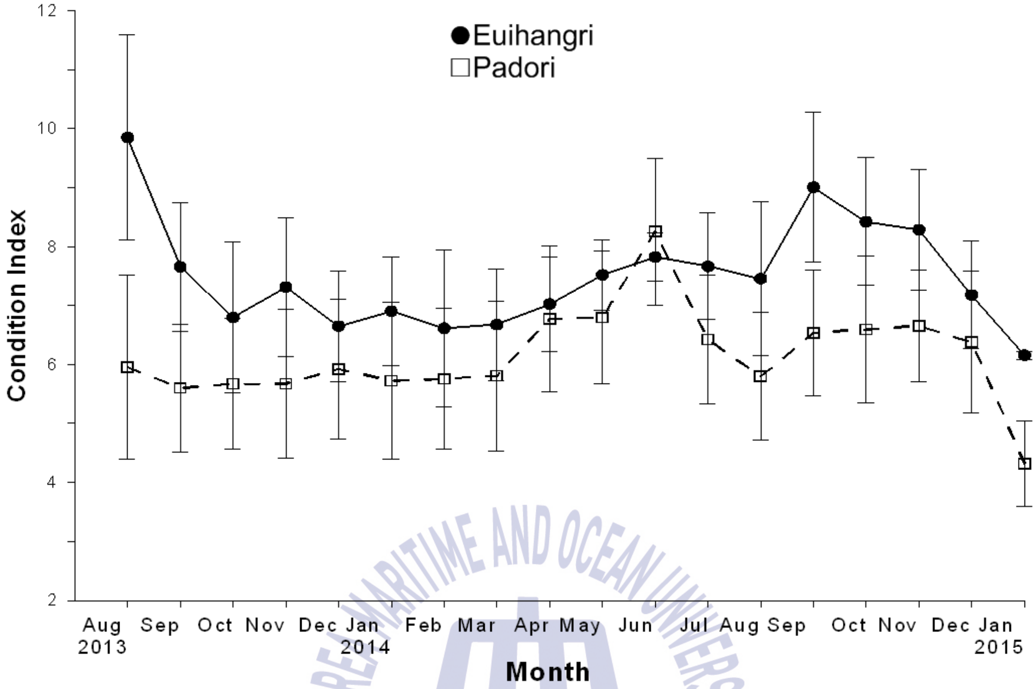

Fig. 13 Monthly variation of the condition indices in *Ruditapes philippinarum* condition indices at Euihangri and Padori on the west coast of Korea

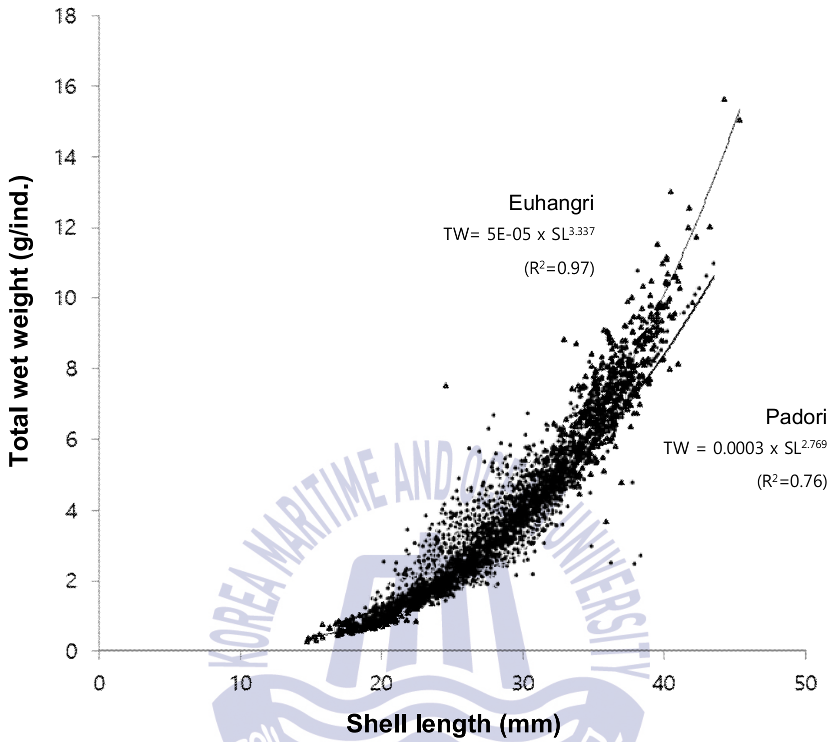

Fig. 14 Relationships between shell lengths and total wet weights of *Ruditapes pilippinarum* at Euihangri and Padori on the west coast of Korea

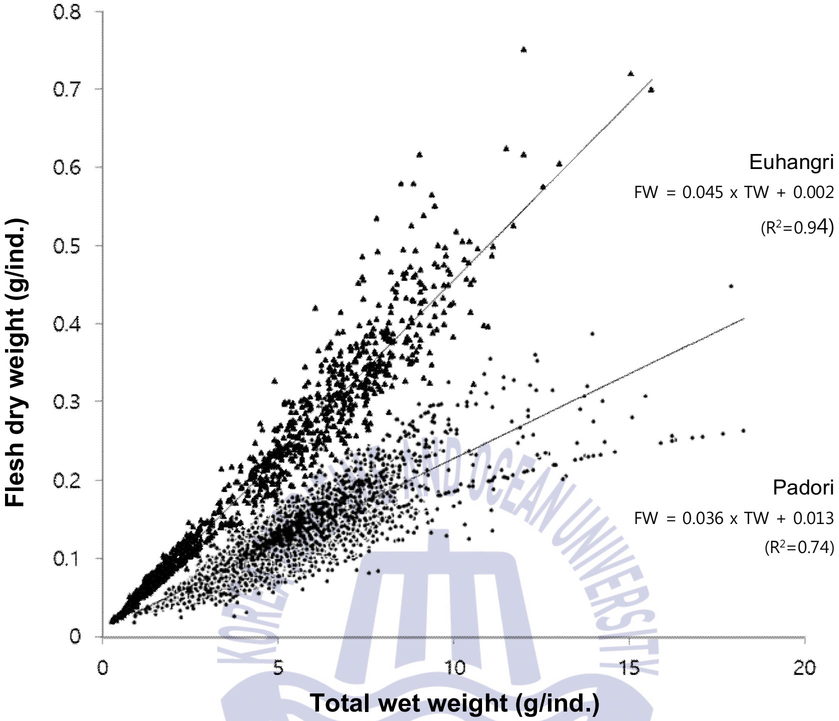

Fig. 15 Relationships between total wet weights and flesh dry weights of *Ruditapes pilippinarum* at Euihangri and Padori on the west coast of Korea

$FW = 0.036 \times TW + 0.013$ ($R^2 = 0.74$)   (6)

2.3.3 Correlations between environmental and biological variables and the density and condition index of *Ruditapes philippinarum*

Water temperature was positively correlated with the salinity and concentration of chlorophyll *a* in the water column. The density of *Ruditapes philippinarum* spat was negatively correlated with salinity, but was positively correlated with mean sediment grain size (Table 2). The density of adults was negatively correlated with mean grain size. The condition index of *R. philippinarum* was negatively correlated with the total density of *R. philippinarum*, the density of adult-sized clams, and the sediment sorting value. At Euihangri, the total density of *R. philippinarum* was positively correlated with the spat density of *R. philippinarum* ($p < 0.001$). The condition index of *R. philippinarum* was negatively correlated with the biomass of *R. philippinarum* ($p < 0.01$) (Table 3). The total density of *R. philippinarum* at Padori was positively correlated with the adult density of *R. philippinarum* and the biomass of macrobenthos ($p < 0.001$) (Table 4).

2.4 Discussion

Sediment conditions affect the feeding of *Ruditapes philippinarum* and its physiological conditions (Lee et al., 1999). Lee (1969) reported that these clams are concentrated at sites where the sediment content includes 10–35% fine sand and 50–90% coarse sand. Icho and Osima (1931) determined that a mix with 50–80% sand content provides a suitable habitat for *R. philippinarum*; thus, our study areas had the appropriate sand contents.

Although the salinity at Padori fell to 17.1 in August 2013, values were generally >19.2, which is the threshold below which the metabolic and survival rates of this species begin to decline (Fig. 3). Therefore, the salinities at the two

Table 2 Spearman rank correlation between the environmental and biological variables using pooled data (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$)

contents	Salinity	Chl-*a* (water)	Chl-*a* (sediment)	Mz	Sorting value	Condition index	TD	AD	SD	DM	BM	BR
Water temperature (℃)	0.43**	0.52**	-0.13	-0.01	-0.18	0.30	0.06	0.02	-0.29	0.06	0.27	-0.02
Salinity		0.09	0.07	-0.27	-0.07	-0.02	-0.16	0.10	-0.38*	-0.04	-0.19	0.03
Water chlorophyll *a* (μg/mg)			-0.08	0.07	-0.05	0.22	0.09	-0.01	-0.07	-0.08	0.24	-0.03
Sediment chlorophyll *a* (μg/mg)				-0.19	-0.23	-0.18	-0.26	-0.01	-0.27	-0.30	-0.33*	-0.18
Mean grain size (Φ)					-0.25	0.49**	-0.05	-0.45**	0.41*	0.13	0.19	-0.39
Sorting value						-0.39*	0.24	0.13	0.09	0.20	0.13	0.23
Condition index of *R. philippinarum*							-0.36*	-0.66***	0.38*	0.10	0.14	-0.67***
Total density of *R. philippinarum* (TD)								0.61***	0.18	0.26	0.17	0.51**
Adult density of *R. philippinarum* (AD)									-0.42*	-0.09	-0.04	0.87***
Spat density of *R. philippinarum* (SD)										0.40*	0.12	-0.46**
Density of Macrobenthos(DM)											0.21	0.01
Biomass of Macrobenthos (BM)												-0.04
Biomass of *R. philippinarum* (BR)												

Table 3 Spearman rank correlation between the environmental and biological variables at Euihangri (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$)

contents	Salinity	Chl-*a* (water)	Chl-a (sediment)	Mz	Sorting value	Condition index	TD	AD	SD	DM	BM	BR
Water temperature (℃)	0.40	0.63**	-0.27	0.18	-0.20	0.70**	-0.03	-0.33	-0.09	-0.03	0.55*	-0.45
Salinity		0.18	-0.01	-0.28	0.09	0.32	-0.05	0.16	-0.20	0.06	0.05	-0.15
Water chlorophyll *a* (μg/mg)			-0.20	0.01	-0.25	0.51*	-0.15	-0.22	-0.23	-0.25	0.11	-0.23
Sediment chlorophyll *a* (μg/mg)				-0.20	-0.11	-0.08	0.04	0.38	-0.11	-0.05	-0.48*	0.06
Mean grain size (Φ)					-0.15	0.20	0.38	-0.28	0.48*	0.36	0.50*	-0.06
Sorting value						-0.36	-0.11	-0.31	0.10	0.34	-0.13	-0.14
Condition index of *R. philippinarum*							0.01	-0.35	-0.02	-0.28	0.41	-0.60**
Total density of *R. philippinarum* (TD)								0.12	0.92***	0.38	0.01	-0.21
Adult density of *R. philippinarum* (AD)									-0.07	0.06	-0.27	0.44
Spat density of *R. philippinarum* (SD)										0.41	0.10	0.29
Density of Macrobenthos (DM)											0.03	0.23
Biomass of Macrobenthos (BB)												0.48*
Biomass of *R. philippinarum* (BR)												

Table 4 Spearman rank correlation between the environmental and biological variables at Padori (*, P<0.05; **, P<0.01; ***, P<0.001)

contents	Salinity	Chl-*a* (water)	Chl-*a* (sediment)	Mz	Sorting value	Condition index	TD	AD	SD	DM	BM	BR
Water temperature (℃)	0.44	0.46	-0.11	-0.16	-0.20	0.33	0.18	0.18	-0.42	0.11	0.03	0.18
Salinity		0.08	0.15	-0.03	-0.22	0.06	-0.39	-0.37	-0.34	-0.22	-0.44	-0.28
Water chlorophyll *a* (μg/mg)			-0.08	0.12	0.20	0.02	0.38	0.34	0.07	0.11	0.40	0.34
Sediment chlorophyll *a* (μg/mg)				0.31	-0.35	-0.17	-0.62**	-0.59*	-0.32	-0.59*	-0.20	-0.75***
Mean grain size (Φ)					-0.28	0.17	-0.05	-0.07	-0.19*	-0.30	-0.08	-0.10
Sorting value						-0.43	0.32	0.32	-0.10	0.17	0.24	0.38
Condition index of *R. philippinarum*							-0.22	-0.28	0.10	-0.04	-0.11	-0.11
Total density of *R. philippinarum* (TD)								0.99***	0.10	0.28	0.44	0.95***
Adult density of *R. philippinarum* (AD)									0.04	0.20	0.40	0.95***
Spat density of *R. philippinarum* (SD)										0.34	0.10	0.06
Density of Macrobenthos(DM)											0.37	0.27
Biomass of Macrobenthos (BB)												0.45
Biomass of *R. philippinarum* (BR)												

sites probably did not influence the survival of the clams. Water temperatures were in the ranges 3.5–26°C and 2.6–26.9°C at Euihangri and Padori, respectively. According to Ryther and Tenore (1976), the equilibrium temperature for the balance between respiration and assimilation is near 13°C. The growth rate *Ruditapes philippinarum* increases up to 25°C, and decreases at higher temperatures (Liang et al. 1987). Our study sites had low water temperatures in winter. The ranges of salinity and temperature that we measured were similar to those in *R. philippinarum* farms at four locations in Kyunggi Bay (Park, 2010). The growth of *R. philippinarum* decreased with rapid declines in water temperature during winter (Bourne, 1982). Hence, winter cooling of the seawater at our study sites may have affected their growth.

The condition index of *Ruditapes philippinarum* at Euihangri was highest in August, declining thereafter to ca. 30% of the highest value by October. The index value at Padori was highest in June, decreasing thereafter to ca. 30% of the highest value by August (Fig. 13). Calculations of the condition index of bivalves based on changes in size and weight can be used to estimate spawning periods; the index changes rapidly through the spawning period (Won and Hur, 1993). Clam gametes develop when individuals have shell lengths of 5−10 mm, and full maturity is reached at lengths of 15–20 mm. All clams >20 mm long are able to spawn (Holland and Chew, 1974). The condition index is highest during the peak of ripeness after the accumulation of resources in preparation for reproduction (Drummond et al., 2006). Condition declines thereafter through the spawning period (Park and Choi, 2004). The condition index of *R. philippinarum* on Seonjaedo Island off the west coast of Korea decreases by ca. 30% through the end of the spawning period (Uddin et al 2010). Thus, based on the condition index of *R. philippinarum* at our sites, the estimated spawning period began after August at Euihangri and after June at Padori. Our counts of clam spat recruiting to the sediment indicated that the population sizes at Euihangri and Padori increased after

October and May, respectively (Figs. 4, 5). In September 2014, reduced adult population of the two sites may be affected to anthropogenic interference as well as a relatively high temperature and salinity.

The condition index of *Ruditapes philippinarum* at Euihangri exceeded that at Padori (Table 1). The slope of the regression equation describing clam growth was also higher for the Euihangri population. The condition index can be used to predict clam growth as well as the spawning period. Higher clam condition indices are indicative of high growth rates (Kim 2005). In stable environments, the shells of this species are prolate in shape, but egg-shaped in unstable environments (Choi 1965). Shell shapes at Euihangri were indicative of better growth than the shell shapes at Padori. Our regression models showed that for a given SL, the TW at Euihangri exceeded that at Padori. Furthermore, the slope of the regression describing the relationship between TW and FW was steeper at Euihangri than at Padori (Fig. 7, 8). Variation in FW is directly related to nutrient ration, and is a good indicator of changes in gonadal growth and spawning over time when the energy balance is favorable (Pérez-Camacho et al. 2003; Delgado and Pérez-Camacho 2003). Our calculated regression slopes indicate that growth rate was higher at Euihangri.

Spat of *Ruditapes philippinarum* generally settle where the mix of particle sizes is diverse and the degree of sediment sorting is high (Park et al., 2013b). The degree of sediment sorting was not different between our two sites. Nevertheless spat abundance was higher at Padori, recruitment was more successful at Euihangri, likely because of reduced intra-specific competition for space in the low-density population. Our biomass correlation analyses indicated that the density of *R. philippinarum* was unrelated to the biomass of other macrobenthic species. Therefore, interspecific competition is likely less important for the recruitment success of *R. philippinarum* than intraspecific competition. Regulation of the density of *R. philippinarum* populations and the food sources available in its habitat may optimize the environment conditions for commercial production of this clam. Although chlorophyll *a* was not different between our two sites, chlorophyll *a* is believed to be because it represents an indirect amount of phytoplankton require a lot of research on the benthic microalgae.

Chapter 3. Macrobenthos community in *Ruditapes philippinarum* aquaculture

3.1 Introduction

The Yellow Sea west of Korea is a semi-enclosed, northwestern Pacific marginal sea into which two of the largest rivers in China flow: the Huanghe (Yellow) and Changjiang (Yangtze) Rivers. The high terrigenous sediment inputs from the surrounding landmasses to the Yellow Sea result in high sediment accumulation in its subaqueous deltas (Alexander et al., 1991, Yang et al., 2003; Yang & Youn, 2007). Along open shores, fine sediments are eroded by frequent storms and the effects of monsoon seasonal winds. By contrast, in the low-energy environment of summer, fine sediments pile up (Chun et al., 1998; Lee et al., 1999). Along semi-enclosed shores like those of the Yellow Sea, sedimentation is primarily under the control of seasonal winds, geological factors (*e.g.*, the form of the back-bay area), and currents in the intertidal zone (Jang & Choi, 1998; Ryu et al., 1999; Ryu et al., 2000; Chu et al., 2000). Along the western shore of Korea, the changes in the sea surface are great, due to the large differences in ebb and flow in the morning and evening and the complex actions of astronomical tides and storm surges. Along the western shore, where the water is shallow and there are wide beaches at low tide, the mean sea level has been changed by seashore development, such as the reclamation of land from the sea (Jung, 2014). In other areas, the sea level has risen continuously due to climate change and other factors (Kang et al., 2005a; KHOA, 2011).

Macrobenthos play a decisive role in the structure and function of marine ecosystems. Benthos are consumed by fish and mammals, providing food for higher trophic levels, and are also important in nutrient and organic matter cycling and serve as a link between the pelagic and benthic divisions of marine ecosystems (Grall & Chauvaud, 2002; Iken et al., 2010; Hill et al., 2011; Brey, 2012; Oug et al., 2012). Benthic communities are susceptible to change over time because of the response of the species' life cycles to environmental variability (Convey, 1996; Sibly & Calow, 1989). They are considered to be good indicators of ecosystem health because of their sedentary nature and longevity, which results in long-term exposure to toxic materials, and are representative taxa that can respond to complex types of stress (Jewett et al., 1999). Studies of the responses of macrobenthos community structure to environmental gradients are popular in community ecology because macrobenthos communities are affected by environmental gradients, salinity, hydrology feeding resources, and habit heterogeneity, especially with seasonal changes that may hide the effects of environmental gradients. Changes in the abundance, biomass and benthic communities after natural disasters, such as droughts and floods, can differ over spatial scale, across habitats, and among benthic animal taxa (Jones, 1990; Grilo et al., 2011; Dittmann, 2015). Moreover, the effects of climate change do not exist in isolation from other anthropogenic influences, such as eutrophication in estuaries (Dolbeth et al., 2011) or river flow eutrophication as a result of artificial obstructions like dams and flood gates, which can affect sediment and nutrient discharge, and influence biota by limiting movement and poor water quality (Dittmann, 2015; Smaal & Nienhuis, 1992; Ritter et al., 2008).

This study examined the nature and dynamics of the intertidal macrobenthos communities by examining the number of species, population densities, and community structure in areas dominated by *Ruditapes philippinarum* at Geunsoman on the west coast of Korea.

3.2 Materials and Methods

3.2.1 Sampling

Sediment samples were collected seasonally from April 2011 to October 2014 at Geunsoman (36°43.575′N, 126°10.269′E), Sowon-myeon, Taean-gun, Chungcheongnam-do (Fig. 16). Two replicate samples for macrobenthic community analysis were collected with a can core (0.1 m^2) to a depth of 30 cm and then sieved througha 1-mm mesh. Residues on the mesh were sorted to collect animals and preserved in 10% formalin in seawater. Simultaneously, the surface sediment (0 –1 cm layer) was collected to analyze sediment grain size and the total organic carbon (TOC) concentration, and these samples were frozen until analysis. In the laboratory, all organisms were sorted from the sediments and identified to the lowest possible taxonomic level under a stereomicroscope. Sediment particle sizes were determined after treating samples with a solution of 10% hydrogen peroxide. Sediment samples were heated to >100°C to evaporate the hydrogen peroxide, and then washed at least three times with distilled water to remove organisms and salts. The washed samples were passed through a 63-μm standard sieve (Ingram, 1971). After drying, the sediments trapped by the sieve were weighed and subjected to automatic particle size analysis using a SediGraph 5120 apparatus following the addition of a dispersing agent (sodium hexametaphosphate). We calculated the average particle sizes and degrees of sorting. The sediments were categorized according to Folk’s classification system (Folk & Ward, 1957). The TOC was analyzed using a CN analyzer.

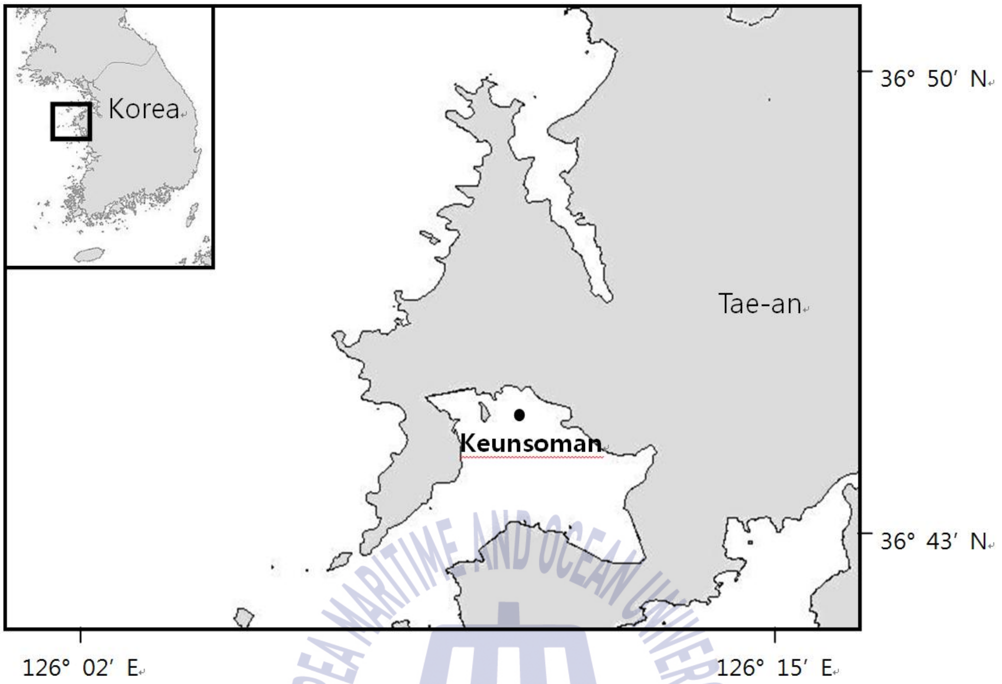

Fig. 16 Locations of sampling stations at Keunsoman on the west coast of Korea

3.2.2 Data analysis

The density and biomass data were reported per square meter. The Shannon–Wiener diversity index H' (Shannon & Wiener, 1963) was calculated from the density data. Cluster analysis and similarity profile (SIMPROF) permutation tests were implemented to illustrate the macrobenthic communities of the sampling sites, using a Bray–Curtis similarity measure based on the 4th root converted group-average linkage and density data. Similarity percentage (SIMPER) analysis was used to determine the contributions of each species to the similarity/dissimilarity of those groups. All analyses were implemented using the PRIMER 10.5 software package (developed at the Plymouth Marine Laboratory) (Clarke & Gorley, 2006).

3.3 Results

3.3.1 Sediment analysis

The mean grain sizes of the sediment in April, July, and October 2011 were 6.46, 3.3, and 4.4 Φ, respectively. In 2012, the average sediment grain size was 4.75 Φ, which did not differ from the value in 2011. This decreased to 3.70 Φ in 2013. In 2014, there was a large decline in the mean grain size to an average of 3.14 Φ. The smallest size (1.72 Φ) was recorded in October 2014 (Table 5). The TOC in April, July, and October 2011 was 0.58, 0.31, and 0.44, respectively. This increased to an average of 0.99 for 2012, with the highest value of 2.15 recorded in October 2012. The average TOC was 0.48 and 0.47 in 2013 and 2014, respectively, and did not differ markedly from the TOC in 2011 (Table 5). On average, the sediment comprised 6.1% gravel, 54.4% sand, 25.7% silt, and 13.9% clay (Fig. 17).

Table 5 Sediment analysis at Geunsoman on the west coast of Korea

Year	Month	Mz	St.De	Skew.	Kurt.	Type	TOC
2011	Apr	6.463	3.057	0.323	0.618	sM	0.58
2011	Jul	3.394	3.515	0.047	1.137	gmS	0.31
2011	Oct	4.397	3.674	0.180	1.002	(g)sM	0.44
2012	Jan	6.147	3.492	0.330	0.651	(g)sM	0.75
2012	Apr	4.585	2.385	0.706	1.913	(g)mS	0.59
2012	Jul	4.156	4.014	0.201	0.833	gmS	0.47
2012	Oct	4.110	3.411	0.068	1.363	gM	2.15
2013	Jan	4.570	3.272	0.362	1.518	(g)mS	0.44
2013	Apr	5.399	2.999	0.572	0.953	(g)mS	0.49
2013	Jul	2.339	3.172	0.840	3.845	gmS	0.58
2013	Oct	2.718	3.256	0.246	1.718	gmS	0.53
2014	Jan	4.342	2.673	-0.415	2.884	gM	0.42
2014	Apr	4.056	2.707	0.086	2.171	zS	0.36
2014	Jul	2.494	3.099	0.086	1.029	gmS	0.47
2014	Oct	1.719	0.383	-0.005	1.230	S	0.31

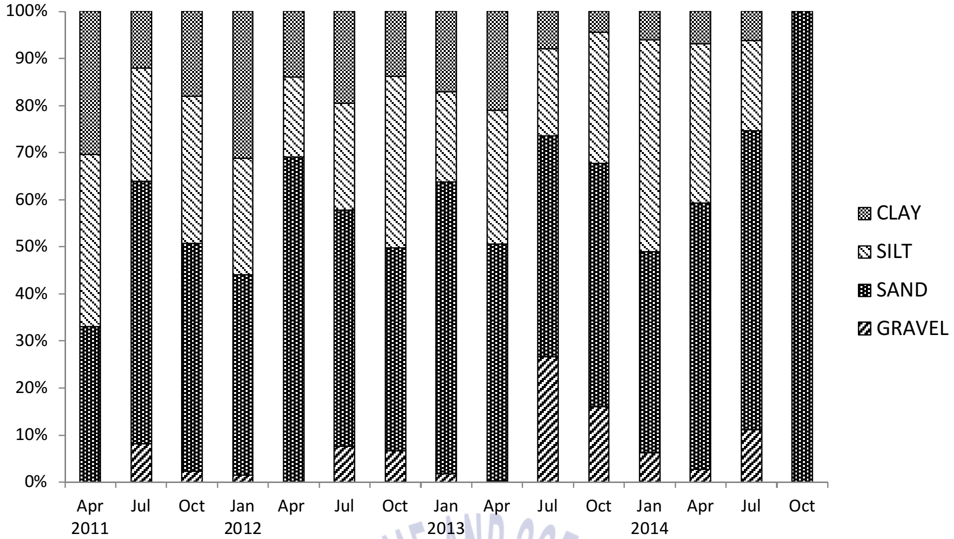

Fig. 17 Seasonal variaton in sediment component at Geunsoman on the west coast of Korea

3.3.2 Species composition

During the research period, the average macrobenthos density was 3548 individuals (ind.)/m^2, of which 44% were mollusks, 41% were polychaetes, 13% were crustaceans, and 1% was others (Fig. 18). The macrobenthos density averaged 3880 ind./m^2 in 2011. It increased markedly to 9470 ind./m^2 in January 2012 and then decreased to 4180, 5040, and 1580 ind./m^2 in April, July, and October, respectively. It was 1959 ind./m^2 in 2013 and, in January 2014, it increased to 3580 ind./m^2 and was 2950, 2905, and 3405 ind./m^2 during April, July, and October, respectively.

From April 2011 to October 2014, 145 macrobenthos species were identified, of which 12% were mollusks, 56% were polychaetes, 26% were crustaceans, and 6% were others. In 2011, 35 and 45 species were found in April and July, respectively. In 2012, the number of species increased from 40 in January to 55 in April and declined to 39 in July and 36 in October. On 2013, an average of 35 species were recorded, which increased to 51 in 2014, but decreased to 34 in April and 32 in July, before increasing to 46 species in October. Polychaetes were the most common species in January 2014, with 31, and this declined to 16 species in April (Fig. 19).

The biomass averaged 4514 g/m^2 in 2011, increased to 9955 g/m^2 in January 2012, and then decreased to 3374 g/m^2 in April. However, the average jumped to 27956 g/m^2 in July 2012, before falling to 8366 g/m^2 in October, 8318 g/m^2 in January 2013, and then 3452 g/m^2 in July 2013. In 2014, the average was 6665 g/m^2, although it was only 793 g/m^2 in April of that year.

3.3.3 Ecological analysis (Diversity index *H'*, Evenness J', Species richness d)

The diversity index was 1.83 in April 2011 and then increased to 2.31 in July and 2.39 in October (Fig. 20). The index was 2.25 in 2012, 2.32 in 2013, and 2.20 in 2014, although it was especially low in April 2014 (1.62). The species

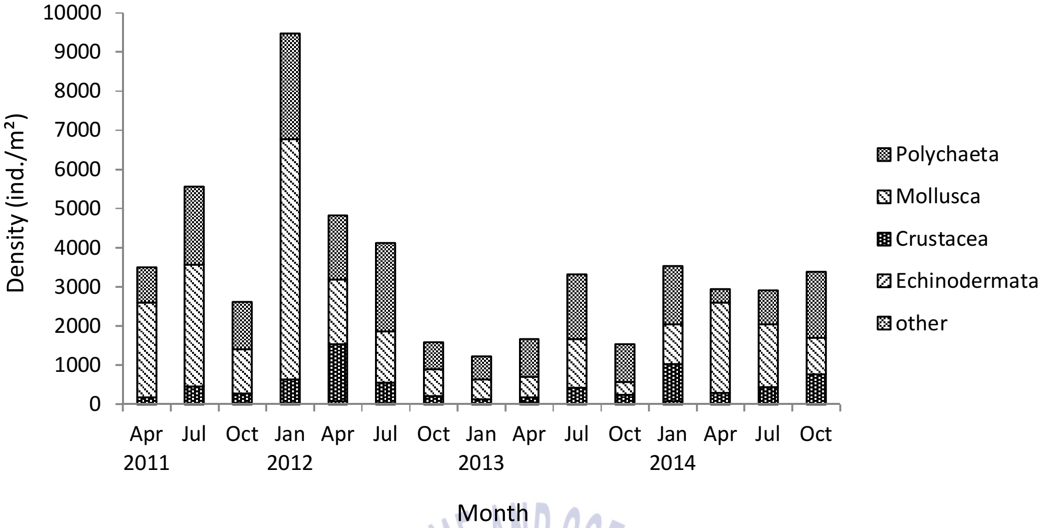

Fig. 18 Seasonal variatonof density in macrobenthos at Geunsoman on the west coast of Korea

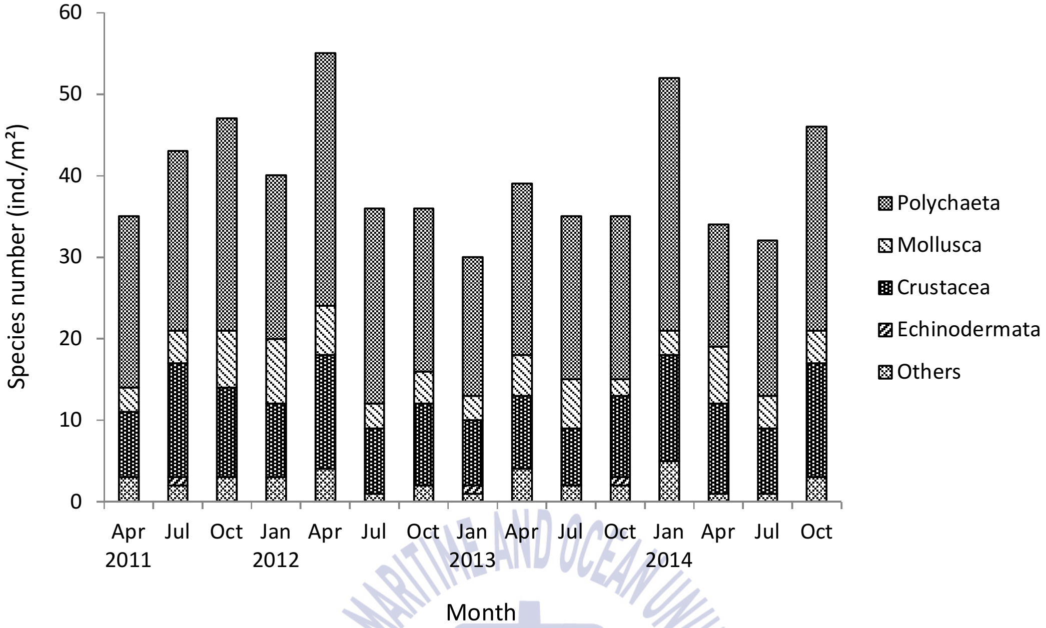

Fig. 19 Seasonal variaton of number of species in macrobenthos at Geunsoman on the west coast of Korea

evenness index averaged 0.58, 0.6, 0.66, and 0.59 in 2011 to 2014, respectively. The index was the lowest in April 2014, at 0.46. The species richness index in 2011 was 4.17, 4.87, and 5.85 in January, July, and October, respectively. In 2012, it averaged 4.96, which was similar to the average of 4.97 in 2011. The average decreased to 4.50 in 2013, but increased to 4.92 in 2014 (Fig. 20).

3.3.4 Variation of dominant species

The most numerous taxa were polychaetes (60 species), followed by crustaceans (53 species) and mollusks (16 species). Dominant species included the mollusks *Ruditapes philippinarum*, *Musculus senhousia*, *Laternula marilina*, and *Bullacta exarata*, the polychaetes *Heteromastus filiformis*, *Ceratonereis erythraeensis*, *Ampharete arctica*, *Lumbrineris longifolia*, *Nephtys polybranchia*, *Glycinde gurjanovae*, and *Glycera chirori*, and crustaceans *Crangon affinis*, *Diastylis paratricincta*, *Cumella somersi*, *Grandidierella japonica*, and *Ilyoplax pingi*. The most abundant species was the mollusk *R. philippinarum*, which had an average of 1335 ind./m^2, followed by *H. filiformis*, *C. erythraeensis*, and *A. arctica*. *R. philippinarum*, *H. filiformis*, *C. erythraeensis*, *N. polybranchia*, *G. gurjanovae*, and *G. chirori* were found throughout the study (Table 6).

3.3.5 Macrobenthic community analysis

A cluster analysis and multidimensional scaling analysis of the Bray–Curtis similarity based on the density of the macrobenthos delineated six groups from April 2011 to October 2014: groups A1, April 2011; A2, July, October 2011, January 2012, April, July, and July 2013; A3, January 2013, April 2014; A4, October 2012; A5, April 2011; and B, April 2014 (SIMPROF test, $P < 0.05$; Fig. 21, 22). The major species contributing to the overall average dissimilarity of the communities, which was 50.67% for A1 & A2, were *C. affinis* and *Iphinoe tenera*,

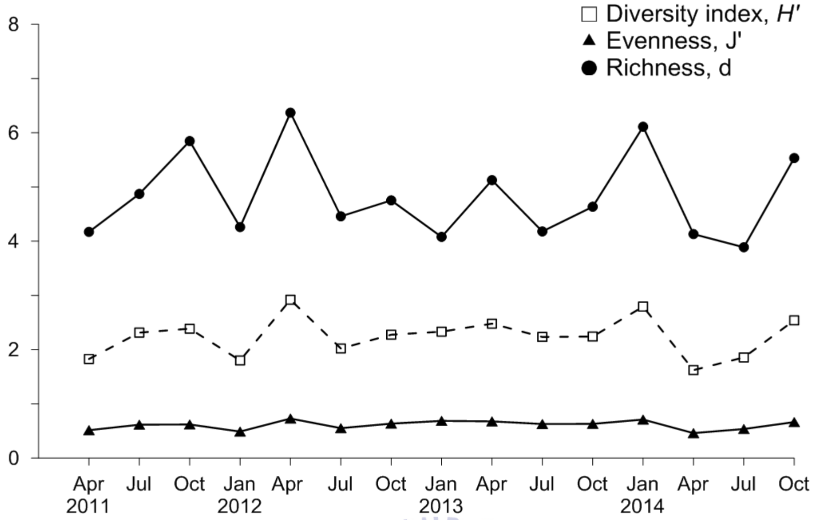

Fig. 20 Ecological analysis at Geunsoman on the west coast of Korea (Diversity index *H'*, Evenness J', Species richness d)

Table 6 Dominant species of macrobenthic fauna at Geunsoman on the west coast of Korea

Taxa	Species	Density (ind./m^2)	%	frequency (%)
MBi	*Ruditapes philippinarum*	1335	37.6	100
Apo	*Heteromastus filiformis*	381	10.7	100
Apo	*Ceratonereis erythraeensis*	289	8.1	100
Apo	*Ampharete arctica*	194	5.5	73.3
MBi	*Musculus senhousia*	189	5.3	80
MBi	*Laternula marilina*	108	3.0	26.7
Apo	*Lumbrineris longifolia*	99	2.8	93.3
CDE	*Crangon affinis*	91	2.6	53.3
Apo	*Nephtys polybranchia*	59	1.7	100
Apo	*Glycinde gurjanovae*	53	1.5	100
Apo	*Glycera chirori*	50	1.4	100
CCU	*Diastylis paratricincta*	50	1.4	80
CCU	*Cumella somersi*	45	1.3	6.7
CAM	*Grandidierella japonica*	40	1.1	93.3
CDE	*Ilyoplax pingi*	38	1.1	73.3
MGa	*Bullacta exarata*	36	1.0	20

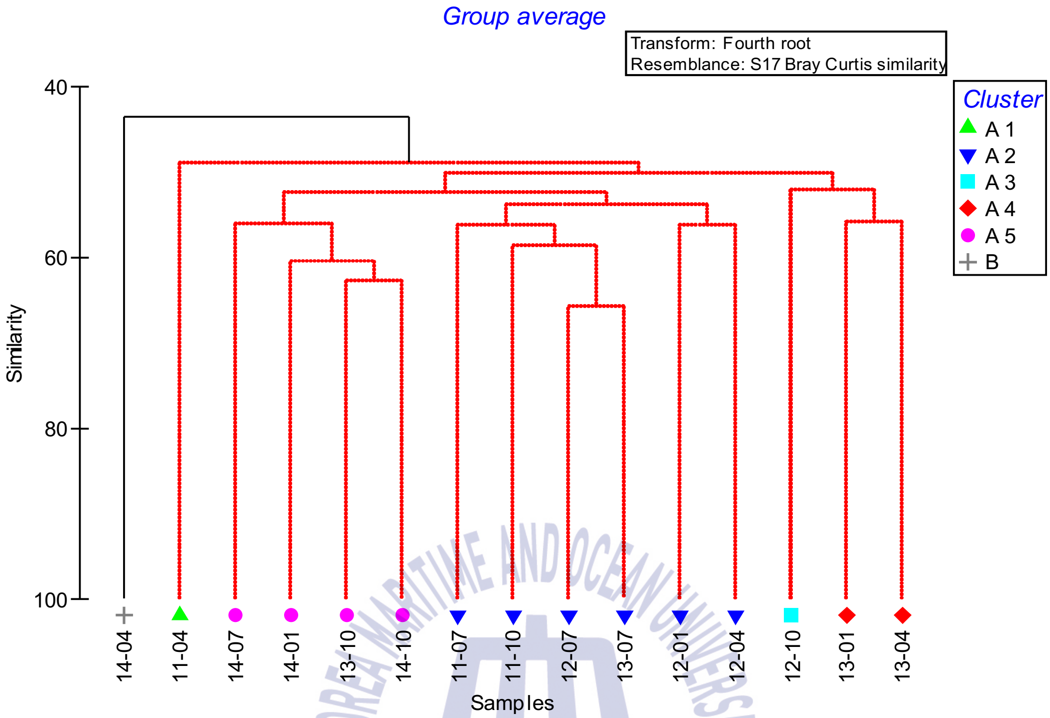

Fig. 21 Clustering analysis of macrobenthic community using Bay-Curtis similarity with 4th transformed data of macrobenthic density.

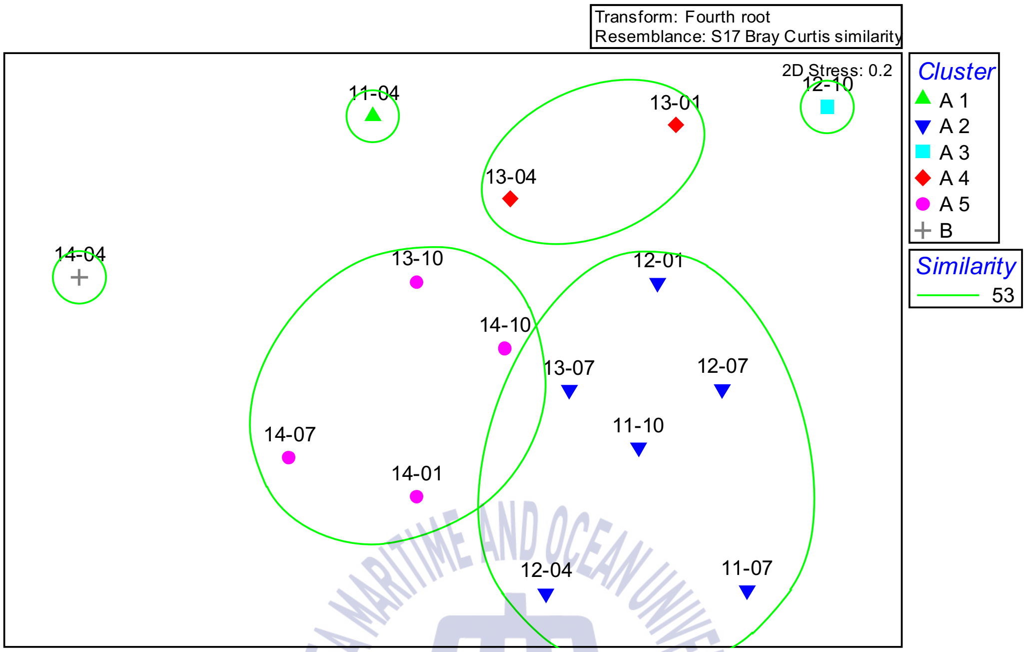

Fig. 22 MDS analysis of macrobenthic community using Bay-Curtis similarity with 4th transformed data of macrobenthic density.

contributing 2.96% and 2.92%, respectively (Table 7). For A1 & A3, the overall dissimilarity was 60.14% and *M. senhousia* contributed 5.50%. For A2 & A3, the overall dissimilarity was 52.17%; *A. arctica* and *M. senhousia* contributed 4.13% and 3.71%, respectively. A & B had the largest dissimilarity of 54.53% and the majorcontributing species were *B. exarata* (3.93%) and *Nippoleucon hinumensis* (3.39%).

3.3.6 Correlation analysis

The correlations among the top three dominant species, sediments, and ecological and environmental factors showed that the species numbers positively correlated with the species richness (Table 8). The diversity showed positive correlations with the evenness and richness. *Ruditapes philippinarum* was positively correlated with the macrobenthos biomass, and negatively correlated with species richness and diversity. Of the dominant species, *G. chirori* was positively correlated with *H. filiformis* and *C. erythraeensis*; *H. filiformis* was positively correlated with *C. erythraeensis*; and *N. polybranchia* was positively correlated with *G. gurjanovae* and *G. japonica*. However, *R. philippinarum* did not have any associations with any species above the top 1% (Table 9).

Table 7 SIMPER analysis of macrobenthic animals, listing the main characterising species at Geunsoman on the west coast of Korea.

Group	Taxonomic group	Average abundance	Average abundance	Average dissimilarity	% Contribution	Cumulative %	Overall average dissimilarity
A 1 & A 2	*Crangon affinis*	0.00	2.41	1.50	2.96	2.96	50.67
	Iphinoe tenera	2.84	0.35	1.48	2.92	5.89	
	Ampharete arctica	1.97	3.44	1.31	2.58	8.47	
	Chone teres	0.00	2.14	1.27	2.51	10.98	
A 1 & A 3	*Musculus senhousia*	4.83	0.00	3.41	5.68	5.68	60.14
	Eteone longa	2.99	0.00	2.11	3.51	9.19	
	Iphinoe tenera	2.84	0.00	2.01	3.34	12.53	
A 1 & A 4	*Musculus senhousia*	4.83	1.06	2.76	5.50	5.50	50.14
	Diastylis paratricincta	0.00	2.20	1.59	3.17	8.67	
	Lumbrineris cruzensis	0.00	2.11	1.52	3.03	11.70	
A 1 & A 5	*Iphinoe tenera*	2.84	0.00	1.82	3.65	3.65	49.8
	Ilyoplax pingi	0.00	2.41	1.54	3.10	6.75	
	Macoma tokyoensis	2.34	0.00	1.50	3.01	9.75	
	Crangon affinis	0.00	2.22	1.49	2.99	12.74	
A 2 & A 3	*Ampharete arctica*	3.44	0.00	2.15	4.13	4.13	52.17
	Musculus senhousia	3.26	0.00	1.94	3.71	7.84	
	Grandidierella japonica	2.68	0.00	1.63	3.12	10.96	
A 2 & A 4	*Ampharete arctica*	3.44	0.00	2.18	4.52	4.52	48.34
	Crangon affinis	2.41	0.00	1.57	3.24	7.76	
	Musculus senhousia	3.26	1.06	1.56	3.23	10.99	
A 2 & A 5	*Philine argentata*	2.12	0.00	1.13	2.38	2.38	47.59
	Ampharete arctica	3.44	2.72	1.12	2.35	4.72	
	Crangon affinis	2.41	2.22	1.08	2.26	6.99	
	Noemysis awatschensis	0.00	1.80	1.00	2.10	9.08	
	Musculus senhousia	3.26	2.57	0.93	1.96	11.05	
A 3 & A 4	*Crangon affinis*	2.84	0.00	2.11	4.40	4.40	47.91
	Helice tridens wuana	2.66	0.00	1.97	4.12	8.51	
	Neomysis japonica	2.34	0.00	1.74	3.62	12.14	
A 3 & A 5	*Ampharete arctica*	0.00	2.72	1.84	3.48	3.48	52.81
	Helice tridens wuana	2.66	0.00	1.75	3.32	6.80	
	Musculus senhousia	0.00	2.57	1.68	3.18	9.98	
	Grandidierella japonica	0.00	2.44	1.58	2.99	12.97	
A 4 & A 5	*Ampharete arctica*	0.00	2.82	1.87	3.82	3.82	48.91
	Crangon affinis	0.00	2.22	1.56	3.19	7.01	
	Chone teres	0.00	1.88	1.25	2.56	9.56	
	Noemysis awatschensis	0.00	1.80	1.19	2.44	12.00	
A & B	*Bullacta exarata*	0.46	3.78	2.08	3.93	3.93	54.53
	Nippoleucon hinumensis	0.19	3.12	1.79	3.39	7.32	
	Eudorella hwanghaensis	0.00	2.43	1.49	2.82	10.14	
	Apocorophium acutum	1.87	2.72	1.44	2.72	12.86	
	Venatomya truncata	0.00	2.11	1.30	2.45	15.31	
	Crangon affinis	1.87	0.00	1.16	2.19	17.50	
	Musculus senhousia	2.62	3.64	1.05	1.97	19.48	

Table 8 Spearman rank correlation between the three dominant species, sediments, ecological and environmental factors (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$)

Content	No.spp.	Density	Biomass	Evenness	Richness	Diversity	RP	HF	CE
Sand	-0.07	-0.23	-0.19	0.02	0.28	0.32	-0.22	-0.12	-0.31
Silt	0.00	-0.11	-0.16	0.05	-0.23	-0.21	0.03	-0.13	-0.19
Mean grain size	0.18	0.16	0.21	0.10	0.03	0.03	0.09	-0.04	0.13
Sorting value	-0.12	0.05	0.47	-0.21	-0.38	-0.34	0.29	0.01	0.02
Skewness	-0.22	-0.06	0.15	-0.20	0.09	-0.10	-0.08	-0.12	0.02
Kurtosis	-0.03	-0.21	-0.44	0.13	0.45	0.30	-0.48	-0.12	-0.11
TOC (%)	0.31	0.14	0.29	0.30	0.25	0.08	-0.14	0.31	0.40
No.spp.		0.52*	0.00	0.93***	0.39	0.65**	-0.02	0.61*	0.71**
Density (ind/m^2)			0.02	0.20	-0.29	-0.13	0.66**	0.49	0.71**
Biomass (ind/m2)				0	0.10	-0.01	-0.05	0.34	0.30
Evenness (J')					0.61*	0.81***	-0.35	0.53*	0.52*
Richness (d)						0.91***	-0.84***	0.11	0.18
Diversity (*H'*)							-0.68**	0.23	0.29
Ruditapes philippinarum (RP)								-0.01	0.18
Heteromastus filiformis (HF)									0.78***
Cratonereis erythraeensis (CE)									

Table 9 Spearman rank correlation among the dominant species >1% of total density (*, P<0.05; **, P<0.01; ***, P<0.001)

Species	HF	CE	AA	MS	LM	LL	CA	NP	GG	GC	DP	CS	GJ
Ruditapes philippinarum (RP)	-0.01	0.18	0.42	0.42	0.44	0.21	0.20	0.13	0.09	0.25	0.04	-0.12	-0.02
Heteromastus filiformis (HF)		0.78***	-0.03	0.13	0.15	0.34	0.10	0.45	0.34	0.75***	0.05	0	0.55*
Cratonereis erythraeensis (CE)			0.16	0.26	0.08	0.51	0.12	0.73**	0.56*	0.81***	0.36	0.12	0.63*
Ampharete arctica (AA)				0.15	0.18	0.14	0.66**	0.18	0.18	0.21	0.13	0.19	0.22
Musculus senhousia (MS)					0.25	0.24	-0.37	0.25	0.04	0.35	0.14	0.37	0.49
Laternula marilina (LM)						0.32	0.19	0.38	0.39	0.30	-0.14	-0.16	0.21
Lumbrineris longifolia (LL)							-0.12	0.68**	0.44	0.72**	0.19	0.37	0.31
Crangon affinis (CA)								-0.01	0.06	0.07	-0.36	-0.26	-0.10
Nephtys polybranchia (NP)									0.70**	0.73**	0.55*	0.40	0.69**
Glycinde gurjanovae (GG)										0.51	0.39	0.25	0.38
Glycera chirori (GC)											0.23	0.31	0.49
Diastylis paratricincta (DP)												0.38	0.48
Cumella somersi (CS)													0.31
Grandidierella japonica (GJ)													
Ilyoplax pingi (IP)													
Bullacta exarata (BE)													

3.4 Discussion

The number of macrobenthos species differs according to the investigation period and sampling method, but the overall diversity of similar areas can be predicted (Jeong, 2006). In this study, 145 species were recorded at Geunsoman, which is similar to values of 154 and 134 reported by Kim (2015), the 135 species Jeong (2006) found at Anmyeon, and the 127 species at Seo (2003). Polychaetes change the quality and size of sedimentary facies through feeding, playing important roles in benthic ecosystems (Jin, 1989). In this research, polychaete species comprised 44% of all species and 55% of the total population. The respective values were 45% and 73% for the Gwangyang tidal flat (Choi, 2003), 48% and 67% for the Hallyeohaesang National Park area (Yoon, 2009), and 43% and 62% for the Gamak tidal flat (Koo et al., 2004). The survey area was dominated by *Ruditapes philippinarum*, but there were no differences in the numbers of species or numbers or proportions of polychaetes in the study areas. The correlation analysis also showed that *R. philippinarum* was not associated with other dominant species. It appears that *R. philippinarum* and other macrobenthos use different spaces, and the competition for food and habitat is not severe.

The spatial patterns of the dominant species give information necessary to understand the structure of target populations. The proportions of the dominant species play a significant role in community structure research interpreting the environment (Pearson & Rosenberg, 1978; Fried et al., 2000; Dobbs & Scholly, 1986; Borja et al., 2003). In this survey, the dominant species were the mollusk *Ruditapes philippinarum*, the polychaetes *H. filiformis*, *C. erythraeensis*, and *A. arctica*, and *R. philippinarum* accounted for 37.6% of the total population.

Ruditapes philippinarum is mainly affected by the composition of sediments (Han et al., 2002). In four areas in Gyeonggi Bay, off the west coast of Korea, in the area with a high *R. philippinarum* density, the sediment grain size was 3.8 ± 0.1 Φ, which is similar to that in our study area. For Seonjaedo, Inchon, Kim (2005) reported that the mean grain size was 3.47 ± 0.45 Φ in the east and 3.60 ± 0.34 Φ in the west, and the TOC differed compared with Geunsoman, and was 2.72 ± 1.01 and 1.97 ± 0.57 for Seonjaedo and Geunsoman, respectively. *R. philippinarum* prefers sediment that is 50~80% sand (Icho & Osima, 1931), and the sediment in our study area was 54% sand, making it a good *R. philippinarum* habitat. The second most dominant species, the polychaete *H. filiformis*, is dominant in other areas, such as Asan Bay (Yu et al., 2011) and Gyeonggi Bay (Lim & Choi, 1998). Lim and Choi (1998) suggested that *H. filiformis* is potentially a pollutant indicator species that occurs in areas where organic pollution has progressed and *H. filiformis* should be monitored. Therefore, the survey area needs continuous monitoring in the future.

Analyzing the community schemes in the multidimensional scaling analysis, the populations have a linked structure instead of clearly divided clusters. The community structure was divided into two main groups, A and B. The SIMPER test indicated that the top five contributing species were *Bullacta exarata*, *Nippoleucon hinumensis*, *Eudorella hwanghaensis*, *Apocorophium acutum*, and *Venatomya truncata*. Specifically, *E. hwanghaensis* and *V. truncata* did not appear in group B. *B. exarata* made a large contribution to group A and very little to group B. Group A was subdivided into five groups, which seem to have been influenced by seasonal changes. In 2011, there was a large change in the cluster, while in 2013 and 2014, the cluster fluctuations were similar. Again, there were marked changes in the clusters in January and April, while the clusters were similar in July and October. Overall, except for Group B (April 2014), it was the same community, which means that community structure in the survey area did not change much from 2011 to 2014. This indicates that the population of the dominant species, *Ruditapes philippinarum*, did not change significantly in the survey area.

Somerfield *et al*. (2009) stated that the species diversity, feeding, and competition depend on geography and the history of the environment. In this study, the diversity had a similar pattern in terms of the evenness and richness indexes, as also shown in the correlation analysis. This was connected to *Ruditapes philippinarum*. Choi (2003) stated that the benthic groups at Gwangyang successfully reflect the degree of dominance and the regional distribution of the dominant species. In our study, the number of species decreased as the population of *R. philippinarum* increased. Higher diversity means more environmentally stable conditions, while if more *R. philippinarum* are present, the diversity is reduced, which could have an adverse ecological impact.

Chapter 4. General Discussion

4.1 Effects of environmental and biological factors on the recruitment and growth of *Ruditapes philippinarum*

Sediment conditions affect the feeding and physiology of *Ruditapes philippinarum* (Lee et al., 1999). According to Ryther and Tenore (1976), the equilibrium temperature for the balance between respiration and assimilation is near 13°C and the growth rate of *R. philippinarum* increases up to 25°C and decreases at higher temperatures (Liang et al. 1987). Our study sites had low water temperatures in winter. Calculations of the condition index of bivalves based on changes in size and weight can be used to estimate spawning periods; the index changes rapidly through the spawning period (Won and Hur, 1993). The condition index of *R. philippinarum* at Euihangri was highest in August, declining thereafter to ca. 30% of the greatest value by October. Clam gametes develop when individuals have shell lengths of 5–10 mm, and full maturity is reached at lengths of 15–20 mm. All clams that are >20 mm long are able to spawn (Holland and Chew, 1974). The condition index is highest during the peak of ripeness after the accumulation of resources in preparation for reproduction (Drummond et al., 2006). Condition declines thereafter throughout the spawning period (Park and Choi, 2004), as shown by the ca. 30% decrease in the condition index of *R. philippinarum* on Seonjaedo Island, off the west coast of Korea, through to the end of the spawning period (Uddin et al 2010). Based on the condition index of *R. philippinarum* at our sites, the estimated spawning period began after August at Euihangri and after June at Padori. Our counts of clam spats recruited to the sediment indicated that the population sizes at Euihangri and Padori increased after October and May, respectively (Figs. 4, 5).

The condition index can also be used to predict clam growth; higher clam condition indices are indicative of high growth rates (Kim 2005). The slope of the regression equation describing clam growth was also higher for the Euihangri population. In stable environments, the shells of this species are prolate in shape, while they are egg-shaped in unstable environments (Choi 1965). The shell shapes at Euihangri were indicative of better growth than the shell shapes at Padori. Our regression models showed that, for a given shell length (SL), the total weight (TW) at Euihangri exceeded that at Padori. Furthermore, the slope of the regression describing the relationship between TW and fresh weight (FW) was steeper at Euihangri than at Padori (Fig. 7, 8). The variation in FW is directly related to the nutrient input, and is a good indicator of the changes in gonadal growth and spawning over time when the energy balance is favorable (Pérez-Camacho et al. 2003; Delgado and Pérez-Camacho 2003). Our calculated regression slopes indicate that the growth rate was higher at Euihangri.

Spat settlement of *Ruditapes philippinarum* generally occurs where the mix of particle sizes is diverse and the degree of sediment sorting is high (Park et al. 2013b) and the degree of sediment sorting did not differ between our two sites. Nevertheless, spat abundance was higher at Padori, while recruitment was more successful at Euihangri, most likely because of reduced intra-specific competition for space in the low-density population. Our biomass correlation analyses indicated that the density of *R. philippinarum* was unrelated to the biomass of other macrobenthic species. Therefore, interspecific competition appears to be less important for the success of *R. philippinarum* recruitment than intraspecific competition. Regulation of the density of *R. philippinarum* populations and the food sources available in its habitat may optimize the environmental conditions for commercial production of this clam.

4.2 Macrobenthos community in *Ruditapes philippinarum* aquaculture

The proportions of polychaetes to the total number of species and total population were 44% and 55%, respectively. The respective values were 45% and 73% for the Gwangyang tidal flat (Choi, 2003), 48% and 67% for the Hallyeohaesang National Park area (Yoon, 2009), and 43% and 62% for the Gamak tidal flat (Koo et al., 2004). The survey area was dominated by *Ruditapes philippinarum*, but there were no differences in the numbers of species or numbers and proportions of polychaetes in the study areas. The correlation analysis also showed that *R. philippinarum* was not associated with other dominant species. The spaces used by *R. philippinarum* and other macrobenthos appear to differ, and competition for food and habitats is not severe.

The dominant species were the mollusk *Ruditapes philippinarum*, and the polychaetes *Heteromastus filiformis*, *Ceratonereis erythraeensis*, and *Ampharete arctica*, and *R. philippinarum* accounted for 37.6%. *R. philippinarum* is mainly affected by the composition of sediments (Han et al., 2002). Comparing the mean grain size of four areas in Gyeonggi Bay (Lim & Choi, 1998), off the west coast of Korea, the highest *R. philippinarum* density was associated with a grain size of 3.8 ± 0.1 Φ, which is similar to that in the study area. *R. philippinarum* prefers sediment that is 50~80% sand (Icho & Osima, 1931), and the sediment in our study area was 54% sand, making Geunsoman a good *R. philippinarum* habitat. The second most dominant species, polychaete *H. filiformis*, is dominant in other areas.

The community structure was divided into two main groups, A and B. Using the SIMPER test, five species made the biggest contributions: *Bullacta exarata*, *Nippoleucon hinumensis*, *Eudorella hwanghaensis*, *Apocorophium acutum*, and *Venatomya truncata*. Specifically, *E. hwanghaensis* and *V. truncata* did not occur in group B. *B. exarata* made a large contribution to group A and very little to group B. Group A was subdivided into five groups, which seem to have been influenced by seasonal changes. Overall, however, except for Group B (April 2014), it was the same community, which means that the community structure of the survey area did not change much from 2011 to 2014. This indicates that the population of the dominant species, *R. philippinarum*, did not change significantly in the survey area.

In this study, the diversity had a similar pattern in terms of the evenness and richness indexes, as also shown in the correlation analysis. This was connected to *Ruditapes philippinarum*. Choi (2003) stated that the benthic groups at Gwangyang successfully reflect the degree of dominance and the regional distribution of the dominant species. In our study, the number of species decreased as the *R. philippinarum* population increased. Higher diversity means more environmentally stable conditions, while if more *R. philippinarum* are present, the diversity is reduced, which could have an adverse ecological impact.

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