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Intersexuality of *Crassostrea gigas* and *Ruditapes philippinarum* in Southern Coastal Waters of Korea

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ABSTRACT

Objectives : The aim of this study was to verify the intersexuality of *Crassostrea gigas* and *Ruditapes philippinarum* in southern coastal waters of Korea.
Methods : Specimens of *Crassostrea gigas* (n=363) were collected from six areas of Tongyeong, Geoje and Yeosu. *Ruditapes philippinarum* (n=221) was collected from five areas of Yeosu. The sex ratio and intersexuality were determined after observing gonad preparations.
Results : The rate of intersexuality in *Crassostrea gigas* was 16.25%, with females (24.79%; n=30/121) exhibiting a higher rate than males (11.98%; n=29/242). The rate of intersexuality in *Ruditapes philippinarum* was 24.43%, with females (37.76%; n=37/98) exhibiting a higher rate than males (13.82%; n=17/123).
Conclusions : The results of this study suggest that intersexuality is induced by aquatic pollutants such as endocrine disrupting chemicals (EDCs).

Key words : *Crassostrea gigas*, Intersexuality, *Ruditapes philippinarum*

INTRODUCTION

There are four main steps in the risk assessment for environmental factors: 1) hazard identification, 2) dose-response assessment, 3) exposure assessment, and 4) risk characterization [1].

The biomarker used for risk assessment is a terminology which designates cells that can measure primary exogenous factors on organisms, or which designates object level's index such as in physiology, biochemistry or in its structure. Out of these physiological biomarkers, reproductive indexes are important items used to judge the long-term and continuous effect by pollutants [2].

Bivalves take in food via filter feeding. Their soft tissues accumulate pollutants easily but their ability to detoxify and release pollutants is low. Thus they are good indicators of the level of pollution in the sediment and of water quality [3].

Endocrine disrupting chemicals (EDCs) disturb the reproductive endocrine system and change the manifestation or function of sex in aquatic animals, as either androgenic or estrogenic effectors [4-7]. In bivalves such as *Mya arenaria* and *Dreissena polymorpha*, EDCs are responsible for reproductive inhibition, an

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imbalanced sex ratio and intersexuality [8,9]. Furthermore, the intersexuality found in bivalves such as *Scrobicularia plana* [10] and *Gomphina veneriformis* [11-13] can be used as a biomarker of reproductive disturbance due to the combined effects of EDCs or nonylphenol, organotin, and zinc.

This study reports the intersexuality of the gonochorism bivalves, *Crassostrea gigas* and *Rupiditapes philippinarum*, which was discovered during the process of investigating the ecological health status of southern coastal waters of Korea.

MATERIALS AND METHODS

The 363 specimens of *Crassostrea gigas* used in the analysis were collected from six areas of Tongyeong, Geoje, and Yeosu, while 221 specimens of *Rupiditapes philippinarum* were collected from wild conditions in five areas of Yeosu.

The samples were measured and dissected. The visceral part that included the gonads was extracted and fixed with aqueous Bouin's solution for 18 hours. Tissues were then embedded in paraffin wax blocks, frozen and prepared for sectioning. The embedded tissues were serially sectioned to 4-6 μm of thickness, mounted on slides and subjected to Mayer's hematoxylin and 0.5% eosin (H-E) double staining.

The sex ratio and intersexuality were determined after observing gonad preparations. Intersexuality, observed in 5-10 sections each of 1 cm^2 on average, was determined only when the opposite germ cell was observed; other sexual characters were not included.

RESULTS

1. *Crassostrea gigas*

The sex ratio (female : male) of *Crassostrea gigas* differed in each of the six areas, being 1 : 1 in the coastal area of Sadeungmyeon. In the coastal areas of Sanyangeub and Sinwoldong, males were more numerous than females, but in the other three areas the females were more numerous (Table 1).

In intersex ovaries, the opposite germ cells were observed either individually or in groups in the interfollicular space and inside the gametogenic follicle (Fig. 1A and C). Oocytes in the intersex testis were at the previtellogenic or initial vitellogenic stage. They were either scattered individually or in groups in the

Table 1. Specimen size and number for analysis and sex ratio of *Crassostrea gigas*

Sampling location		Sampling date	Number	Size (SH, mm)	Sex ratio (F : M)
Tongyeong	Sanyangeub	August 2009	185	37.1	1 : 6.10
	Dosanmyeon	June 2010	42	55.0	1 : 0.75
Geoje	Dundeokmyeon	June 2010	40	42.6	1 : 0.67
	Sadeungmyeon	June 2010	48	72.8	1 : 1
Yeosu	Sinwoldong	May 2010	23	47.5	1 : 1.30
	Janggundo	May 2010	25	43.0	1 : 0.92

Table 2. Specimen size and number for analysis and sex ratio of *Ruditapes philippinarum*

Sampling location		Sampling date	Number	Size (SL, mm)	Sex ratio (F : M)
Yeosu	Mogdo Hwayangmyeon	May 2010	44	27.8	1 : 1.10
	Samdo Hwayangmyeon	May 2010	42	28.8	1 : 1.63
	Imogri Hwayangmyeon	June 2010	43	34.6	1 : 1.53
	Songdo Myododong	June 2010	53	31.4	1 : 1.30
	Chosamdo Nammyeon	June 2010	39	27.7	1 : 0.86

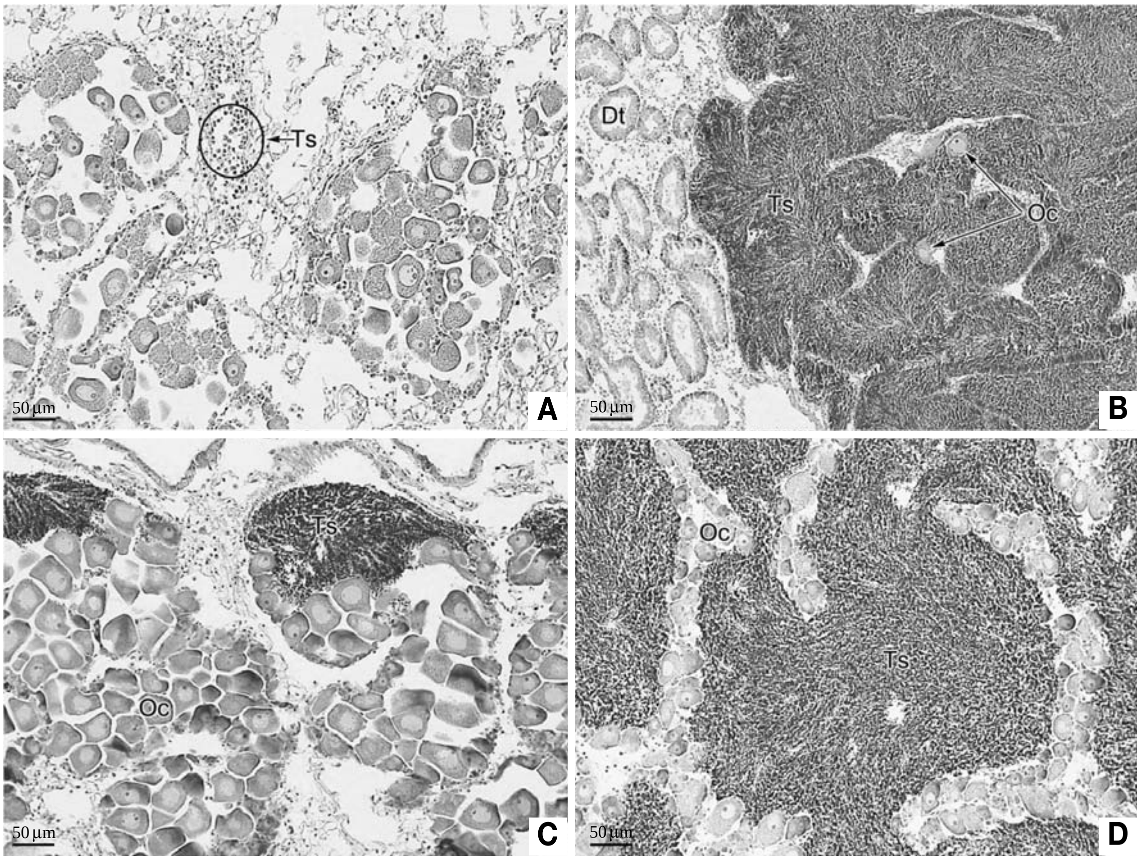

Fig. 1. Intersex gonad of *Crassostrea gigas*.
A and C: Female, B and D: Male. Dt: digestive tubule, Oc: oocyte, Ts: testicular tissue.

interfollicular space and inside the gametogenic follicle (Fig. 1B and D). However, germ cells of the opposite sex were not observed in the exterior connective tissue of the gonad. In the intersex female, degenerations of oocytes and digestive gland tubules were observed (Fig. 1B).

The rate of intersexuality in *Crassostrea gigas* was 16.25% (n=59/363): 24.79% (n=30/121) in females and 11.98% (n=29/242) in males. Out of the six areas, Janggundo had the highest rate of intersexuality at 44.0%, and Sanyangeub had the lowest at 5.95%. The rate of intersexuality in males was higher than in females in Dosanmyeon and Sadeungmyeon at 44.44% and 25.0%, respectively. Intersexuality in the other four areas was also more common in females, and was particularly high in Janggundo with a rate of 53.85% (Fig. 2).

2. *Ruditapes philippinarum*

The sex ratio (female : male) of *Ruditapes philippinarum* differed in all five areas. With the exception of Chosamdo, all had more males (Table 2).

The histological characteristics of intersexuality in *Ruditapes philippinarum* were similar to those of *Crassostrea gigas* (Fig. 3). Germ cells of the opposite sex were not observed in the exterior connective tissue of the gonad. In the intersex ovary, the opposite germ cells were observed either individually or in groups in the interfollicular space and inside the gametogenic follicle (Fig. 3A and C). In the intersex testis, the

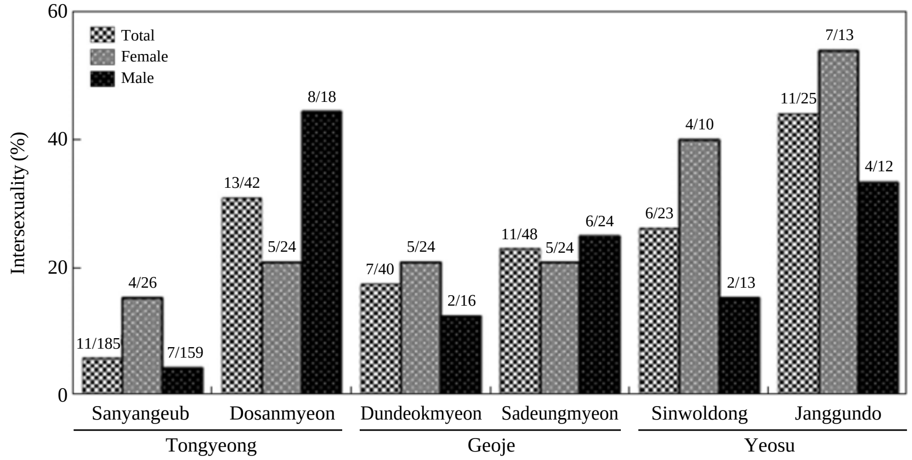

Fig. 2. Intersexuality of *Crassostrea gigas*.

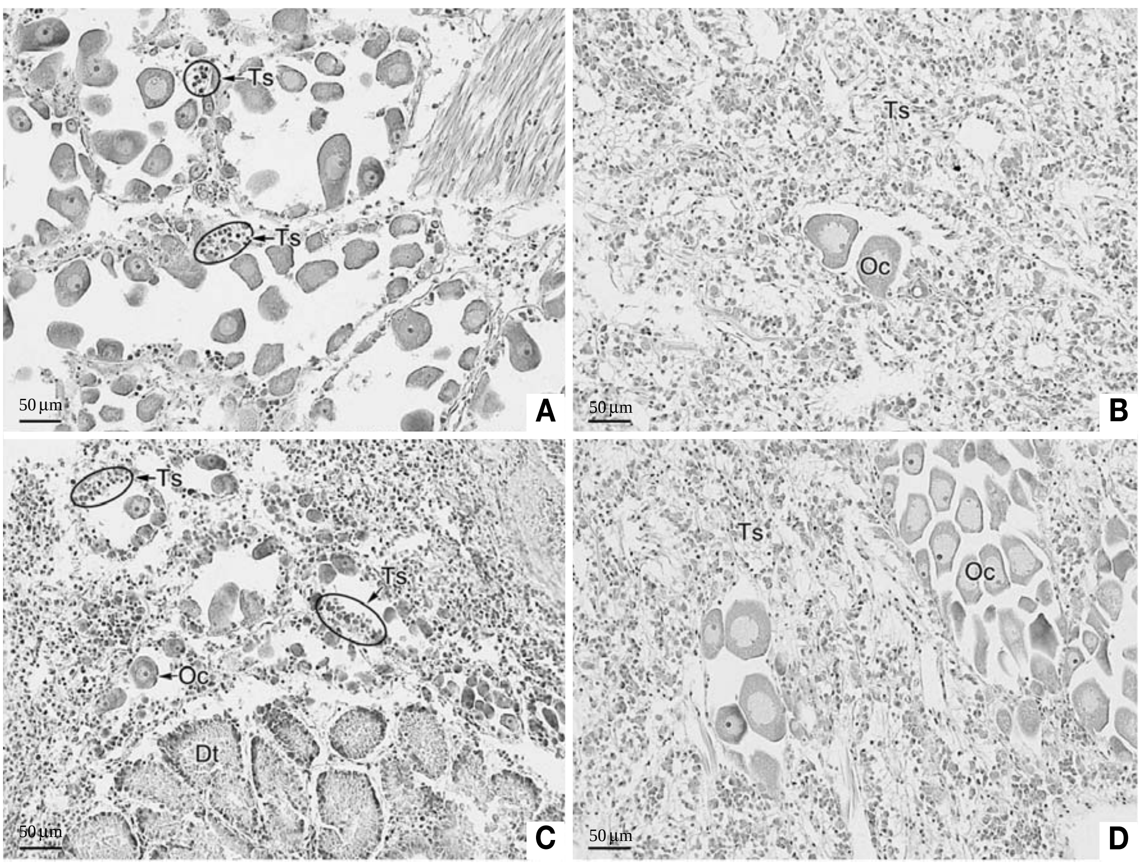

Fig. 3. Intersex gonad of *Ruditapes philippinarum*.
A and C: Female, B and D: Male. Dt: digestive tubule, Oc: oocyte, Ts: testicular tissue.

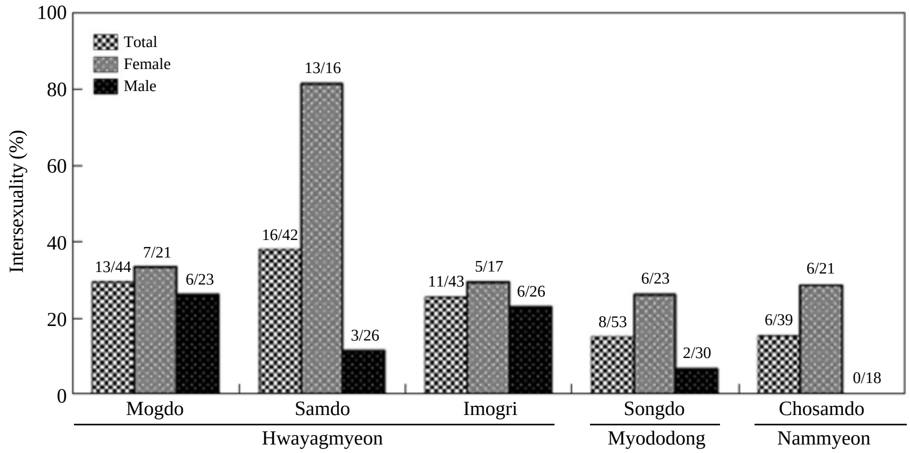

Fig. 4. Intersexuality of *Ruditapes philippinarum*.

oocytes were observed either individually in the connective tissue or in groups in the gametogenic follicle (Fig. 3B and D). In the intersex female of *Ruditapes philippinarum*, as in *Crassostrea gigas*, degenerations of oocytes and digestive gland tubules were observed (Fig. 3C).

The rate of intersexuality in *Ruditapes philippinarum* was 24.43% (n=54/221) and was higher than that of *Crassostrea gigas*. The rate of intersexuality in females was 37.76% (n=37/98), and in males it was 13.82% (n=17/123). Thus, as in *Crassostrea gigas*, intersexuality was more prevalent in females. The rate of intersexuality of *Ruditapes philippinarum* differed between areas, being highest in Samdo at 38.1%, and lowest in Chosamdo at 15.38%. The rate of intersexuality was higher in females than males in all five areas, the highest rate being 81.25% in Samdo. The rate of intersexuality in males was the highest in Mokdo at 26.09%, with no intersexuality in Chosamdo (Fig. 4).

DISCUSSION

In the aquatic ecosystem, the results of risk assessments are very important for the safety of organisms, and the maintenance and preservation of the ecosystem at the level of the individual or population.

Of the many biomarkers that react to environmental factors, the sex ratio, intersex, imposex, gonad development, histopathological markers, changes in sex hormones and others are used to understand their effects on reproductive biology. These are important biomarkers for understanding long-term environmental effects, and are well-utilized for monitoring ecological effects [2,14-18].

Among the various effects of pollutants on aquatic organisms, the physiological and reproductive changes induced by endocrine disrupting chemicals (EDCs) have been widely investigated [19].

EDCs have a reproductive effect on the sex ratio and gonad development of bivalves. It has been reported that the male of *Mya arenaria* makes up 63% of the population in regions of high organotin concentrations within estuaries of the Saint Lawrence River in Canada [20]. Nonylphenol, depending on its concentration, changes the sex ratio of *Gomphina veneriformis* and *Tapes philippinarum* [11,21]. Likewise, organotin and zinc increase the male ratio of *Gomphina veneriformis* [12,13].

The induction of imposex and intersex in fish and shellfish has also been attributed to EDCs. They have been reported to induce intersex in mosquitofish, *Gambusia affinis* [22], Japanese medeka, *Oryzias latipes* [23], *Cyprinus carpio* [24], *Rutilus rutilus* [17,25], *Scaphirhynchus albus* [26], *Gobio gobio* [27], and

Leiognathus nuchalis [28]. Organotin compounds induce imposex in gastropods such as the dog-whelk, *Nucella lapillus*, *Thais clavigera, T. bronniin, T. luteostoma*, and *Haliotis madaka* [29-32].

Like the above-mentioned reports, this study showed that the intersex of *Crassostrea gigas* and *Ruditapes philippinarum* is related to aquatic pollutants such as EDCs. However, this study was limited to the hazard identification stage, which is an environmental risk assessment stage suggested by the NRC [1]. In order to find out more about the pollutants and the causal relationship identified in this study, further research is needed.

Sadovy and Shapiro [33] classified the gonad structure of hermaphrodite fish as delimited or undelimited. In the delimited type the testicular tissue and ovarian tissue are divided by a connective membrane. In the undelimited type the testicular tissue and ovarian tissue are divided, but 1) the connective membrane does not exist and 2) the testicular tissue and ovarian tissue are mixed.

Chesman and Langston [10] reported the intersex of the ovotestis in the male *Scrobicularia plana* and classified this in five stages according to the number of oocytes and the level of occupation. According to Sadovy and Shapiro [33], the intersexuality of *Crassostrea gigas* and *Ruditapes philippinarum* is of the undelimited type, as the testicular tissue and ovarian tissues are mixed. There was a small amount of variation with area, but all five levels of intersex suggested by Chesman and Langston [10] were observed.

The effect that EDCs have on ecology varies according to the species, age, and lifestyle [34]. These chemicals are mostly lipid-soluble, which allows great persistence within the ecosystem, and thus medium levels of transportation and biological accumulation through the food chain. Even when the EDCs are at low concentrations, they can have a harmful influence on humans or other animals that are at higher levels of the food chain [35-37]. *Crassostrea gigas* and *Ruditapes philippinarum* play important roles in the food chain in the marine ecosystem. In addition, they are important biological resources for fisheries in Korea. From this perspective, management of the marine ecosystem and fishery resources in general is essential.

CONCLUSIONS

This study reports the intersex of *Crassostrea gigas* and *Ruditapes philippinarum* in southern coastal waters of Korea. Specimens of *Crassostrea gigas* were collected from six areas of Tongyeong, Geoje and Yeosu. *Ruditapes philippinarum* was collected from five areas of Yeosu. The rate of intersexuality in *Crassostrea gigas* was 16.25%, while females (24.79%; n=30/121) exhibited a higher rate than males (11.98%; n=29/242). The rate of intersexuality in *Ruditapes philippinarum* was 24.43%, and females (37.76%; n=37/98) again exhibited a higher rate than males (13.82%; n=17/123). The results of this study indicate that intersex is induced by aquatic pollutants such as endocrine disrupting chemicals (EDCs).

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