Animal Cells and Systems 13: 223-227, 2009

Animal Cells
and
Systems

http://www.AnimalCells.or.kr

Reproductive Disrupting Effect of Organotin Compound in the Ark Shell, *Scapharca broughtonii* (Bivalvia: Arcidae)

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Abstract: This study was carried out on the ark shell *Scapharca broughtonii* in order to examine if organotin compounds, which are known to induce reproductive abnormalities in gastropods, have the same affect on bivalves. The research was conducted during seven weeks in spring season through a field transplantation experiment in one reference area (Ra) and two organotin-polluted areas (Opa) near a shipyard complex. Sex ratio in the Ra was 1:1.6 (female:male). Sex ratio in Opa I and Opa II were 1:0.49 and 1:1.03, respectively, illustrating slightly higher proportion of females. Gonad activity exhibited a sequence of Opa II>Ra>Opa I. Intersex individuals of 3.33% (n=4/120) were confirmed in Opa. Intersex gonads were observed only in females. The results show that organotin compounds caused reproductive disruption in *Scapharca broughtonii*.

Key words: organotin compounds, *Scapharca broughtonii*, sex ratio, gonad activity, intersex

INTRODUCTION

Among various affects of pollutants on aquatic organisms, physiological and reproductive changes induced by endocrine disrupting chemicals (EDCs) have been widely investigated (Gibbs et al., 1990; Gray and Metcalfe, 1997; Quinn et al., 2004). Organotin compounds not only impart effects on reproduction of aquatic organisms but also affect the structure of tissues and cells by directly acting on each organ system (Gibbs and Bryan, 1986; Wester and Canton, 1987; Gibbs et al., 1990; Chin et al., 1999). In particular, organotin compounds induce imposex in gastropods, such as the dog-whelk *Nucella lapillus* (Gibbs et al., 1988). However, there are very few reports of similar reproductive changes in bivalves. Organotin compounds such as TBT (tributyltin) and TPT (triphenyltin), which are known to cause impediment in endocrine systems, are often used as insecticides, or in application as preservative for timber or anti-fouling materials (Maguire et al., 1986).

The aim of this study was to investigate the effects of organotin compounds on the reproductive biology of the ark shell *Scapharca broughtoni*. Ark shells, usually inhabiting silty sediments at a depth of 15-50 m, are abundant species in the southern coastal waters of Korea (Yoo, 1988).

MATERIALS AND METHODS

Specimens

In total, 120 individual ark shells, with shell length of approximately 7 cm (adult) were used. Microscopic analysis revealed that gonadal phase of the clams was early active stage I at the beginning of the experiment.

Transplantation experiment

This study was conducted in three locations within one reference area (Ra-Goheung: N 34°28.25', E 127°27.29'), and two organotin-polluted areas (Opa), namely Opa I (Jinhae: N 35°06.08', E 128°42.88') and Opa II (Jinhae: N 35°05.81', E 128°42.07') near a shipyard complex (Fig. 1). Specimens were transplanted in the two organotin-polluted areas over seven-weeks during spring season. A cage was installed to prevent the bivalves from escaping. The bottom of the cage was securely inserted into the sediment in order to allow the organisms to be able to burrow.

Analysis of organotin compounds

Sediment analysis: Chemical analysis of organotin (butyltin and phenyltin) compounds in sediment was conducted by the method described in MOMAF (2005). Homogenized samples were extracted with HCl and 1:1 EtOAc:ethanol, and then extracted to hexane. The extracts were derivatized

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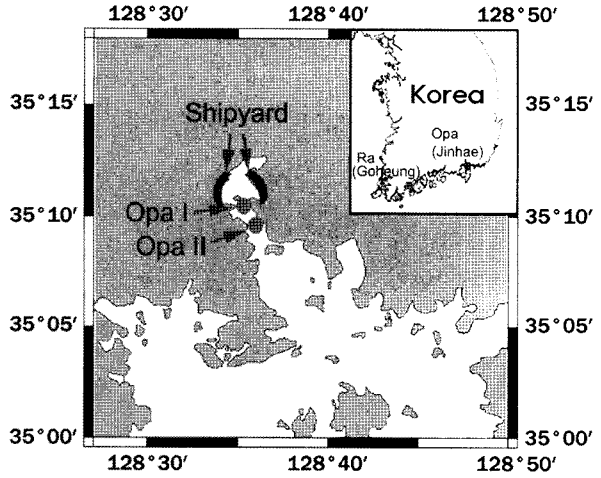

Fig. 1. Map of study area. Opa, organotin-polluted area; Ra, reference area.

with propylmagnesium bromide, cleaned by silica gel column chromatography and quantified by gas chromatography with mass spectrometric detection. The detection limit of the method was 0.01 ng/g, while certified reference material of NO. 12 (National Institute for Environmental Studies, Japan) and PACS-2 (National Research Council, Canada) were used for quality assurance and quality control.

Biota analysis: Chemical analysis of organotin (butyltin and phenyltin) compounds in soft tissues (whole body) of each specimen was conducted according to methods described in Horiguchi et al. (1994). Briefly, tissues were extracted with 0.1% tropolone/benzene and 1 N HBr/ethanol by ultrasonification, derivatized with propylmagnesium bromide, cleaned by silica gel column chromatography and quantified by gas chromatography with mass spectrometric detection. The detection limit of the method was 0.01 ng/g, and certified reference material of NIES CRM NO. 11 (National Institute for Environmental Studies, Japan) was used for quality assurance and quality control.

Histological analysis: Samples were fixed in aqueous Bouin's fluid on site and washed before it was dissected to segregate mantle, gill, foot and visceral mass. Sampled organs went through several stages of dehydration with alcohol and embedded onto paraplast (McCormick, USA). Embedded tissues were sectioned at 4-5 μm thickness using a microtome (RM2235, Leica, Germany). Tissues were stained with Mayer's hematoxylin-0.5% eosin (H-E).

Gonadal maturity was divided into five phases: an inactive stage (Ia), early active stages I (Ea I), II (Ea II), III (Ea III) and a growing stage (G) by observing the gonad preparations (Table 1). The gonad index (GI) of each individual was computed by a modified Eversole's method (1997).

Statistical analysis: Data obtained were statistically tested with a Student t-test on averages using SPSS statistical program (version 12) and significance ($P<0.05$) was examined with least-significant difference test as means of multiple comparison (Tukey test).

RESULTS

Concentration of organotin compounds

Sediment: Concentration of organotin compounds at the beginning of transplantation was 12.87 ng/g in the sediment of the reference area (Ra), while those in the organotin-polluted area I (Opa I) and Opa II were 381.90 ng/g and 120.77 ng/g, respectively (Table 2).

Body tissue: Concentration of organotin compounds in soft tissues (whole body) of the ark shell from Ra at the beginning of the experiment was 4.46 ng/g, while those of Opa I and Opa II at the end-point of the experiment were 184.92 ng/g and 145.86 ng/g, respectively (Table 3).

Sex ratio: At the completion of the experiment, sex ratio in the reference area was 1:1.6 (female:male) illustrating larger proportion of males, while those of Opa I and Opa II were 1:0.49 and 1:1.03, respectively, illustrating slightly higher proportion of females (Fig. 2).

Table 1. Histological criteria for scoring of gonadal development phase of the ark shell, *Scapharca broughtonii*

Gonad phase	Score	Sex	Histological features
Inactive (Ia)	1	Non-sexualization	Non detected gametogenic follicles or beginning of gametogenic follicles appearance
Early active I (Ea I)	2	Non-sexualization	Expansion of gametogenic follicles and single layered arrangement of non-sexualized germ cells
Early active II (Ea II)	3	Female	Basophilic oocytes of egg diameter about 15 μm
		Male	Multi-layered arrangement of spermatogonia and spermatocytes
Early active III (Ea III)	4	Female	Eosinophilic oocytes of egg diameter 20~30 μm
		Male	Multi-layered arrangement of spermatocytes
Growing (G)	5	Female	Eosinophilic oocytes of egg diameter above 30 μm
		Male	Multi-layered arrangement of spermatids

Table 2. Organotin concentration in sediment of the study areas

Concentration (ng/g WW) /Station		Study area		
		Ra	Opa I	Opa II
Sediment	TBT	10.65	363.53	116.40
	ΣBT	12.87	381.90	120.77

*Opa, organotin-polluted area; Ra, reference area.

Table 3. Organotin concentration in body tissue of the ark shell *Scapharca broughtonii*

Concentration (ng/g WW) /Station		Beginning	End-point		
			Ra	Opa I	Opa II
Body tissue	TBT	3.39	3.79	159.52	125.95
	ΣBT	4.46	10.88	184.92	145.86

*Opa, organotin-polluted area; Ra, reference area.

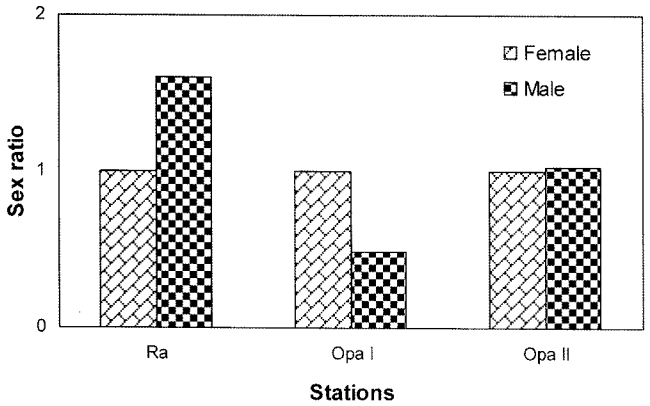

Fig. 2. Sex ratio of the ark shell, *Scapharca broughtonii*. Opa, organotin-polluted area; Ra, reference area.

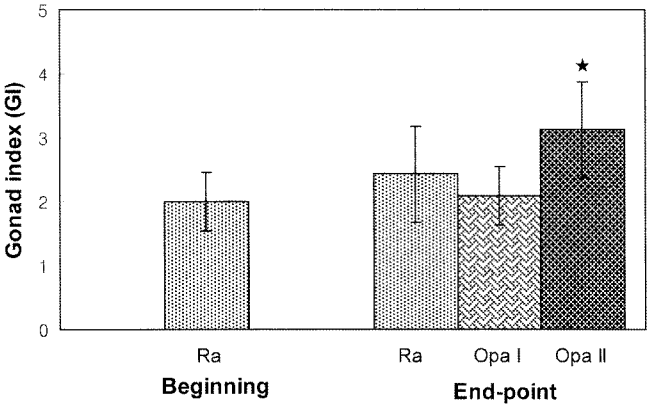

Fig. 3. Gonad index (GI) of the ark shell, *Scapharca broughtonii*. Opa, organotin-polluted area; Ra, reference area. (★) Significant differences (P<0.05) from reference area.

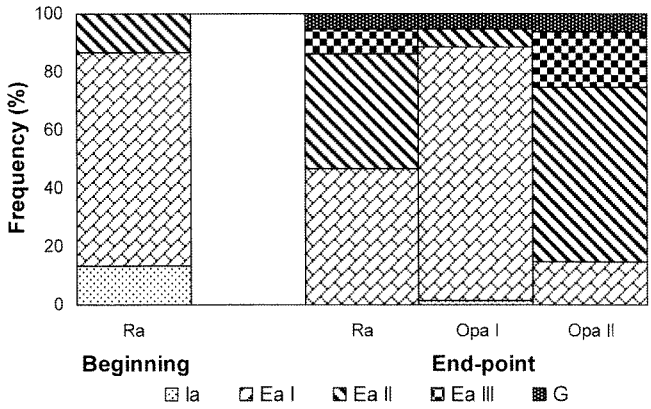

Fig. 4. Gonadal development after transplantation experiment. Ea, early active stage; G, growing stage; Ia, inactive stage; Opa, organotin-polluted area; Ra, reference area.

Gonadal activity: Gonad index (GI) showed slight differences depending on the study area, exhibiting the following sequence; Opa II>Ra>Opa I. GI in the initial stage of the experiment was 2 for Ra, while at the end-point, the Opa II was significantly increased from Ra (Fig. 3).

In relation to the developmental stage of gonads during the initial stages of the experiment, early active stage I was most dominant with 73.3%. At the end-point of the experiment, early active stage I and II accounted for 46.6% and 39.7% of specimens in Ra, respectively. In Opa I, the early active stage I gonads was dominant with 86.7%, while in Opa II, early active stage II and III gonads accounted for 60% and 19% of specimens, respectively (Fig. 4).

Intersexuality: Intersex individuals were not observed at Ra. However, 4 individuals (n=4/120, 3.33%) were confirmed in Opa, among which 3 from Opa I (n=3/61, 4.92%) and 1 from Opa II (n=1/59, 1.69%). Intersexuality was observed only in females (Fig. 5).

DISCUSSION

Biological indicators such as survival rates, growth, respiration, reproduction, histopathology, hormone metabolism and biochemical change are often used in risk assessment of aquatic pollutants. Among these, sex ratio, gonadal activity and intersexuality are being used as indicator to assess the effects of pollution on reproductive characteristics (Bortone and Davis, 1994; Munkittrick and Van Der Kraak, 2000; Lee and Park, 2007).

EDCs, including TBT impart influence on sex hormone metabolism and sex ratio of aquatic animals. Tributyltin, by acting on the androgen metabolism of *Ruditapes decussata*, intervenes with their masculinization (Morcillo et al., 1998). Furthermore, they inhibit changes in sex hormone and gonad development in the soft-shell clam, *Mya arenaria* (Siah et al., 2003).

It has been reported that the male species of *Mya arenaria* accounts for 63% in regions of high organotin compound

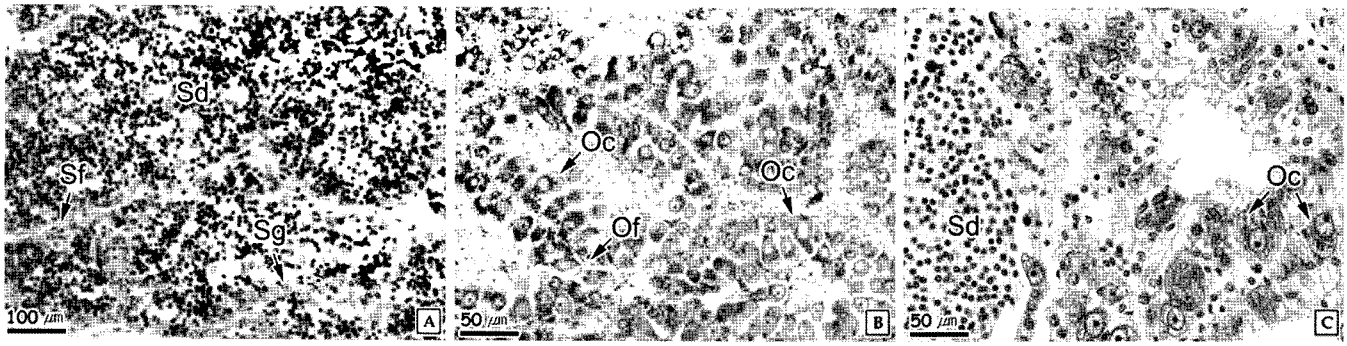

Fig. 5. Photomicrographs on gonad of the ark shell *Scapharca broughtonii*. A, growing testis, showing the spermatids (Sd) within the spermatogenic follicle (Sf). B, early growing ovary, showing the numerous oocyte (Oc) attaching the oogenic follicle (Of). C, intersex at organotin-polluted area. Sg, spermatogonium.

concentrations within intertidal zones of estuaries in Canada (Gagné et al., 2003). In the case of *Tapes philippinarum,* sex ratio (female:male) of females was high at 1:0.77, 0.64, and 0.66 in nonylphenol exposure concentrations of 0.025, 0.05 and 0.1 mg/L respectively, while the ratio of male was higher at 1:1.66 in concentration of 0.2 mg/L (Matozzo and Marin, 2005). The sex ratio of *Gomphina veneriformis* was similar at 1:1 and 1:1.03 in both wild condition and indoor control groups, respectively. However, after exposing these clams to nonylphenol for 24 weeks, a bigger ratio of males were observed. In particular, sex ratio in the highest concentration group, of 5.0 μg/L, was 1:3.50 (female : male) (Lee and Park, 2007).

In this study, a higher proportion of males in the reference area (Ra) was observed, while organotin-polluted areas I and II (Opa I and II) contained higher proportion of females.

Organotin compounds also affect reproductive cycle and development stages in terms of gonad maturity. Horiguchi et al., (2000) reported that GI differences in *Haliotis madaka* became very large as a result of exposure to organotin compound, for 7 months. Inhibition of gonad development in zebra mussel, *Dreissena polymorpha* has also been attributed to organotin compounds (Regoli et al., 2001).

In this study, gonad development was more inhibited in ark shells from Opa I in comparison to specimens from Ra. However, gonad development was more active in ark shells from Opa II in comparison to those from Ra. These results may indicated that high concentration of organotin compounds inhibit gonad development, while low concentration of organotin compounds, in contrast, promote gonad development.

The induction of intersexuality has also been attributed to EDCs. They have been reported to induce intersexuality in mosquitofish, *Gambusia affinis* (Drysdale and Bortone, 1989) and testis-ova in the Japanese medaka, *Oryzias latipes* (Gray and Metcalfe, 1997). Intersex individuals constituted 23.6% of a sample population in *Gomphina veneriformis,* which was exposed to nonylphenol for 24 weeks (Lee and Park, 2007).

Organotin compounds induce imposex in dog-whelk, *Nucella lapillus* (Gibbs et al., 1988), *Thais clavigera*, *T. bronniin* (Horiguchi et al., 1994), *T. luteostoma* (Kahng et al., 1996), and *Haliotis madaka* (Horiguchi et al., 2000).

Although intersex individuals were not observed in Ra, 4 individuals (n=4/120, 3.33%) were histologically confirmed in Opa, among which, 3 came from Opa I for which the concentration of organotin compound was the highest at 381.40 ng/g. Organotin compounds have been reported as an EDCs with the strongest androgenic effects in molluscs (Gibbs and Bryan, 1986; Gibbs et al., 1988, 1990; Horiguchi et al., 1994, 2000). In relation to intersexuality, organotin compounds displayed similar effect in this study. However, analysis of sex ratio revealed the effects to be more estrogenic based. As a result, it was difficult to conclude whether organotin compounds have androgenic or estrogenic effects on the reproduction of *Scapharca broughtonii.*

REFERENCES

Bortone SA and Davis WP (1994) Fish intersexuality as indicator of environmental stress: Monitoring fish reproductive systems can serve to alert humans to potential harm. *BioScience* 44: 165-172.

Chin P, Lee JA, Shin YK, and Lee JS (1999) Environmental tolerance for pollutants in *Littorina brevicula* (Philippi). 2. The growth, metabolism and histological changes exposed to TBTCl and heavy metals in *Littorina brevicula. J Korean Fish Soc* 32: 593-600.

Drysdale DT and Bortone SA (1989) Laboratory induction of inter-sexuality in the mosquitofish, *Gambusia affinis*, using paper mill effluent. *Bull Environ Contam Toxicol* 43: 611-617.

Eversole AG (1997) Gametogenesis of *Mercenaria mercenaria, M. campechiensis* and their hybrids. *Nautilus* 110: 107-110.

Gagné F, Blaise C, Pellerin J, Pelletier E, Douville M, Gauthier-Clerc S, and Viglino L (2003) Sex alteration in soft-shell

clams (*Mya arenaria*) in an intertidal zone of the Saint Lawrence River (Quebec, Canada). *Comp Biochem Physiol C* 134: 189-198.

Gibbs PE and Bryan GW (1986) Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *J Mar Biol Assoc UK* 66: 767-777.

Gibbs PE, Bryan GW, Pascoe PL, and Burt GR (1990) Reproductive abnormalities in female *Ocenebra erinacea* (Gastropoda) resulting from tributylin-induced imposex. *J Mar Biol Assoc UK* 70: 639-656.

Gibbs PE, Pascoe PL, and Burt GR (1988) Sex change in the female dog-whelk *Nucella lapillus* induced by tributyltin from antifouling paints. *J Mar Biol Assoc UK* 68: 715-731.

Gray MA and Metcalfe CD (1997) Induction of testis-ova in Japanese medaka *Oryzias latipes* exposed to *p*-nonylphenol. *Environ Toxicol Chem* 16: 1082-1086.

Horiguchi T, Shiraishi H, Shimizu M, and Morita M (1994) Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan. *J Mar Biol Assoc UK* 74: 651-669.

Horiguchi T, Takiguchi N, Cho HS, Kojima M, Kaya M, Shiraishi H, Morita M, Hirose H, and Shimizu M (2000) Ovo-testis and disturbed reproductive cycle in the giant abalone *Haliotis madaka*: possible linkage with organotin contamination in a site of population decline. *Mar Environ Res* 50: 223-229.

Kahng SH, Je JE, Oh JR, Shim WJ, and Shim JH (1996) Imposex of *Thais clavigera* and *T. luteostoma* (Muricidae) as an evidence of organotin pollution in Chinhae Bay. *Korean J Malacol* 12: 123-131.

Lee JS and Park JJ (2007) Risk assessment of nonylphenol using the sex ratio, sexual maturation, intersex and lipofuscin accumulation of the equilateral venus, *Gomphina veneriformis* (Bivalvia: Veneridae). *J Korean Fish Soc* 40: 16-23.

Maguire RJ, Tkacz RJ, Chau YK, Bengert GA, and Wong PTS (1986) Occurrence of organotin compounds in water and sediment in Canada. *Chemosphere* 15: 253-274.

Matozzo V and Marin MG (2005) Can 4-nonylphenol induce vitellogenin-like proteins in the clam *Tapes philippinarum* ? *Environ Res* 97: 43-49.

MOMAF (2005) Marine environment experimental progress manuals. Ministry of Maritime Affairs and Fisheries of Korea, Seoul, pp 127-130.

Morcillo Y, Ronis MJJ, and Porte C (1998) Effects of tributyltin on the phase I testosterone metabolism and steroid titers of the clam *Ruditapes decussata*. *Aquat Toxicol* 42: 1-13.

Munkittrick KR and Van Der Kraak G (2000) Appropriate uses of physiological techniques for endocrine studies. In: Henshel DS, Black MC, and Harrass MC (eds), Environmental toxicology and risk assessment: Standardization of biomarkers for endocrine disruption and environmental assessment, 8th Vol, ASTM, West Conshohocken, pp 95-118.

Quinn B, Gagné F, Costello M, McKenzie C, Wilson J, and Mothersill C (2004) The endocrine disrupting effect of municipal effluent on the zebra mussel (*Dreissena polymorpha*). *Aquat Toxicol* 66: 297-292.

Regoli L, Chan HM, de Lafontaine Y, and Mikaelian I (2001) Organotins in zebra mussels *Dreissena polymorpha* and sediments of the Quebec City Harbour area of the St. Lawrence River. *Aquat Toxicol* 53: 115-126.

Siah A, Pellerin J, Amiard J-C, Pelletier E, and Viglino L (2003) Delayed gametogenesis and progesterone levels in soft-shell clams (*Mya arenaria*) in relation to in situ contamination to organotins and heavy metals in the St. Lawrence River (Canada). *Comp Biochem Physiol C* 135: 145-156.

Wester PW and Canton JH (1987) Histopathological study of *Poecilia reticulata* (guppy) after long-term exposure to bis(tri-*n*-butyltin)oxide (TBTO) and di-*n*-butyltinchloride (DBTC). *Aquat Toxicol* 10: 143-165.

Yoo JS (1988) Korean shells in colour. Iljisa Publighing Co, Seoul, pp 105-166.

[Received April 1, 2009; accepted May 28, 2009]