Effects of environmental endocrine disruptors on the sex differentiation in Korean rockfish, *Sebastes schlegeli*


* Department of Aquaculture, Yosu National University, Chonnam 550-749, Korea (E-mail: wklee@yosu.ac.kr; lk2262@hanmail.net; kwakej@yosu.ac.kr; wklee@yosu.ac.kr)
** Yeonggwang Fisheries Technology Institute, Chonnam 513-800, Korea (E-mail: yang0219@hanmail.net)
*** College of Medicine, Seonam University, Chonbook 590-711, Korea (E-mail: pjcoli@tiger.seonam.ac.kr; ecoju@tiger.seonam.ac.kr)
**** Department of Civil and Environmental Engineering, Yosu National University, Chonnam 550-749, Korea (E-mail: wjlee@yosu.ac.kr; wooble@yosu.ac.kr)

**Abstract** The effects of estradiol-17β, methyltestosterone, tributyltin, bisphenol A and 2,4-dinitrophenol on sex differentiation were investigated in the Korean rockfish, *Sebastes schlegeli*. The 51 day-old fry with undifferentiated gonads were fed with food containing the chemicals at 0.05, 0.5, 5, 50 and 100 µg/diet for 29 days. Sex ratio and gonadal abnormality after the chemical treatment were determined by histological examination of gonads. In control group, the sex ratio of fry was 1:1.3 (female:male), and more males than females appeared. The sex of fry fed with estradiol-17β at 5, 50 and 100 µg/diet was all females. Feminization of the fry was induced by exogenic estradiol-17β. In the methyltestosterone group, sex ratio of fry was 1:10.2 in 0.05 µg/diet, 1:2.4 in 0.5 µg/diet, 1:3.1 in 5 µg/diet, 1:1.2 in 50 µg/diet and 1:2.3 in 100 µg/diet. Feminization of the fry was induced by methyltestosterone at the lowest concentration. Furthermore, intersex of the fry was observed. The number of intersex fry was 23.3, 25.0 and 35.3 at 0.5, 5 and 50 µg/diet of methyltestosterone, respectively. Sex ratio of bisphenol A group showed no difference with that of the control. The sex of fry fed with 2,4-dinitrophenol at 5, 50 and 100 µg/diet was all females. 2,4-dinitrophenol should be considered as a new member of endocrine disruptors. In the tributyltin group, the sex ratio of fry at 50 µg/diet of tributyltin was 5.4:1. Tributyltin induced the feminization of the fry at the highest dose. Tributyltin showed an estrogenic effect in the Korean rockfish in contrast to an androgenic effect in gastropods.

**Keywords** Bisphenol A; 2,4-dinitrophenol; endocrine disruptor; fish; sex differentiation; tributyltin

**Introduction** During the last decade, there has been growing concern about environmental chemicals, that disturb normal endocrine functions in animals, thereby causing reproductive disorders and abnormalities in wildlife (Colborn and Clement, 1992). These chemicals are defined as environmental endocrine disruptors. Many of these endocrine disruptors have estrogenic activity and can disrupt normal functions of sex steroids (Colborn et al., 1993). Feminization of animals derived from areas polluted by estrogenic chemicals has been observed in nearly all classes of vertebrates including fish (Munkittrick et al., 1991), reptiles (Guillette and Crain, 1996), birds (Fry and Toone, 1981), and mammals. Some endocrine disruptors have androgenic activity and can mimic the action of androgens. Imposer of gastropods derived from the coastal areas polluted by tributyltin has been reported in many countries (Tan, 1997; Curtis and Kinley, 1998). The disturbance of wildlife by endocrine disruptors has mainly occurred in aquatic ecosystems, because sewage including many synthetic chemicals is released into the river and the coastal area.

Contamination of coastal environments is of particular concern because seafood is one of the major sources of diet in Korea. However, the pollution of coastal water and sediment...
is a growing problem in Korea as in other countries. The environmental endocrine disruptors such as bisphenol A, tributyltin and polyaromatic hydrocarbons have been detected in water and sediment, and high incidence of imposex of the rock shell, *Thia clavigera*, in several coastal areas (Kang, 1995). The control of pollution in the coastal area is very important for fisheries and aquaculture because the coastal area serves as spawning, juvenile growth and cultivation places of marine organisms. The management of coastal environmental conditions is essential for the sustainable production of fisheries and aquaculture.

It is well known that the sex differentiation of fish like that of other vertebrates is under the control of sex steroids (Nakamura *et al*., 1998). The undifferentiated gonad is developed into ovary by estrogens or testis by androgens during ontogenesis. The high incidence of sex change in fish with exogenous sex steroids during early development has been known for many decades. Generally, feminization of the male is induced by estrogen and its derivates are administered orally or via the water to fish at the sexually undifferentiated stage through the period of sexual differentiation, and masculinization of the female is induced with the treatment of androgen and its derivates. Furthermore, the sex reversal of fish can be induced by the treatment of environmental endocrine disruptors. The sex reversal of fish is considered a good biomarker like vitellogenine for the assay of environmental endocrine disruptors. The Korean rockfish, *Sebastes schlegeli*, inhabit the rocky bottom in the coastal area of Korea, and is one of the two major species in Korean mariculture. For the development of an endocrine disruptor assay system in marine fish, the effects of bisphenol A, 2,4-dinitrophenol and tributyltin on sex differentiation were investigated in the Korean rockfish, *Sebastes schlegeli*.

**Materials and methods**

The fry of the Korean rockfish, *Sebastes schlegeli*, used in the present investigation, were obtained from 3 year-old broodstock that was cultured in floating net cages in the south coast of Yosu, Chonnam in Korea. They were reared in the aquaria (1 ton) at 19–22°C during experiments. The rearing water was artificially filtered, aerated and exchanged every day.

The 51 day-old fry with undifferentiated gonads were divided into six groups including one control and five chemical treatment groups. The control group was fed with the diet containing only ethanol. The chemical treatment group were fed with the food containing estradiol-17β, methyltestosterone, bisphenol A, 2,4-dinitrophenol or tributyltin at the dose of 0.05, 0.5, 5, 50 and 100 µg/diet for 29 days. The number of fry was 70 individuals per test and the experiment was done in triplicate. The chemicals were dissolved in 95% ethanol, added to powdered fish diet at adequate dosage levels, dried at room temperature and stored in a refrigerator until used. The fry were fed with 2.52 g of the diets containing the chemicals per day. At the end of cultivation, the sex ratio and gonadal development of fry were investigated by histological methods. The gonad were fixed in Bouin’s fluid, dehydrated in alcohol and embedded in paraffin or Technovit resin. Serial paraffin sections were cut transversely at 5 µm in thickness, and stained with Mayer’s hematoxylin and counterstained with eosin. Technovit resin was cut transversely at 2 µm in thickness, and stained with 1% toluidine blue. The histological morphology of gonad was examined under the light microscope.

Data were analyzed statistically using the Student’s *t*-test. Statistical significance was inferred at *P* < 0.05.

**Results and discussion**

The sex of fry in the Korean rockfish can be identified by the morphology of gonads. The ovary in the female showed ovarian cavity in the lateral side of its gonad, and the testis in
the male showed the rudiment of sperm duct in the center of its gonad (Figure 1a and 1b).

Table 1 showed the sex ratio and the gonadal development of fry with treatment of the various exogenic chemicals for 29 days. In the control group, there was no abnormality in morphology of the differentiated gonads (ovary and testis), and the sex ratio of fry was 1:1.3 (female:male). More males than females appeared in the control. In estradiol-17β group, the sex ratio of fry was 1:1.1 in 0.05 μg/g diet and 2.7:1 in 0.5 μg/g diet. However, the sex of fry fed with estradiol-17β at 5, 50, 100 μg/g diet was all females (P<0.05). Feminization of the Korean rockfish was induced by exogenic estradiol-17β, and was more sensitive than that of other fish, where ovarian development of genetic males was induced by 50 μg/g diet of estradiol-17β (Nakamura and Tanakshi, 1973).

In the methyltestosterone group, the sex ratio of fry was 1:10.2 in 0.05 μg/g diet, 1:2.4 in 0.5 μg/g diet, 1:3.1 in 5 μg/g diet, 1:1.2 in 50 μg/g diet and 1:2.3 in 100 μg/g diet. Masculinization of the fry was induced by the methyltestosterone at the lowest concentration (P<0.05). Furthermore, intersex of the fry was observed. The number of intersex fry was 23.3, 25.0 and 35.3 at 0.5, 5 and 50 μg/g diet of methyltestosterone, respectively. Some intersex fry showed the lateral growing of somatic tissue that is evidence of ovarian development in differentiated testis (Figure 1c). The others showed the ovarian cavity and sperm duct in the same gonad (Figure 1d). The exogenic methyltestosterone was changed to 17α–methylestradiol by the aromatase (Piferrer and Dornaldson, 1991; Piferrer et al., 1993), which could be a reason why the number of intersex fish increased with higher doses of methyltestosterone in the Korean rockfish.

Bisphenol A is a chemical used in the production of many valuable products including epoxy resins, polycarbonate and is well known as an endocrine disruptor. The estrogenic activity of bisphenol A has been identified over the past several decades (Smeets et al., 1993), which could be a reason why the number of intersex fish increased with higher doses of methyltestosterone in the Korean rockfish.

Bisphenol A is a chemical used in the production of many valuable products including epoxy resins, polycarbonate and is well known as an endocrine disruptor. The estrogenic activity of bisphenol A has been identified over the past several decades (Smeets et al., 1993), which could be a reason why the number of intersex fish increased with higher doses of methyltestosterone in the Korean rockfish.

**Table 1** Effects of chemicals on sex differentiation of *Sebastes schlegeli*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Number of individuals</th>
<th>Total length (cm)</th>
<th>Number of individuals</th>
<th>Sex ratio (female:male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>0.05</td>
<td>4.68±0.09</td>
<td>51.00±6.98</td>
<td>2.0:1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.68±0.11</td>
<td>51.00±6.98</td>
<td>2.0:1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.68±0.11</td>
<td>70.00±0.00</td>
<td>0 :1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.68±0.11</td>
<td>70.00±0.00</td>
<td>0 :1</td>
</tr>
<tr>
<td>Methyltestosterone</td>
<td>0.05</td>
<td>4.49±0.59</td>
<td>6.33±1.33</td>
<td>61.00±0.00</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0.05</td>
<td>4.51±0.18</td>
<td>5.21±0.21</td>
<td>1.3:1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.51±0.18</td>
<td>5.21±0.21</td>
<td>1.3:1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.51±0.18</td>
<td>7.00±0.00</td>
<td>0 :1</td>
</tr>
<tr>
<td>2,4-dinitrophenol</td>
<td>0.05</td>
<td>4.48±0.54</td>
<td>6.33±1.33</td>
<td>37.33±5.00</td>
</tr>
<tr>
<td>Tributyltin</td>
<td>0.05</td>
<td>4.17±0.13</td>
<td>50.00±6.00</td>
<td>25.00±6.00</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.17±0.13</td>
<td>50.00±6.00</td>
<td>25.00±6.00</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.17±0.13</td>
<td>70.00±0.00</td>
<td>0 :1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.59±0.53</td>
<td>70.00±0.00</td>
<td>0 :1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
0.05 µ/g diet, 1:1.1 in 0.5 µ/g diet, 1.3:1 in 5 µ/g diet, 1:1.3 in 50 µ/g diet and 1:1 in 100 µ/g diet. There was no significant difference compared with control (P>0.05). The estrogenic effect of bisphenol A on sex differentiation in the Korean rockfish did not appear.

2,4-dinitrophenol is used in the manufacture of dyes and wood preservatives, and acts as an uncoupler between the electron transport system and oxidative phosphorylation in the mitochondria. The effects of 2,4-dinitrophenol on development were fetal growth inhibition in animal studies. In the Korean rockfish, the sex ratio of fry was 1:2 in 0.05 µ/g diet and 2.7:1 in 0.5 µ/g diet of 2,4-dinitrophenol. However, the sex of fry fed with 2,4-dinitrophenol at 5, 50 and 100 µ/g diet was all females (P<0.05). Generally, a number of compounds with endocrine disrupting properties have significantly lower potencies than estradiol-17β, and showed 1/50 to 1/10,000 activity of estradiol-17β (Arnold et al., 1996). However, 2,4-dinitrophenol was similar to estradiol-17β in the induction of feminization, and showed strong estrogenic activity. Therefore, 2,4-dinitrophenol should be considered as a new member of endocrine disruptors.

Tributyltin is used as one of the components in antifouling paints on ships, boats and offshore installations, as a fungicide in agriculture and a protectant against microbial decomposition (textiles, dispersion paints, PVC and other plastics). The androgenic activity of tributyltin as an endocrine disruptor has been identified by the induction of imposex in

Figure 1 Photographs of the differentiated gonad in Sebastes schlegeli. The bar is 50 µm. (a) Aspect of a normal ovary. This is characterized by the presence of ovarian cavity (oc) in the lateral side of the ovary. (b) Aspect of a normal testis. This is characterized by the presence of sperm duct (sd) in the middle of the testis. (c) Aspect of an intersex gonad. This is characterized by the presence of the sperm duct and the growing somatic tissue in the same gonad. (d) Aspect of a further progressed intersex gonad. This is characterized by the completion of the ovarian cavity formation.
many gastropods (Horiguchi et al., 1997). However, the action of tributyltin as an androgenic endocrine disruptor is not clear in other animals. In the tributyltin group in the Korean rockfish, the sex ratio of fry was 1.8:1 in 0.05 µg/g diet, 1.1:1 in 0.5 µg/g diet, 2:1 in 5 µg/g diet and 1.3:1 in 50 µg/g diet. There was no significant difference compared with the control ($P>0.05$). However, the sex ratio of fry at 100 µg/g diet of tributyltin was 5.4:1 (female:male, $P<0.05$). Tributyltin induced the feminization of the fry at the highest dose. In marine gastropods, imposex can be induced at concentration of only 1 ng/L of tributyltin, which is far below what causes larval abnormalities (Gibbs et al., 1987). Tributyltin is thought to be an aromatase inhibitor in gastropods. The inhibition of aromatase with tributyltin leads to an imbalance in steroid hormone titers in gastropods, which consequently result in the formation of male sexual characteristics in female specimens (Oehlmann et al., 1993; Stroben, 1994; Bettin et al., 1996). Tributyltin showed an estrogenic effect in the Korean rockfish in contrast to an androgenic effect in gastropods. Further study of the difference of the tributyltin effects between the fish and the gastropods is necessary. Imposex induction of fish with treatment of an aromatase inhibitor was reported in salmonid (Piferrer et al., 1994). More studies on the action of tributyltin as an aromatase inhibitor is necessary in more studies in fish.

**Conclusion**

The sex differentiation of rockfish was very sensitive to chemical treatment. Furthermore, it was very effective in detecting a chemical whether it is an endocrine disruptor or not. We showed that the 2,4-dinitrophenol could be a new member of endocrine disruptors. The sex differentiation of the fish, like the vitellogenine detection system, can be applied for the assay of environmental endocrine disruptors.

**Acknowledgements**

This work was supported by the research program of Jeonnam Regional Environment Technology Development Center.

**References**


