The Chinese rare minnow (*Gobiocypris rarus*) as an in vivo model for endocrine disruption in freshwater teleosts: a full life-cycle test with diethylstilbestrol

Xueping Zhong, Ying Xu*, Yong Liang, Tao Liao, Jianwei Wang

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

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**Abstract**

Chinese rare minnow (*Gobiocypris rarus*), a freshwater teleost, was exposed to diethylstilbestrol (DES) at 0.05, 0.5, 1 and 5 μg/L from fertilized eggs for up to mature period under flow-through condition. Several endpoints that related to development, reproductive fitness and transgenerational effects were evaluated. It was found that body length and body weight were significantly reduced and vitellogenin (Vtg) levels were significantly increased for fish exposed to DES. Histological examination showed that the sex ratios of F0 fish skewed to female and about 2% of the fish exposed to 0.05 μg/L DES developed testes–ova. The reproductive success, as determined from data on egg production, was reduced in female fish exposed to 0.05, 0.5, 1 and 5 μg/L DES. The lowest-observed-effect concentrations (LOEC) for changes of sex ratios, reproductive success and histology alteration of F0 are 0.05 μg/L. In the offspring, transgenerational effects on egg hatching rate, egg fertilization and Vtg levels of juvenile individuals were not observed. However, survival of F1 generation fry significantly declined. The analysis of sex steroid levels revealed a significant decrease of testosterone (T) in the whole body homogenates (WBH) of male progeny and somewhat elevation of estradiol (E2) in the WBH of female offspring. These findings indicate that exposure to DES causes a variety of developmental, reproductive and transgenerational effects.

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**Keywords:** Diethylstilbestrol; Endocrine-disrupting chemicals; Life cycle; Reproductive success; Transgenerational effect

1. Introduction

In recent years, global concern has been raised about natural steroid hormones and other hormone mimics present in the environment. Many of these compounds have been shown to modulate endocrine response, alter sexual development, or reduce reproductive success in fish at environmental concentrations much lower than those causing acute or chronic toxicity (Gimeno et al., 1996; Jobling et al., 1998; Gronen et al., 1999). Thus, in vivo test methods, which are based on definitive indicators and could show a direct...
cause-and-effect relationship for endpoints of ecological concern, are needed. Because of this background, many organizations have involved in toxicological risk assessment, such as the Organization for Economic Cooperation and Development (OECD, 2001; Paris, France), and developed test methods to identify the chemicals possessing hormonal activity in aquatic environment. Among the methods, the fish full life-cycle test (FFLC), in which fish are exposed to the test chemicals throughout their whole lives including their next generation, has been proposed as a confirmatory test because endocrine-disrupting chemicals (EDCs) can profoundly disturb the early development period (especially during sexual differentiation) and the reproductive stage (Koge et al., 2000; Devlin and Nagahama, 2002). Moreover, this test is considered useful because a concern exists that exposure of parental fish to EDCs may have adverse effects on the next generation.

Medaka (Oryzias latipes), zebrafish (Danio rerio) and fathead minnow (Pimephales promelas) have been recommended and used for the FFLC test. Länge et al. (2001) conducted an FFLC for ethynylestradiol (EE2) with fathead minnow, based on the standard protocol supplemented with vitellogenin (Vtg) analysis, and histology of liver, kidney, and gonad. Yokota et al. (2001) and Seki et al. (2003) detected the effects of 4-nonylphenol and 4-tert-pentylphenol on productive potential of medaka and indicate the FFLC test with medaka is applicable to evaluation of weak estrogens. However, to date, no in vivo screening tests that are relevant to exposure of Chinese native fish to EDCs have been identified and validated. Since many potential EDCs are likely to be released directly into ecosystem in China, a need exits to develop in vivo screening tests for EDCs using Chinese native species.

Rare minnow, Gobiocypris rarus, is a Chinese freshwater cyprinid (Re and Fu, 1983). It has many attractive features that make it a suitable organism in aquatic toxicity tests. These advantages include sensitivity to chemicals, small size (adult 2–8 cm), wide temperature range (0–35 °C), easily being cultured in laboratory, large amount of eggs (average 266 eggs per hatch and continuous batch spawner), short duration of embryonic development (72h at 26 °C) and short life cycle (about 4 months) (Wang, 1999). Meanwhile, it has been approved to be sensitive to heavy metals and xenosterogens (Zhou et al., 1995; Lu and Shen, 2002). So, in the present study, a full life cycle experiment was performed with rare minnow from a few hours post-fertilization to maturation.

The aim of this study was to investigate the responses of rare minnow to diethylstilbestrol (DES) in the development and reproduction. DES is a synthetic non-steroid estrogen. It has been used as an estrogen supplement before its carcinogenic effects was recognized (Smith and Smith, 1948; Herbst et al., 1971). DES is chosen as the test compound for this study because it has been widely used as a model compound and its effects on reproduction of human and animals have been well characterized (Giusti et al., 1995; Martin et al., 2002). An understanding of the responses of rare minnow to DES will help us to determine whether rare minnow is suitable for fish full life cycle test in assessing the environmental impact of EDCs.

2. Materials and methods

2.1. Experiment animals

The Chinese rare minnow (Gobiocypris rarus) were raised in 24 L flow-through chambers with dechlorinated tap water. Fish were maintained in a light/dark cycle of 14:10 h at 23–26 °C and fed once a day. To start the FFLC test with DES, embryos <24 h post-fertilization were collected from at least three spawnings as required by standard protocol. Batches of embryos were examined, pooled, and randomly allocated to each exposure group. The process was repeated until each group contained 100 embryos.

2.2. Test chemicals

DES (>97% purity) was purchased from Sigma. The stock solution (100mg/L) was prepared by dissolving in ethanol. It was further diluted in ethanol and the dilutions were stored at 4 °C. These dilutions were used to prepare the renewal test solutions in the aquaria.

2.3. Exposure design

A laboratory-constructed flow-through system was employed. Each unit of the system was composed of three chambers. Two separate chambers with a volume of 24L were used for raising fish. One chamber with a volume of 50L, in which there were peristaltic pump
and magnetic stirrers, was used for mixing and cycling of the test solution. DES test solution was pumped from the cycling chamber and injected into the raising chamber in an appropriate flow rate through two spouts. The raising chamber was equipped with overflow tubes to maintain test chamber volumes. The redundant test solution in the raising chamber flew back the cycling chamber. So the DES test solution was flowing and cycling in the system. The exposure system was run for several hours prior to introduction of test organism in order to saturate all surfaces with the test substance. Nominal concentrations of DES at 0.05, 0.5, 1 and 5 \( \mu \text{g/L} \) were selected for FFLC study based on a 28 days preliminary exposure study. The control received dechlorinated tap water added with carrier solvent. The photoperiod was a light/dark cycle of 14:10 h. Water temperature was controlled in the range from 23 to 26 \(^\circ\)C. During the experiment, residual food and faeces in the test chamber were removed every day and 80% of the test volume was renewed once in 2 days. The test equipment and chambers were cleaned once a week.

2.4. Biological protocols

2.4.1. Embryological phase

Exposure was initiated at <24 h after fertilization. The 300 embryos used for each treatment were randomly separated into three groups. In each group, the embryos were placed in a cylindrical glass cup containing about 1 L test solution and the test solution was renewed every day. The developing embryos were observed daily and the dead embryos were discarded once discovered. This procedure was repeated until all the living embryos hatched.

2.4.2. Larval-juvenile phase

After hatching, the larvae were fed with adequate amount of Artemia nauplii (<24 h after hatching) twice a day. The mortality, abnormal behavior and appearance of the larvae were recorded until 30 days post-hatch. At 30 days post-hatch, fish were transferred to the continuous-flowing system and fed with Limnodrilus spp. At 64 days post-hatch, fish were sampled to measure body weight, body length and then stored at \(-80\) \(^\circ\)C for Vtg analysis and total thyroxine (TTv) measurement. At 179 days post-hatch, 20 fish were sacrificed. Gonads were removed and fixed with Bouin’s solution for histological observation.

2.4.3. Reproductive phase

Six mating pairs were selected from control group at 200 days post-hatch for examination of fecundity and fertility. No male from 0.05, 0.5, 1 and 5 \( \mu \text{g/L} \) treatment groups could be obtained because sex ratio completely skewed to female. So, six females from these groups were transferred to clean dilution water and paired with non-exposed males. Each pair was assigned to a test chamber. The water temperature was controlled in the range between 25 and 28 \(^\circ\)C to stimulate spawning. The eggs spawned from each female were counted and assessed for viability over 7 consecutive days. At the end of reproductive phase, fish remained were sacrificed and measured body weight and body length.

2.4.4. F1 embryo phase

The eggs spawned from the females during the first 7 days were kept in clean dilution water and subjected to the study of F1 generation. To evaluate their hatchability and hatching time, the fertilized eggs collected from each group were placed in a glass cup under the same condition as those for F0 generation.

2.4.5. F1 larval-juvenile phase

The newly hatched larval in each treatment group and the control group were randomly separated into four test chambers, respectively and kept in the chambers until 30 days post-hatch. The mortality, abnormal behavior, and appearance of the F1 larval were observed and recorded. At 30 days post-hatch, the larval were randomly separated into four groups of 30 and transferred into the continuous-flow system described previously for the F0 generation. At 118 days post-hatch, the fish were sampled from each group for Vtg, TTv and total triiodothyronine (TT3) measurements. At 182 days after hatch, four females and four males were sampled for sex steroids measurement. At the end of the experiment, fish remaining were weighed and gonads were removed for gonadal somatic index (GSI) calculation.

2.5. Vtg analysis

Fish were individually homogenized in ice-cold phosphate-buffered saline (PBS) (pH 7.3) with a 1:2 ratio of wet mass: buffer volume in a glass homogenizer. The homogenate was then centrifuged at 15,000 \( \times \) g for 15 min at 4 \(^\circ\)C, and the supernatant was withdrawn and immediately frozen at \(-80\) \(^\circ\)C.
Whole body homogenate samples were assayed for Vtg using a semi-quantitative heterologous carp enzyme-linked immunosorbant assay (ELISA), which has been shown to be suitable for determination of Vtg concentration in the rare minnow (Zhong et al., 2004).

Carp Vtg was purified from the plasma of a mature female using a procedure described by Liang et al. (2002). Details of the preparation on polyclonal antibody against carp Vtg are described elsewhere (Zhong et al., 2004).

2.6. Histological analysis

Fish sampled at 179 days post-hatch were sacrificed by MS-222 anaesthetic. The gonads of treated and untreated F0 fish were removed and fixed in Bouin’s solution for analyzing abnormalities. After washing with 50% ethanol, samples were dehydrated, embedded in paraffin, serially sectioned (7 μm) transversely, and stained with hematoxylin and eosin. Sections of gonads not available for histological examination seldom spanned more than 35 μm and never more than 84 μm. All sections of gonads were examined by light microscopy.

2.7. Thyroid hormone assays

Fish were homogenized in 3 mL ice-cold PBS (pH 7.3). The whole body homogenate were then centrifuged at 13,000 × g for 15 min at 4 °C, and the supernatant was withdrawn and immediately frozen at −80 °C. The TT3 in the tissue and serum were measured by radioimmunoassay (RIA) using commercially available 125I kits (Institute of Shanghai Atomic Nuclear, Chinese Academy of Science) according to the method of Gharib (1970). The TT4 levels were measured by chemiluminescence immunoassay (CLIA) according to the method described by Christofides and Sheehan (1995).

2.8. Steroid hormone radioimmunoassay (RIA)

The concentration levels of 17β-estradiol (E2) and testosterone (T) in the whole body homogenate of rare minnow were measured by the RIA method described by Abraham et al. (1974).

2.9. Statistical analysis

Values are expressed as mean ± S.D. One way analysis of variance (ANOVA) followed by Student’s t-test was used to determine the effects of DES on fish exposed to DES and its offspring. P-values below 0.05 were set as significant.

3. Results

3.1. F0 generation

3.1.1. Hatchability of F0 embryos and survival of F0 larvae

In the experiment, hatching success of the control embryos was 84.67 ± 8.69%. It ranged from 75.82 to 86.35% in the DES exposure groups. There was no evidence of a dose response in hatching success versus DES exposure. Statistical analysis indicated that none of the exposure concentrations were significantly different from controls. At 30 days post-hatch, survival of F0 larvae was all >80%, except in the highest DES concentration of 5 μg/L where it was 51%.

3.1.2. Growth of F0 juvenile fish and F0 adult

The data for growth of F0 juvenile are summarized in Table 1. Statistical analysis of the body length, body weight and TT4 level at 64 days post-hatch indicated a significant reduction in growth at 5 μg/L DES exposure. The body length and body weight data were also analyzed for females in the control and DES exposure groups at 200 days post-hatch since males were absent in 0.5, 1 and 5 μg/L DES-treated groups. For females, Table 1

Table 1: Effects of DES exposure on growth of F0 generation at 64 days post-hatch

<table>
<thead>
<tr>
<th>Test concentration (μg/L)</th>
<th>Endpoints</th>
<th>Body length (mm)</th>
<th>Body weight (mg)</th>
<th>TT4 (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.11 ± 2.83</td>
<td>84.67 ± 30.31</td>
<td>54.35 ± 12.30</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>20.39 ± 2.91</td>
<td>40.00 ± 37.34</td>
<td>49.25 ± 16.38</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>18.89 ± 3.78</td>
<td>73.57 ± 45.44</td>
<td>66.20 ± 25.51</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.10 ± 3.43</td>
<td>73.69 ± 44.39</td>
<td>58.50 ± 26.14</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12.95 ± 8.35</td>
<td>21.50 ± 8.08</td>
<td>266.70 ± 15.50</td>
<td></td>
</tr>
</tbody>
</table>

n=8–20, * P < 0.05.
there was a significant reduction in body weight and body length in 0.5, 1 and 5 μg/L groups compared to the controls (\(P < 0.05\)) (Table 2).

3.1.3. Gross morphological changes in F₀ fish

Fish from the 1 and 5 μg/L exposure groups exhibited physical deformities. The most prominent findings were malformed spine in the early developmental stage and pericardial edema in the adult stage. Many fish exposed to 5 μg/L also showed hemorrhaging. Fish from exposure groups (≥0.05 μg/L) began to show sexual differentiation after 179 days post-hatch, in that fish were observed to be female or immature individuals and no males were seen in the high concentration exposure groups (0.5, 1, 5 μg/L). Consequently, the initiation of the spawning phase of the study had been conducted with the wild males.

3.1.4. Sex ratios of F₀ generation

Due to the complete masculization of males in 0.5, 1 and 5 μg/L DES exposure groups, wild male fish were supplemented to mate with the females exposed to DES for assessing the reproductive effects of DES exposure. The results of the fecundity of F₀ generation are shown in Fig. 2. In fish exposed to DES for up to 200 days post-hatch, there were significant reductions in spawning per female daily (with six individual breading pairs) in 0.5 and 5 μg/L DES-treated groups and in the number of eggs per hatch in 5 μg/L DES-treated group. However, fertilization rate and hatching rate of eggs in the treatments was not significantly different from that of control. In the experiment, hatching success of the control embryos was 78.58 ± 9.29%. It ranged from 67.34 to 87.89% in the DES exposure groups. Therefore, the

**Table 2.** Effects of DES on growth of F₀ generation at 200 days post-hatch and sex ratio as determined by gonadal histology

<table>
<thead>
<tr>
<th>Test concentration (μg/L)</th>
<th>Female</th>
<th>Male</th>
<th>Percentage of fish with</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body length (cm)</td>
<td>Body weight (g)</td>
<td>Body length (cm)</td>
<td>Body weight (g)</td>
<td>Testis</td>
<td>Ovary</td>
<td>Testis–ova</td>
</tr>
<tr>
<td>Control</td>
<td>5.13 ± 0.57</td>
<td>1.71 ± 0.82</td>
<td>4.78 ± 0.34</td>
<td>1.17 ± 0.85</td>
<td>44</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>0.05</td>
<td>4.61 ± 0.35</td>
<td>2.31 ± 0.34</td>
<td>4.25 ± 0.34</td>
<td>1.1 ± 0.32</td>
<td>11</td>
<td>87</td>
<td>2</td>
</tr>
<tr>
<td>0.5</td>
<td>4.11 ± 0.41</td>
<td>1.01 ± 0.38</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4.7 ± 0.54</td>
<td>1.16 ± 0.34</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3.89 ± 0.52</td>
<td>1.16 ± 0.34</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

\(n=32-56.\)

* \(P<0.05.\)

Fig. 1. Representative hematoxylin and eosin-stained gonad section of rare minnow at 179 days post-hatch, ×100. (up) Ovary of control female; (down) testis of a rare minnow exposed to 50 ng/L showing testis–ova, arrows indicate oocytes within the testicular tissue.
Fig. 2. Reproduction by rare minnow females exposed to DES from 200 to 336 days post-hatch (n = 6). (a) Egg numbers per batch in each treatment; (b) egg numbers per female per breeding day in each treatment.

LOEC values for F0 generation reproduction at 200 days post-hatch were considered as 0.05 μg/L.

3.1.6. Vtg concentration in the whole body homogenate of F0 juvenile rare minnow

At 64 days post-hatch, Vtg concentrations in the whole body homogenates of rare minnow exposed to DES increased significantly and the Vtg levels were almost the same as that of the females in the reproductive phase (16,718 μg/g fish; Fig. 3).

3.2. F1 generation

3.2.1. Hatchability of F1 embryos

The hatching rate of control fish was 83.0 ± 10.1% (Table 3). For fish exposed to DES, hatching rates were 54.45 ± 31.42% at 50 ng/L, 67.45 ± 17.01% at 0.5 μg/L, 70.82 ± 19.75% at 1 μg/L, 56.85 ± 17.49% at 5 μg/L. These exposure groups had no statistically significance from the controls.

3.2.2. Survival of F1 fish

Survival of control fish was 91.59 ± 5.8% at 30 days post-hatch (Table 3). For the fish exposed to 50 ng/L DES, survival was 83.27 ± 17.19% and was not statistically significant different from the control. However, for the fish exposed to 0.5, 1 and 5 μg/L

Table 3

<table>
<thead>
<tr>
<th>Test concentration (μg/L)</th>
<th>Endpoints</th>
<th>Hatchability (%)</th>
<th>Fertility (%)</th>
<th>Survival (%)</th>
<th>Deformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.01 ± 10.3</td>
<td>78.38 ± 9.29</td>
<td>91.59 ± 5.8</td>
<td>1.33 ± 2.34</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>54.45 ± 31.42</td>
<td>67.34 ± 7.0</td>
<td>83.72 ± 17.19</td>
<td>10.17 ± 10.7</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>67.45 ± 15.07</td>
<td>87.89 ± 4.82</td>
<td>51.59 ± 14.73</td>
<td>18.53 ± 4.12</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70.82 ± 19.75</td>
<td>71.01 ± 16.70</td>
<td>42.44 ± 21.86</td>
<td>28.67 ± 39.27</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>56.85 ± 17.49</td>
<td>83.93 ± 12.97</td>
<td>64.00 ± 15.00*</td>
<td>15.66 ± 11.53</td>
<td></td>
</tr>
</tbody>
</table>

n = 700–1000.

* P < 0.05.
DES survivals were significantly reduced. Survival was 51.59 ± 14.73% at 0.5 µg/L, 42.44 ± 21.86% at 1 µg/L and 64 ± 16.06% at 5 µg/L.

3.2.3. Growth of F₁ fish

The growth of F₁ fish was evaluated at 118, 182 and 246 days post-hatch. At 118 days post-hatch, F₁ juvenile fish increased significantly in body length and body weight at 1 and 5 µg/L when compared with control (P < 0.05). Results of RIA measurement showed that TT₃ level had no significant difference between the control and treatment groups and TT₄ level was significantly reduced in the group exposed to 5 µg/L (Fig. 4). At 182 days post-hatch, all differences on growth were not statistically significant. At 246 days post-hatch, the growth of adult fish was significantly reduced in the middle concentrations (Fig. 5).

3.2.4. GSI and adult sex ratio of F₁ fish

At 246 days post-hatch, significant reduction was observed in the GSI of adult male, but not in female (Fig. 6). No significant change on sex ratio was found in all DES treated groups compared to the controls (Fig. 7).

3.2.5. Vtg concentration in the whole body homogenate of offspring

At 118 days post-hatch, offspring of control and the fish exposed to DES did not differ significantly from...
each other with regard to their whole body homogenate Vtg levels (Fig. 8).

3.2.6. Sex steroid levels

In the whole body homogenates of male offspring of fish exposed to DES, the analysis of T concentration revealed a significant decrease compared to control values. E2 levels also showed decrease (Fig. 9). In the whole body homogenates of female progeny of DES-exposed fish, E2 levels were, although not significantly, elevated compared to control fish. T levels remained unchanged (Fig. 9).

4. Discussion

The present study was carried out to assess the developmental and reproductive impairment of rare minnow by DES and to investigate whether maternal DES exposure could exert adverse effect to progeny generation. The results showed that DES exerted numerous estrogenic and non-estrogenic effects on each life stage of rare minnow and its progeny. It suggests that rare minnow were sensitive to DES and rare minnow possessed several advantages for use in research on EDCs.

In the F0 generation, body length and body weight has been reduced significantly for fish under continuous exposure to DES. The reduction of growth, induced by an exposure to xenobiotics, has been reported in a number of fish. Länge et al. (2001) has demonstrated effects on body length and body weight in juvenile fathead minnow exposed to 4, 16, 64 ng/L EE2 for 56 days and in female adults exposed to 1 ng/L EE2 for 301 days. Belt et al. (2003) has also shown a reduced growth in zebrafish (Danio rerio) exposed to EE2 concentrations ranging from 1 to 25 ng/L for three months. The reduction of body weight and body length may be due to the effects of xenoestrogens on Vtg synthesis. Vitellogenesis has been demonstrated to compromise growth in fish by diverting energy stores as well as producing a burden of protein for the kidneys (Herman and
Kincaid, 1988). In the present study, the change of TT4 and the retardation of maturation are also confirmations of growth or development defects.

Xenoestrogen has been also reported to affect the sexual differentiation of fish. Gimeno et al. (1988) exposed genetically male common carp to 4-tert-pentylphenol and then observed the formation of oviducts in the gonads. Hermaphroditic gonads (testis–ova) in fathead minnow are induced by treatment with EE2 (Aerle et al., 2002). Yokota et al. found the sex ratio of medaka skewed toward female after exposure to 51.5 μg/L 4-nonylphenol for 60 days (Yokota et al., 2001). In the present study, the result has shown that 2% test–ova existed in the 0.05 μg/L group and sex ratios skewed completely to female at higher concentrations. Although the mechanism of the development of test–ova in rare minnow with estrogen is unknown, the elevated Vtg level and completely skewed sex ratios of F0 fish suggested that the abnormal development in the gonads were induced by the estrogenic activity of DES.

Fecundity has been considered to be a key parameter affecting the stabilization of fish populations. The impacts of xenoestrogen on the reproductive health of fish populations have become a major concern. So, the evaluation of the reproductive effects of xenoestrogen has been an important goal in the FFLC study with fish (Lange et al., 2001). In recent years, many studies have found that the female reproductive success of fish has been impaired after exposed to EDCs. Lange et al. (2001) demonstrated that there was a general trend toward a decrease in spawning in fathead minnow after EE2 long-term exposure at 0.2 and 1 ng/L. Scholtz and Gutzeit (2000) found that the egg number laid by female Medaka decreased significantly after exposed to EE2 from hatch to the age of 2 months. Similar effects have also been reported in female sheepshead minnow exposed to 200 ng/L EE2 for up to 59 days (Zillionix et al., 2001). In the present study, a reduced egg production has been also observed in the 0.5 and 5 μg/L DES treated females after breeding with non-exposed males. The number of eggs per batch in each treatment has been also found to decrease although only 5 μg/L DES treatment is significantly different from control. This indicates that DES exposure impair the female reproductive success of rare minnow. In this study, the egg number produced by females exposed to DES at 1 μg/L has also been found to decrease, but not significant. This is probably due to the large variation in egg number produced by different individual pairs of rare minnow. However, the number of abnormal fry and the percentage of survival fry of F1 generation af-
The impact of maternal DES exposure on progeny has also been evaluated. It has been found that concentrations of T and E2 in WBH of male offspring has also been significantly reduced and that E2 levels in WBH of female offspring have been somewhat elevated. The imbalance of sex steroid level in F0 generation and/or their progeny, induced by xenoestrogens, has been reported in many other fish. Ankely et al. (2001) found that both methoxychlor and methyltestosterone exposure reduced plasma concentration of T and KT in male fathead minnow and reduced T and E2 in female fathead minnow. Schweiger et al. (2002) reported that nonylphenol exposure caused plasma E2 and T increased by 2- and 13-fold in male and female offspring of rainbow trout. Since it has been shown that the homeostasis between E2 and T/KT concentrations could affect phenotypic sex characteristics, brain and behavior differentiation, and development of reproductive organs, the hormonal imbalances detected in the offspring of exposed fish indicate a transgenerational effect mediated by the endocrine disruption (Hunter and Donaldson, 1983).

In this study, the gonadal examination of the offspring in DES-exposed and control fish has not given any significant differences between exposed and non-exposed groups with respect to the sex ratios. This is in agreement with the findings of Schweiger et al. (2002), who found no significant change on sex ratio in the offspring of rainbow trout exposed to 1 and 10 μg/L nonylphenol. However, reduced GSI and reduced growth have been detected in the male offspring. This also indicates the effects on the progeny of DES-exposed fish. Meanwhile, our data for an altered hormone levels in males are also a confirmation of growth and development defects in male offspring.

In conclusion, not only growth, sexual differentiation and reproductive success of F0 generation have been affected after continuous exposure to DES, but also growth, sex steroid level and GSI of its offspring have been influenced. These results indicate that exposure to xenoestrogens causes a broad variety of developmental, reproductive and transgenerational effects.

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