Plasma vitellogenin levels in male common carp *Cyprinus carpio* and crucian carp *Carassius cuvieri* of Lake Kasumigaura

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ABSTRACT: In order to investigate the possible influence of estrogenic environmental endocrine disruptors on the reproductive activity of fish in Lake Kasumigaura, plasma levels of vitellogenin (VTG), a biomarker of estrogen exposure, were measured in wild and cultured male common carp *Cyprinus carpio* and wild crucian carp *Carassius cuvieri*. Testicular histology and plasma steroid hormone levels were also examined. Fish were collected from June 1998 to August 1999. Plasma VTG levels in most fish examined were below a detection limit (40 ng/mL) throughout the sampling period, and a small amount of VTG (43.5 ng/mL–1680 ng/mL) compared to that in females was detected in some fish. Active spermatogenesis in the testis and increased levels of plasma sex steroids were observed in most of the fish examined. Thus, no marked influence of estrogenic chemicals was detected in the reproductive activity of male common carp and crucian carp in Lake Kasumigaura collected from the sampling sites in the present investigation.

KEY WORDS: *Carassius cuvieri*, carp, *Cyprinus carpio*, endocrine disruptors, estradiol, 11-ketotestosterone, testosterone, vitellogenin.

INTRODUCTION

It is recently considered that some chemicals in the environment have the potential to adversely affect endocrine systems of humans and wild animals.1,2 These chemicals are called endocrine disruptors and include plasticizers, detergents, pesticides and herbicides. Among these chemicals, many of them are shown to have estrogenic effects and presumed to influence the reproductive activity of humans and wild animals by disrupting endocrine control of reproduction. The sources of these synthetic chemicals are primarily discharges from industry, effluent from sewage-treatment works and agricultural run-off. Besides these synthetic estrogenic chemicals, it is reported that natural estrogens from human and domestic animals and phytoestrogens from pulp mill effluent are released into the environment.3–5 As these synthetic and natural estrogenic chemicals eventually enter the aquatic environment, concerns have been raised that these chemicals may have adverse effects on sex differentiation and reproductive activity of aquatic animals, including fish.

There are some reports on fish showing examples of abnormalities that are presumably induced by estrogenic effects. Male roach *Rutilus rutilus* that had oocytes in the testis were found in rivers in the United Kingdom, and the incidence of intersexuality was higher in rivers that received more sewage-treatment works effluent than in rivers receiving less effluent.6 Plasma vitellogenin (VTG), a precursor protein of egg yolk, was also detected in these male fish. Elevated levels of VTG were observed in male common carp *Cyprinus carpio* that were collected from effluent channels of sewage-treatment plants or from rivers receiving effluents from sewage-treatment plants in the USA and Japan.7–10 Moreover, plasma VTG was detected

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in males of marine fish. Male flounders *Platichthys flesus* and *Pleuronectes yokohamae* collected in an area that received a large amount of effluent from sewage-treatment works and industrial discharge had higher levels of plasma VTG than fish collected in sites that received low levels of effluent and discharge. Some of these male flounder with higher levels of plasma VTG showed testicular abnormalities, such as testicular malformation and development of oocytes. These examples indicate that populations of wild fish in some areas are being exposed to estrogenic chemicals and suggest that effluent from sewage-treatment works and industrial discharges contain estrogenic chemicals. Although it is not investigated whether these abnormalities cause a decline in the population of wild species, endocrine disruption in fish by estrogenic chemicals is a matter of great concern in fisheries.

Vitellogenin is a precursor protein of egg yolk in oviparous animals and produced in the liver under regulation of estrogens in vertebrates. Female fish show high plasma levels of VTG during ovarian development when large amounts of estrogens are produced in the ovary. Plasma VTG levels in male fish is normally very low or non-detectable as the amount of estrogens produced in the testis is much smaller than that produced in females. However, VTG production can be elicited in males by administration of exogenous estrogens. Thus, plasma or hepatic VTG levels in male fish have been used as a specific biomarker for the detection of estrogen exposure.

Lake Kasumigaura is one of the important lakes in freshwater fisheries and aquaculture of Japan, and it is significant to survey whether fish in this lake receive any estrogenic effects and whether the effects are strong enough to disrupt the endocrine reproductive system and, hence, the influence on the sustainability of the population. In the present study, we sampled wild and cultured common carp *Cyprinus carpio* and wild crucian carp *Carassius cuvieri* in Lake Kasumigaura, and measured plasma levels of VTG in these species, in which the VTG assay system is available. We also investigated testicular histology and plasma sex steroid levels of common carp and crucian carp in order to examine the reproductive activity of these two species.

### MATERIALS AND METHODS

**Fish**

Wild male common carp *Cyprinus carpio* and crucian carp *Carassius cuvieri* caught by fixed nets in the southern part of Lake Kasumigaura near Futto, Sakuragawa Village (Fig. 1) were obtained from a local fisherman. After fish were caught, they were kept in a holding net and were sampled on the same day or on the following day. Fish were sampled from June 1998 to August 1999 (common carp *N*=53, body weight (BW) 580–4700 g; crucian carp *N*=29, BW 67–760 g). Age of these fish was unknown.

Male common carp, which were cultured by a local fish farm in the northern part of Lake Kasumigaura near Ushiwata, Kasumigaura Village (Fig. 1) were obtained through a local fish market in Saitama Prefecture. After fish were transferred from the fish farm to the fish market in Saitama Prefecture, they were kept in a holding tank and sampled as mentioned above. Fish were sampled from June 1998 to August 1999 (common carp *N*=37, BW 770–1500 g). Age of these fish was unknown.

In order to sample fish from different sites of Lake Kasumigaura, 1-year-old-male common carp cultured in Ibaraki Prefectural Freshwater Experimental Station in Tamatsukuri Town (Fig. 1) were additionally sampled in June 1999 (*N*=10, BW 110–270 g). These fish were cultured with the water pumped up from Lake Kasumigaura.

Female common carp and crucian carp were also collected from the same sources as males: wild common carp, *N*=21, BW 380–4300 g; wild crucian carp, *N*=28, BW 120–1200 g; cultured common carp from the fish farm, *N*=23, BW 750–1600 g;
cultured common carp from Ibaraki Prefectural Freshwater Experimental Station, N=8, BW 180–310 g. Spawning periods of common carp and crucian carp in Lake Kasumigaura are from April to June and from April to May, respectively (Kumamaru A, pers. comm., 1999).

After anesthesia with 2-phenoxyethanol (0.3 mL/L), fish were weighed and blood samples were taken from the caudal vasculature with a heparinized syringe and needle. Blood samples chilled on ice were transferred to the University of Tokyo and centrifuged at 3000 \( \times g \) for 10 min. Plasma samples were frozen and stored at –20°C until analysis.

The gonads were dissected and weighed for calculation of gonadosomatic index (GSI: gonad weight \( \times 100 \)/body weight). The middle part of the testis was excised and fixed with 10% formalin for histological examination. The tissues were dehydrated in ethanol, embedded in paraffin and sectioned at 4 \( \mu \)m thickness. The sections were stained with hematoxylin and eosin, and observed with a microscope (Nikon E800, Tokyo, Japan).

**Measurement of plasma vitellogenin levels**

**Preparation of carp lipovitellin antibody**

Common carp VTG and lipovitellin (Lv) were purified from plasma and the ovary of females, respectively, according to the method for the purification of VTG in the Japanese eel *Anguilla japonica*.24 Antiserum against common carp Lv (a-Lv) was raised in a rabbit. The antilipovitellin immunoglobulin G (a-Lv IgG) was purified from a-Lv. F(ab')\(_2\) of a-Lv IgG (a-Lv F(ab')\(_2\)) was prepared from a-Lv IgG as previously described.24 a-Lv F(ab')\(_2\) was labeled with acridinium ester as described by Fukada et al.25

**Chemiluminescent immunoassay**

Plasma VTG levels of common carp and crucian carp were measured by chemiluminescent immunoassay (CLIA) for carp VTG (Fukada *et al.*, unpubl. data, 1998). The microtiter plates were coated with purified a-Lv IgG. A volume of 150 \( \mu \)L of 40 \( \mu \)g/mL a-Lv IgG in 0.01 M phosphate buffer containing 0.15 M NaCl, pH 7.0 (PBS) was dispensed into each well and incubated overnight at 4°C. After washing twice with PBS containing 1% Tween 20 (PBS-T), the microtiter plates were blocked with PBS containing 1% bovine serum albumin (BSA) and 0.1% NaN\(_3\) (PBS-BSA) for 1 day at 4°C. Aliquots (100 \( \mu \)L) of samples or purified common carp VTG (standard) diluted with PBS-BSA were applied and incubated in coated wells for 4 h at room temperature. After washing as described above, each well received 100 \( \mu \)L acridinium ester-labeled a-Lv F(ab')\(_2\) diluted to 0.2 \( \mu \)g/mL with 0.2 M phosphate buffer, pH 7.0, containing 0.1% BSA, 1% Tween and 0.1% NaN\(_3\) (PB-BSA-T) and incubated for 4 h at room temperature. After washing as described above, chemiluminescence of acridinium ester-labeled a-Lv-F(ab')\(_2\) was counted in Luminescencer-JNR AB-2100 (Atto, Tokyo, Japan), which automatically injects the two reagents (Reagent 1, 0.5% H\(_2\)O\(_2\) and 0.1 N HNO\(_3\); Reagent 2, 0.25 N NaOH and surfactant, Chiron Co., Emeryville, CA, USA) necessary to initiate the chemiluminescent reaction. The light emission was expressed as the photon counts accumulated during 2 s.

The CLIA developed in the present study using anticommon carp Lv IgG and its F(ab')\(_2\) cross-reacted with plasma VTG of common carp and crucian carp, and purified common carp VTG was used as standard for the measurement of both common carp and crucian carp VTG. Details of the validation of the CLIA will be described by Fukada *et al.* (Fukuda A., unpubl. data, 2001). The detection limit of VTG was 40 ng/mL in the present study.

**Measurement of plasma sex steroid levels**

Plasma 17β-estradiol (E\(_2\)) and testosterone (T) levels were measured by radioimmunoassay (RIA) as described by Aida *et al.*26 The detection limits of E\(_2\) and T were 80 pg/mL and 120 pg/mL, respectively. Plasma 11-ketotestosterone (11-KT) levels were measured by enzyme immunoassay according to the method of Zairin *et al.*27 The detection limit of 11-KT was 30 pg/mL. Plasma 11-KT levels in some fish were not measured because of limited amounts of plasma samples.

**RESULTS**

**Plasma vitellogenin levels**

Plasma VTG levels in most of the male wild common carp were below the detection limit (40 ng/mL) (Fig. 2a), but a considerable amount of VTG was detected in some of the fish. VTG was detected in 17 out of 53 fish and the levels ranged from 88.0 ng/mL to 1680 ng/mL. Plasma VTG levels in wild female common carp ranged from the non-detectable level (below 40 ng/mL) to 13.4 mg/mL, but high levels were observed in February.
Gonadosomatic index

In male wild common carp, cultured common carp and wild crucian carp, most fish were spermiated throughout the sampling period, and milt was confirmed by gently pressing the abdomen of the fish. Expressible milt was observed in the most of the wild and cultured common carp in April, May and June (spawning period) except cultured carp from Ibaraki Prefectural Freshwater Experimental Station because these fish were immature. At the time of dissection, no abnormality of the testis was observed by their external appearance. High GSI values were observed throughout the sampling period in male wild common carp and cultured common carp from the fish farm when compared to GSI values of young cultured common carp from Ibaraki Prefectural Freshwater Experimental Station (Fig. 3a–c). No clear seasonal changes were observed in GSI values of male wild crucian carp.

Plasma VTG levels in male cultured common carp from the fish farm were mostly below the detection limit (40 ng/mL) (Fig. 2b) but four out of 37 fish showed detectable VTG levels that ranged from 43.5 ng/mL to 197 ng/mL. All male carp from Ibaraki Prefectural Freshwater Experimental Station had no detectable levels (below 40 ng/mL) of VTG (N=10). Plasma VTG levels in female cultured common carp from the fish farm ranged from the non-detectable level (below 40 ng/mL) to 6.35 mg/mL, but high levels were observed in February (pre-spawning period) and May (spawning period). Plasma VTG levels in female common carp from Ibaraki Prefectural Freshwater Experimental Station ranged from the non-detectable level to 3.29 mg/mL.

Plasma VTG levels in male wild crucian carp were mostly undetectable (below 40 ng/mL) (Fig. 2c), but six out of 29 fish showed detectable VTG levels that ranged from 137 ng/mL to 644 ng/mL. In female wild crucian carp, high levels of plasma VTG were observed throughout the sampling period. The level ranged from 22.2 μg/mL to 11.9 mg/mL.

Gonadosomatic index

In male wild common carp, cultured common carp and wild crucian carp, most fish were spermiated throughout the sampling period, and milt was confirmed by gently pressing the abdomen of the fish. Expressible milt was observed in the most of the wild and cultured common carp in April, May and June (spawning period) except cultured carp from Ibaraki Prefectural Freshwater Experimental Station because these fish were immature. At the time of dissection, no abnormality of the testis was observed by their external appearance. High GSI values were observed throughout the sampling period in male wild common carp and cultured common carp from the fish farm when compared to GSI values of young cultured common carp from Ibaraki Prefectural Freshwater Experimental Station (Fig. 3a–c). No clear seasonal changes were observed in GSI values of male wild crucian carp. Although female wild common carp showed no clear seasonal change in GSI, high GSI values were observed in February and May in cultured common carp and from January to May in crucian carp.
Testicular histology

Based on histological observations, the testes of common carp and crucian carp were classified into six developmental stages (Fig. 4): Stage I, seminiferous lobules in the testis are undeveloped, and germ cells mainly consist of spermatogonia. Stage II, seminiferous lobules are developing, but no or few sperm is present in the lumens. Stage III, seminiferous lobules and cysts are moderately developed and spermatogenesis is in progress. Stage IV, the testis is in active spermatogenesis, evidenced by the presence of various stages of germ cells in the cysts and numerous sperm in the lumens. Stage V, the swollen lumens are filled with numerous sperm and spermatogenesis is still in progress. Stage VI, numerous sperm is present in the swollen lumens, but few cysts are recognized in the lobules.

In wild common carp, most testes examined were fully matured with numerous sperm in the lumens (Fig. 5a). In these testes, active spermatogenesis was in progress with various developmental stages of germ cells in the cysts (stages IV and V) or the swollen lumens were filled with numerous sperm (stage VI). In August, however, some wild common carp had regressed (stage I) or maturing (II) testes.

The testes of cultured common carp were mostly matured (stages IV, V and VI), containing a large number of sperm in the lumens throughout the year (Fig. 5b). However, in September and February, some fish had only maturing testes with no or few sperm in the lumens (stages II and III).

In wild crucian carp, the testes were either maturing (stage III) or matured (stages IV, V and VI) and contained sperm more or less in the lumens throughout the sampling period (Fig. 5c). No fish had testes at regressed (stage I) and early maturing stage (II), although the number of fish examined was limited.

As far as we examined, no apparent abnormality was observed in the testicular tissues of wild and cultured common carp and crucian carp.

Plasma steroid levels

Plasma E2 levels did not show clear seasonal changes in wild common carp, cultured common carp and wild crucian carp in either sex (Fig. 6a–c).
plasma T levels were as follows: wild common carp; male, below detection limit (0.12 ng/mL)–6.77 ng/mL, female, <0.12–1.46 ng/mL; cultured common carp; male, <0.12–11.10 ng/mL, female, <0.12–19.90 ng/mL; wild crucian carp; male, <0.12–6.08 ng/mL, female, <0.12–3.43 ng/mL. No significant correlations were observed between plasma T and VTG levels in the fish examined except wild male common carp (wild common carp, male, $r = 0.356$, $P = 0.009$, female, $r = -0.115$, $P = 0.626$; cultured common carp, male, $r = -0.026$, $P = 0.879$, female, $r = 0.374$, $P = 0.079$; wild crucian carp, male, $r = -0.034$, $P = 0.862$, female, $r = -0.137$, $P = 0.492$).

Plasma T levels were generally higher in males than in females in common carp and crucian carp (Fig. 7a–c). Elevated levels of T were observed during most of the sampling period in males, but there was a tendency towards a decrease in August and September (post-spawning period). In some female cultured carp, elevated levels of T were observed in May (spawning period). The ranges of plasma T levels were as follows: wild common carp; male, below detection limit (0.12 ng/mL)–6.77 ng/mL, female, <0.12–1.46 ng/mL; cultured common carp; male, <0.12–11.10 ng/mL, female, <0.12–19.90 ng/mL; wild crucian carp; male, <0.12–6.08 ng/mL, female, <0.12–3.43 ng/mL. No significant correlations were observed between plasma T and VTG levels in the fish examined except female cultured carp (wild common carp, male, $r = -0.147$, $P = 0.296$, female, $r = -0.061$, $P = 0.796$; cultured common carp, male, $r = -0.245$, $P = 0.145$, female, $r = 0.671$, $P = 0.0003$; wild crucian carp, male, $r = -0.019$, $P = 0.922$, female, $r = 0.157$, $P = 0.249$).

Plasma 11-KT levels did not show clear seasonal changes in wild common carp, cultured common carp and crucian carp in either sex (Fig. 8a–c). The ranges of plasma 11-KT levels were as follows: wild common carp; male, below detection limit (0.03 ng/mL)–15.1 ng/mL, female, <0.03–1.25 ng/mL; cultured common carp; male, <0.03–16.6 ng/mL, female, <0.03–12.6 ng/mL; wild crucian carp;
male, <0.03–6.24 ng/mL, female, <0.03–5.38 ng/mL. No significant correlations were observed between plasma 11-KT and VTG levels in the fish examined (wild common carp, male, $r=0.203$, $P=0.149$, female, $r=-0.321$, $P=0.158$; cultured common carp, male, $r=-0.013$, $P=0.942$, female, $r=-0.057$, $P=0.803$; wild crucian carp, male, $r=-0.011$, $P=0.954$, female, $r=0.274$, $P=0.160$).

**DISCUSSION**

In the present study, the possible influence of estrogenic endocrine disruptors on reproductive activity was investigated by measuring plasma VTG levels as a biomarker in male wild common carp, cultured common carp and wild crucian carp in Lake Kasumigaura. In most male carps, including both common carp and crucian carp, plasma VTG levels were under the detection limit. In some male carps, however, small amounts of VTG were detected in plasma. The highest concentrations of VTG in male wild common carp, cultured common carp and wild crucian carp were 1.68 $\mu$g/mL, 197 ng/mL, and 644 ng/mL, respectively, four or five orders of magnitude lower than the highest concentration in females (13.4, 6.38 and 11.9 mg/mL for wild common carp, cultured common carp and wild crucian carp, respectively). In contrast, it is reported that elevated levels of VTG were observed in male common carp that were collected from effluent channels below sewage-treatment plants in the USA and Japan, and some fish showed VTG levels over 1 mg/mL. Therefore, it is conceivable that wild common carp, cultured common carp and wild crucian carp in Lake Kasumigaura have not received such a strong effect of estrogenic endocrine disruptors.

Although the cause of VTG production in the male carps has not been identified, the following
three possibilities are considered: (i) action of endocrine disruptors with estrogenic effect; (ii) action of endogenous steroids produced in the testis of male fish; and (iii) action of phytoestrogen in food. Regarding the first possibility, it is probable that the fish received the effect of natural estrogens from the urine of women and domestic animals via sewage plants that release effluent to the lake. Furthermore, chemicals such as pesticides and herbicides, used in farmland around the lake might have some estrogenic effect. These chemicals released into the environment may have stimulated production of the considerable amount of VTG in the male carps in Lake Kasumigaura.

In contrast, known estrogenic chemicals, such as nonylphenol and bisphenol A, have not been detected at concentrations that are high enough to affect fish reproduction in the water of Lake Kasumigaura according to the survey by the Environment Agency and the Ministry of Construction, Government of Japan. Therefore, it is unlikely that each of these endocrine disruptors induced the production of VTG in the male carps. However, a possibility remains that a mixture of low concentrations of those endocrine disruptors or unidentified estrogenic chemicals induced the production of VTG in the male carps.

Regarding the second possibility, because male fish produce a considerable amount of E₂ in the testis, it is conceivable that the endogenous E₂ stimulated production of VTG in their hepatopancreas. In laboratory-reared male common carp which were unlikely to receive endocrine disruptors, plasma VTG was detected, and it is suggested that testicular E₂ stimulates the production of VTG. Furthermore, there are reports that administration of high doses of T to male fish induced VTG production in the liver. Therefore, it is possible that testicular estrogens and androgens are involved in the production of VTG in the male carps. In fact, E₂ was detected in male fish in the present study at levels that were comparable to those of females. Although clear correlations were not observed between plasma VTG levels and plasma sex steroid levels in the male carps in the present study, it seems difficult to correlate the amount of sex steroids and VTG in the plasma due to their different clearance rates. When E₂ was administered to fish at low doses, although E₂ was cleared in the plasma in several days, plasma VTG produced by the E₂ remained in the plasma for a month or longer (Hara A, unpubl. data, 2000).

Regarding the third possibility, it is conceivable that phytoestrogens ingested with food stimulated
the production of VTG in male fish. Because common carp is an omnivorous feeder and crucian carp *Carassius cuvieri* is a phytoplankton feeder, it is highly possible that the carps ingest phytoestrogens. Pelissero *et al.*36,37 reported that plasma VTG levels rose when a soybean-based diet was fed to the Siberian sturgeon *Acipenser baeri*, and that a large amount of phytoestrogens such as daidzein and genistein are contained in the soybean. Furthermore, in male goldfish, the plasma VTG levels rose when fish were fed commercial fish feed containing soybean, whereas the VTG levels remained low when fish were fed a formulated diet without soybean (Kobayashi M, unpubl. data, 2000). Therefore, it is suggested that phytoestrogens could be one of the factors that induce the production of VTG in the male carps.

Although most cultured common carp in Japan are fed a formula food usually containing soybean, plasma VTG levels of cultured common carp in the present study were mostly undetectable. As there are no other reports on plasma VTG of cultured common carp in fish farms at present, it is not yet known whether the low plasma VTG level is the case with other cultured common carp. One of the characteristics of carp cultured in Lake Kasumigaura is that fish are fed a special diet of lower protein content than that of a regular diet for common carp in order to prevent eutrophication of the lake (Kumamaru A, pers. comm., 1999). The content of phytoestrogens in this special diet may be reduced during the manufacturing process.

Regarding histological observations, no apparent abnormality was observed in the testicular tissue of wild common carp, cultured common carp and crucian carp sampled in the present study. Moreover, active spermatogenesis was observed in most of these fish. It is reported that development of ovotestis could be induced by the treatment of E2 or estrogenic chemicals38,39 and the fish that had ovotestis were found in the rivers that received sewage-treatment works effluent.2,9 Although sample size and sampling sites were limited in the present study, no abnormality and malformation of the testis were observed in male common carp and crucian carp. The results of active spermatogenesis in the testis by histological observation suggest that testicular development is normal in common carp and crucian carp of Lake Kasumigaura.
stimulates the early stage of spermatogenesis and also stimulates production of the small amount of VTG, although biological function of VTG in males is unknown. Furthermore, it is not known why plasma VTG levels in females are much higher than those of males, although plasma E2 levels between males and females did not show such a big difference.

In summary, the possible influence of estrogenic endocrine disruptors on male wild and cultured common carp and wild crucian carp in Lake Kasumigaura was investigated. These fish species showed low levels of plasma VTG and no apparent testicular abnormality in the testis but active spermatogenesis. These results suggest that fish in Lake Kasumigaura have not received estrogenic effects that are strong enough to disrupt normal gonadal development. However, as the present study focused on gonadal development and has not examined a whole range of reproductive activity of fish including spawning behavior and subsequent

Kasumigaura and that these fish receive no marked effect of estrogenic endocrine disruptors.

Plasma sex steroid levels in the male common carp and crucian carp were measured in the present study as one of the parameters for sexual maturity of the fish. Although steroid levels in these fish were considered to be mostly in the normal range\textsuperscript{30,40,41} and be involved in spermatogenesis, a clear seasonal cycle of plasma sex steroids was not observed with the progression of gonadal maturation. The variation of the steroid levels in the present study may be partly due to variations of age and body size of the sampled fish. Therefore, it seems difficult to estimate reproductive activity of fish with the changes in plasma sex steroid levels in the present study. However, it should be noted that E2 was detected in male fishes at levels that are comparable to those of females. It is recently reported that E2 promotes proliferation of spermatogonia in the testis of male fish.\textsuperscript{42} It is possible that E2 observed in male carps in the present study stimulates the early stage of spermatogenesis and also stimulates production of the small amount of VTG, although biological function of VTG in males is unknown. Furthermore, it is not known why plasma VTG levels in females are much higher than those of males, although plasma E2 levels between males and females did not show such a big difference.

In summary, the possible influence of estrogenic endocrine disruptors on male wild and cultured common carp and wild crucian carp in Lake Kasumigaura was investigated. These fish species showed low levels of plasma VTG and no apparent testicular abnormality in the testis but active spermatogenesis. These results suggest that fish in Lake Kasumigaura have not received estrogenic effects that are strong enough to disrupt normal gonadal development. However, as the present study focused on gonadal development and has not examined a whole range of reproductive activity of fish including spawning behavior and subsequent

![Fig. 8](image-url) Plasma 11-ketotestosterone levels in (a) wild common carp, (b) cultured common carp and (c) wild crucian carp in Lake Kasumigaura. The detection limit was 30 pg/mL.
larval development, it is necessary to conduct further studies on the effects of endocrine disruptors on fish.

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