Endocrine Disruption and Altered Gonadal Development in White Perch (*Morone americana*) from the Lower Great Lakes Region

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High prevalences of gonadal intersex have been observed in wild fish populations in areas affected by domestic and industrial effluents. For this study, fish were collected in 1998 from the Cootes Paradise region of Hamilton Harbour in western Lake Ontario, Canada, to determine whether gonadal abnormalities, including intersex, were present in young of the year (YOY) fish. No gonadal abnormalities were observed in goldfish (*Carassius auratus*), common carp (*Cyprinus carpio*), gizzard shad (*Dorosoma cepedianum*), brown bullhead (*Ictalurus ameiurus*), pumpkinseed (*Lepomis gibbosus*), and bluegill (*Lepomis macrochirus*). However, intersex gonads were observed in 8 of 16 male white perch (*Morone americana*) examined in this survey. Subsequently, in 1999 and 2000 white perch estimated to be YOY to approximately 2 years of age were collected from Cootes Paradise and from two other sites in the lower Great Lakes region. Gonadal intersex was observed in male white perch collected from the Bay of Quinte (22–44%) and Lake St. Clair (45%), although the prevalence and the extent of the intersex condition were lower relative to the 83% prevalence in white perch collected in Cootes Paradise. Intersex was not observed in hatchery-reared white perch or in white perch collected from an uncontaminated reference site (i.e., Deal Lake) in the United States. An analysis of plasma collected in the spring of 2002 from male adult white perch in Cootes Paradise revealed high concentrations of vitellogenin, ranging from 49 to 1,711 μg/mL. These observations indicate that male white perch are exposed to estrogenic endocrine-disrupting substances that may be responsible for the induction of gonadal intersex. *Key words:* endocrine disruption, intersex, vitellogenin, white perch. *Environ Health Perspect* 112:898–902 (2004). doi:10.1289/ehp.6514 available via *http://dx.doi.org/* [Online 12 February 2004]

A number of substances have been detected in the environment that can affect the endocrine system (Tyler et al. 1999). These endocrine-disrupting substances (EDSs) include natural estrogens, such as 17β-estradiol (E_2) and estrone, and the synthetic estrogen 17α-ethinylestradiol (EE_2), which are discharged in the effluents of sewage treatment plants (Harries et al. 1999). Other EDSs of industrial origin, such as alkylphenol surfactants, bisphenol A, and phthalate plasticizers, have also been detected in sewage treatment plant effluents (Fürhacker et al. 2000; Spengler et al. 2001; Thomas et al. 2001). E_2, EE_2, alkylphenols, and bisphenol A have also been found in the bile of fish exposed to sewage treatment plant effluents (Larsson et al. 1999).

Alterations to the gonadal development of wild fish populations have been associated with exposure to effluents originating from industrial (Larsson et al. 2000) and domestic sources (Sumpter 1998; Tyler and Routledge 1998). Intersex, a condition where both testicular and ovarian tissues are present in the same gonad, has been observed in fish from a number of locations where EDSs are suspected of being present. In roach (*Rutilus rutilus*) from the sewage-contaminated Nene and Aire Rivers in the United Kingdom, intersex was observed in 100% of male fish compared with 4% of male fish from laboratory controls and reference sites (Jobling et al. 1998). A high prevalence of intersex has also been observed in gudgeon (*Gobio gobio*) captured in locations in the United Kingdom that receive discharges from sewage treatment plants (van Aerle et al. 2001). Intersex has also been observed in 20% of male flounder (*Platichthys flesus*) in the contaminated Mersey estuary in the United Kingdom (Allen et al. 1999) and in barbel (*Barbus plebejus*) captured downstream of the confluence of a polluted tributary in Italy (Viganò et al. 2001). A low level of intersex (two of seven mature males) was also observed in shovelnose sturgeon (*Scaphirhynchus albus*) captured in the Mississippi River, south of St. Louis, Missouri, USA (Harshbarger et al. 2000).

Among populations of fish with gonadal intersex, significant levels of vitellogenin (VTG) were reported in male roach, gudgeon, and flounder in the United Kingdom (Allen et al. 1999; Jobling et al. 1998; van Aerle et al. 2001). The egg precursor protein VTG has been frequently used as a biomarker of exposure of male fish to estrogenic substances. VTG is normally only present in the plasma of mature female fish, but exposure to estrogenic substances can induce VTG synthesis in male fish and in juvenile females (Harries et al. 1997; Larsson et al. 1999; Nichols et al. 1999; Thorpe et al. 2001).

In this study, fish were surveyed in the lower Great Lakes region of Canada to determine whether alterations to gonadal development occur in wild fish populations. Immature fish were surveyed in order to histologically evaluate gonadal structure without the gross changes in structure and size caused by natural breeding cycles. A preliminary investigation was conducted in 1998 with seven fish species collected from the Cootes Paradise region, an embayment of Hamilton Harbour in the western part of Lake Ontario, Canada, that receives discharges of treated domestic sewage. A follow-up investigation of only white perch (*Morone americana*) was conducted in 1999 and 2000 at several sites in the lower Great Lakes that are affected by domestic and industrial discharges. White perch were also collected at two reference sites in the United States. After observations of high prevalences of gonadal intersex in male white perch from several sites in the lower Great Lakes, we hypothesized that these gonadal abnormalities were induced in white perch by exposure to estrogenic EDSs. To confirm that white perch are being exposed to estrogenic substances, plasma was collected from adult white perch from Cootes Paradise and analyzed for the presence of the egg yolk protein VTG.

Materials and Methods

Histologic evaluation of gonadal tissue. An initial survey of young of the year (YOY) individuals of seven fish species was conducted in August 1998 in the Cootes Paradise region in the western part of Lake Ontario. YOY goldfish (*Carassius auratus*), common carp (*Cyprinus carpio*), gizzard shad (*Dorosoma cepedianum*), brown bullhead (*Ictalurus*

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ameiurus), pumpkinseed (*Lepomis gibbosus*), bluegill (*Lepomis macrochirus*), and white perch were collected by seining in Cootes Paradise. Fish were collected in the late summer so that the YOY would be as large as possible and thus would have larger and more differentiated gonads.

After the putative identification of gonadal intersex in white perch from Cootes Paradise, male white perch were collected once again from Cootes Paradise as well as two other regions of the lower Great Lakes (Bay of Quinte and Lake St. Clair, Ontario) to evaluate alterations to gonadal development. In October 1999, white perch (YOY to 2–3 years of age) were collected by electrofishing and trap netting from the Bay of Quinte in eastern Lake Ontario. YOY white perch were also collected in August 2000 by electrofishing in Cootes Paradise and by trawling in the Bay of Quinte and Lake St. Clair.

White perch were also collected at two reference sites in the United States. In July 2002, hatchery-reared white perch (1 to 2–3 years of age) were obtained from the Pamlico Aquaculture Field Laboratory (PAFL) hatchery (Aurora, NC, USA), for reference purposes. At this hatchery, white perch hatchlings had been stocked into 0.1 hectare freshwater ponds and held there for several months until transferred to 26,000 L pools, from which they were collected. The ponds and pools are supplied with well water that is pumped from a 91-m deep aquifer. It was difficult to identify a reference site in the lower Great Lakes that could be regarded as pristine and possessing a resident white perch population; therefore, we selected a reference site—a small, shallow lake that receives no domestic or industrial effluents—in Deal Lake, New Jersey (USA). YOY white perch were collected from this location by seining in September 2002.

All fish were measured for mass and fork length and were then euthanized using an overdose of tricaine methane sulfonate (MS-222; Sigma-Aldrich, Toronto, Ontario, Canada) anesthetic. The ages of the fish were estimated from age versus length and age versus mass relationships for white perch published by Scott and Crossman (1975). The body cavities of the fish were opened, and they were fixed whole in 10% buffered formalin and then stored in 70% ethanol. Gonadal tissues were then removed, dehydrated via a graded series of alcohol concentrations, and embedded in paraffin. Gonads were step-sectioned at a thickness of 6 µm (~10 sections/gonad), stained with hematoxylin and eosin, and examined by light microscopy. Gonadal development was classified according to the stages described by Jackson and Sullivan (1995) and the criteria established by Grier (1981) and Lambert (1970) for testicular and ovarian development, respectively.

We compared intersex prevalence among the sites by Fisher exact tests using SISA statistical software (Uitenbroek 1997). Prevalence data for fish from the Cootes Paradise 1998 sample and each of the sampling periods in 2000 were compared with the prevalence data for fish from the Deal Lake reference site. Because of the larger size and greater age (i.e., 2–3 years) of the white perch from the Bay of Quinte collected in 1999, we compared the intersex prevalence in this group with the prevalence data for fish from the PAFL reference site.

VTG analysis. To confirm that white perch were being exposed to estrogenic substances, adults of this species were collected from Cootes Paradise in 2002. Using a single radial-immunodiffusion assay developed for striped bass (*Morone saxatilis*) to analyze VTG in white perch, Jackson (1992) has shown that VTG is normally absent in males. Thus, this protein would not be expected to be present in male white perch unless the fish had been exposed to xenoestrogens. No ELISA assay is commercially available for quantitative analysis of VTG in white perch, so analysis was conducted by gel electrophoretic separation of protein bands, followed by quantitation of the amount of VTG present in the appropriate band (identified from purified white perch VTG) using densitometry.

Adult white perch (22 males, 5 females) were collected in May 2002 in Cootes Paradise by electrofishing. Because white perch from Hamilton Harbour enter Cootes Paradise in the spring to spawn, May is the best time to collect adult white perch in Cootes Paradise. Observations at a fishway designed to prevent carp from entering Cootes Paradise indicated that white perch had been entering Cootes Paradise for several weeks before collection.

Upon capture, fish were immediately transported from Cootes Paradise in 100-L aerated containers to Trent University, where blood was collected. For comparison, a previously collected group of male white perch (n = 9) that had been held at Trent University for 1–3 years was used as a reference group. These fish had been held in 350-L aerated circular tanks with a flow-through system of water taken from the Otonabee River (Ontario, Canada). Their diet consisted of commercially bought frozen fish as well as occasional live aquarium fish. Blood was collected from the caudal peduncle using heparinized syringes and centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was removed and stored at –80°C in a cryovial containing phenylmethylsulphonyl fluoride (PMSF; Sigma-Aldrich), which was added to inhibit VTG degradation.

The protein samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) at 200 V using the Mini-Proean II Electrophoresis Cell (Bio-Rad, Hercules, CA). SDS gels were divided into a stacking gel [4.0% (vol/vol) 30% acrylamide and 0.8% bisacrylamide; Gibco Invitrogen, Burlington, Ontario, Canada] and a running gel [7.5% (vol/vol) acrylamide/bisacrylamide]. Using the methods described by Gallagher (1998), we ran 10 µL plasma from each sample on a 7.5% SDS-polyacrylamide gel along with *a*) a positive control (mature female white perch captured in Cootes Paradise, just before spawning); *b*) a negative control (male white perch held at Trent University for 1–3 years); *c*) the protein standard SeeBlue (Helixx Technologies, Toronto, Ontario, Canada); and *d*) a purified white perch VTG standard provided by C. Sullivan (North Carolina State University, Raleigh, NC, USA). The gels were stained with Coomassie brilliant blue R250 (Bio-Rad). The VTG band in the samples was identified by comparing band locations to the purified VTG standard (i.e., the 170–180 kDa mark). The gels were scanned, and Alphaease computer software (Version 5.5; Alpha Innotech Corporation, San Leando, CA, USA) was used to analyze the optical density of the lanes. The total protein content (100%) was represented by summing the optical density of all bands. The percentage contribution of VTG to total protein was calculated from the optical density of the VTG band. In samples where a VTG band was detected, the presence of VTG was confirmed by Western blotting.

For Western blot analysis, we used mouse anti–striped bass VTG (ND-1C8; Cayman Chemical Co., Ann Arbor, MI, USA) as the primary antibody. Striped bass and white perch VTG appear to be identical in their biochemical and immunologic characteristics (Tao et al. 1996). The Western blot was conducted as described by Gallagher et al. (1998). Briefly, after SDS-PAGE, the proteins were transferred to a nitrocellulose membrane (Bio-Rad) using the Bio-Rad Mini Trans-Blot cell. The proteins were transferred to the membrane at 100 V for 2.5 hr. The membrane was then placed in a 10% blocking solution [3 g instant skim milk powder dissolved in 30 mL phosphate-buffered saline (PBS)] and incubated on a shaker at 100 rpm overnight. After approximately 12 hr,

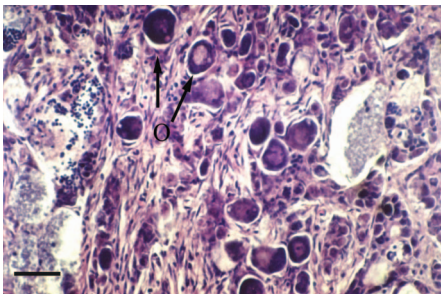

Figure 1. Testis of a male white perch from Coote's Paradise. Numerous primary oocytes (O) are present in the testicular tissue. Hematoxylin and eosin. Magnification, 400×; bar = 30 µm.

the primary antibody was added to the blocking solution. Initially, we used a 1:3,000 dilution of the primary antibody to determine whether VTG was present in the samples. Upon confirmation, we used a 1:10,000 dilution to emphasize the predominant VTG band(s). The membrane was incubated in the primary antibody solution for 2 hr on a shaker at 100 rpm. The primary antibody solution was drained, and the membrane was washed three times in 30–50 mL PBS and two times for 5 min in PBS on a shaker at 100 rpm. The secondary antibody (alkaline phosphatase–goat anti-mouse IgG; Zymed Laboratories, San Francisco, CA, USA) was added to a 10% blocking solution at a dilution of 1:4,000, and the membrane was incubated in this solution for 1 hr on a shaker at 100 rpm. The membrane was washed five times in 30–50 mL PBS and twice for 5 min in PBS on a shaker at 100 rpm. The membrane was developed using an alkaline phosphatase conjugate substrate kit (Bio-Rad).

To quantify the concentrations of VTG, samples in which VTG was detected were run again on a 7.5% SDS-polyacrylamide gel, but this time with the white perch VTG standard at three different dilutions so that a smooth cubic spline calibration curve could be constructed. We used the Alphaease software and spot densitometry to determine the amount of VTG in each sample. We used the predominant VTG band at 170–180 kDa for this estimation in all cases. For comparison, we used the same procedure for the plasma samples from five mature females collected in Cootes Paradise. A one-sample *t*-test was used to determine whether the VTG levels observed in male white perch from Cootes Paradise differed significantly from the male control fish that had been held at Trent University.

Results and Discussion

In the initial survey of YOY fish collected in 1998 from Cootes Paradise, no gonadal abnormalities were observed in the goldfish (7 males, 13 females), carp (10 males, 6 females), gizzard shad (4 males, 3 females), brown bullhead (5 males, 5 females), pumpkinseed (5 males, 5 females), bluegill (23 males, 7 females), and the 11 female white perch that were histologically examined. However, among the male YOY white perch ($n = 16$), an intersex condition of the testis was observed in 8 males. In normal YOY males, testicular tissue mainly consisted of primordial germ cells surrounded by interstitial tissue, whereas in YOY females, the ovaries consisted of previtellogenic oocytes. Gonadal intersex was characterized by many primary oocytes distributed throughout normal testicular tissue (Figure 1).

Subsequently, in 1999 and 2000 white perch were collected from Cootes Paradise and several other sites in the lower Great Lakes. There were some differences in the size (and age) of the male white perch (Table 1). These fish were collected at approximately the same time of year (i.e., around August), with the exception of fish collected in October from the Bay of Quinte. In studies of hatchery-raised white perch in North Carolina, Jackson and Sullivan (1995) observed that the testes of males were at the prespermatogenic stage of development during the months of July and August. This was consistent with our observations of the stage of testicular development in Great Lakes white perch collected in August, because these testes were at very early stages of differentiation, with mainly germ cells and some spermatogonia. However, among the male white perch collected in the Bay of Quinte in October 1999, the testes were in later stages of spermatogenesis, containing spermatocytes and spermatids.

In the survey of white perch from Cootes Paradise conducted in 2000, the prevalence of gonadal intersex in male fish was 83% (Table 2). In white perch collected from the Bay of Quinte and Lake St. Clair, intersex was also observed in male fish but at prevalences much lower than those found at Cootes Paradise (Table 2). The degree of intersex development in individual gonads was also reduced relative to Cootes Paradise fish because the intersex consisted of only a few primary oocytes distributed throughout the testicular tissue (Figure 2). We observed no gonadal intersex in reference white perch from the PAFL hatchery or Deal Lake. The prevalence of intersex in white perch collected from the Bay of Quinte in October 1999 approached but did not reach significance when compared with the PAFL white perch reference group (Fisher exact test, $p = 0.0513$), but a larger sample size may have yielded a significant result. The prevalence of intersex among white perch collected in August 1998 from Cootes Paradise and in August 2000 from all three sampling sites differed significantly from the prevalence in Deal Lake white perch (Fisher exact test, $p < 0.0125$).

Laboratory exposures of Japanese medaka (*Oryzias latipes*) to estrogenic compounds induce gonadal intersex in male fish (Metcalfe et al. 2001). Intersex in wild fish may occur as a result of exposure to estrogenic compounds present in industrial or domestic sewage effluents. However, in certain species, a low level of gonadal intersex in fish may also be a natural phenomenon. For example, Jobling et al. (1998) determined that gonadal intersex occurs naturally in approximately 4% of male roach and in approximately 5% of common carp. A natural occurrence of gonadal intersex has been reported in one individual banded killifish, *Fundulus diaphanous* (Porter and Fivizzani 1983).

In white perch, hermaphroditism was described by Bishop (1920) and intersex was described by Dorfman and Heyl (1976). In both cases, these abnormalities occurred in one

Table 1. Time of collection, sex, length, mass, and estimated age of white perch collected at study sites in the lower Great Lakes and at reference sites (PAFL hatchery, Deal Lake) in 1998–2002.

Location	Month/year	Sex	No.	Fork length (mean ± SD, mm)	Mass (mean ± SD, g)	Estimated age
Cootes Paradise	Aug 1998	M	16	83.7 ± 8.7	6.4 ± 1.5	YOY
		F	11	88.9 ± 5.1	7.5 ± 1.3	YOY
Bay of Quinte	Oct 1999	M	37	148.8 ± 30.6	55.9 ± 26.2	YOY to 2–3 years
		F	38	150.9 ± 43.1	62.1 ± 39.5	YOY to 2–3 years
Bay of Quinte	Aug 2000	M	16	56.4 ± 3.9	2.5 ± 0.5	YOY
Cootes Paradise	Aug 2000	M	12	68.1 ± 7.3	4.2 ± 1.1	YOY
Lake St. Clair	Aug 2000	M	11	54.7 ± 3.8	2.3 ± 0.5	YOY
PAFL	Jul 2002	M	15	169.0 ± 31.5	70.3 ± 25.4	1 to 2–3 years
Deal Lake	Sep 2002	M	13	87.0 ± 6.3	7.7 ± 1.7	YOY

Abbreviations: F, female; M, male.

Table 2. Prevalence of gonadal intersex in male white perch collected from sites in the lower Great Lakes and reference sites (Ref).

Site/year	Total no. of males	No. of males with testis-ova	Percent prevalence in male fish
Bay of Quinte			
1999	37	8	22
2000	16	7	44
Cootes Paradise			
1998	16	8	50
2000	12	10	83
Lake St. Clair			
2000	11	5	45
PAFL Hatchery (Ref)			
2002	15	0	0
Deal Lake (Ref)			
2002	13	0	0

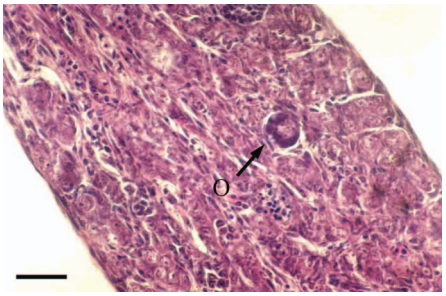

Figure 2. Testis of a male white perch from Lake St. Clair. A single primary oocyte (O) can be observed. Hematoxylin and eosin. Magnification, 400×; bar = 30 µm.

individual fish. However, in the present study, 43–83% of male white perch examined at sites in the lower Great Lakes exhibited gonadal intersex. It seems unlikely that these high prevalences of gonadal intersex are a natural occurrence. It is not clear why gonadal alterations were not observed in other fish species collected in Cootes Paradise in the initial survey in 1998. For instance, common carp have a low natural prevalence of gonadal intersex (Jobling et al. 1998), and this species is sensitive to alterations to gonadal development by exposure to EDSs (Gimeno et al. 1997). It is possible that the prevalence of alterations to gonadal development in the other fish species collected at Cootes Paradise was too low to be detected with the small sample sizes (i.e., < 30 fish). It may be that the life history of white perch brings the sensitive, early life stages of this species into contact with high concentrations of the EDSs discharged in domestic or industrial effluents.

The results of the VTG analysis are consistent with the hypothesis that the intersex observed in white perch is the result of exposure to estrogenic EDSs. Plasma samples from adult white perch (22 males, 5 females) collected from Cootes Paradise in May 2002 were analyzed by both gel electrophoresis (Figure 3) and Western blot analysis (Figure 4). VTG was present in 11 of the male fish collected from Cootes Paradise but was absent in control males that had been held in river water for 1–3 years at Trent University.

We observed two major bands in both the plasma samples and the white perch VTG standard (Figure 4). The prominent VTG band was in the 170–180 kDa range and a second band around 110 kDa. Tao et al. (1996) reported the molecular weight of white perch VTG to to be approximately 170 kDa; therefore, the second band is likely due to degradation. VTG bands at the 170 kDa and 110 kDa mark have been reported in cod (*Gadus gadus*) (Silversand et al. 1993), striped bass (Tao et al. 1993), and flounder (Allen et al. 1999) after SDS-PAGE; these authors concluded that the second, lower-molecular-weight band was the result of VTG degradation. In the present study, we also observed other bands in the plasma samples, which were most noticeable in fish that had high VTG concentrations. Using Western blots, we did not observe any of these bands in control males; therefore, the bands were not the result of cross-reactivity with normal serum proteins. Because VTG is a highly unstable molecule, these bands may have been degraded protein. We used PMSF to inhibit degradation of VTG in the white perch plasma samples. However, Specker and Anderson (1994) discovered that this protease inhibitor was not entirely effective in preventing VTG degradation in striped bass. Because striped bass and white perch VTG share the same biochemical and immunologic characteristics (Tao et al. 1996), it seems likely that this inhibitor may not have been totally effective in preventing VTG degradation. Alternatively, it is possible that these minor bands represent some other female-specific proteins.

Among the 22 male white perch collected from Cootes Paradise, 11 males had detectable VTG, comprising between 0.7% and 13.8% of total protein (Table 3). We also estimated the concentration of VTG present in each sample using spot densitometry. We observed considerable variability among individuals, with VTG concentrations ranging from 49 to 1,711 µg/mL. No VTG was detected in any of the control fish. The high variability in VTG plasma concentrations in Cootes Paradise fish may have been due to fish mobility, resulting in variable exposure to EDSs. However, high variability in VTG also occurs when fish are exposed to EDSs under laboratory conditions (Ackermann et al. 2002), indicating high biologic variability in responsiveness. In several of the male white perch, VTG levels were elevated to concentrations similar to those observed in the mature female white perch (Table 3). Allen et al. (1999) reported VTG concentrations in male flounder that were an order of magnitude higher than those found in mature females that were about to spawn, and exposure of male rainbow trout to xenoestrogens has been reported to produce VTG levels higher (> 100 mg/mL) than those observed in fully mature females (Purdom et al. 1994).

The presence of elevated plasma concentrations of VTG in male white perch indicates that these fish have been exposed to estrogenic

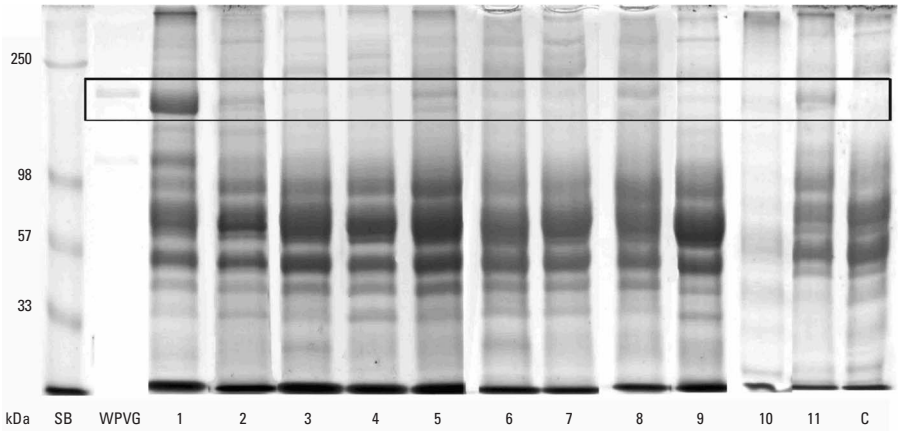

Figure 3. SDS-polyacrylamide gel of 11 white perch samples containing VTG. Abbreviations: C, control male; SB, SeeBlue; WPVG, purified white perch VTG. The predominant VTG band is indicated by the rectangle.

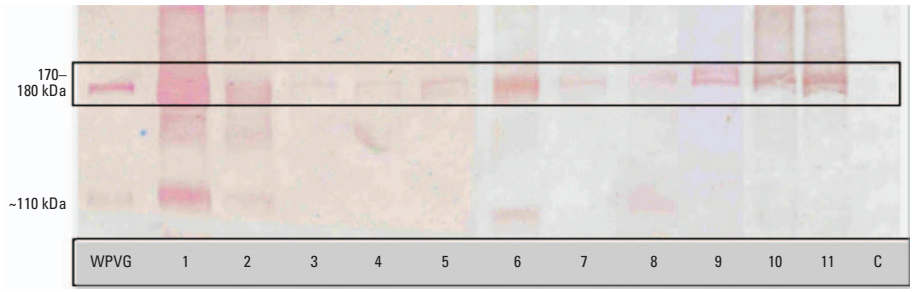

Figure 4. Western blot of 11 white perch samples containing VTG. Abbreviations: C, control male; WPVG, purified white perch VTG. The predominant VTG band is indicated by the rectangle.

Table 3. Estimated concentrations of VTG (percentage of total protein and µg/mL plasma) in VTG-positive adult male white perch (n = 11) and adult female white perch (n = 5) collected in Cootes Paradise in May 2002.

Sample	Sex	VTG (percent of total protein)	Estimated VTG in sample (µg/mL)
1	M	13.8	1,711
2	M	2.5	391
3	M	0.7	51
4	M	0.7	49
5	M	2.0	163
6	M	1.2	76
7	M	1.2	114
8	M	2.7	220
9	M	0.5	79
10	M	5.9	347
11	M	11.3	1,226
12	F	7.6	1,851
13	F	3.1	807
14	F	8.8	2,043
15	F	7.2	1,925
16	F	3.8	994

Abbreviations: F, female; M, male. No VTG was detected in 11 other male white perch collected from Cootes Paradise, and no VTG was detected in 9 male white perch held at Trent University in river water for 1–3 years (controls).

substances. White perch spawn in the spring, and it is during this time that fish, including adult white perch, congregate near a combined sewer overflow that discharges into Cootes Paradise. The discharge from the sewage treatment plant for the municipality of Dundas, Ontario, Canada, may be a source of estrogenic EDSs in the Cootes Paradise region. This treatment plant serves the 27,000 inhabitants of West Hamilton and Dundas and has a conventional activated sludge process with tertiary treatment and a rated capacity of 18.2 million L/day (McMaster University 2001).

We analyzed sediments from Cootes Paradise for concentrations of alkylphenol compounds using the analytical methods of Bennett and Metcalfe (2000). Concentrations of nonylphenol ranged from 1 to 5 μg/g dry weight and those of octylphenol ranged from 0.01 to 0.6 μg/g dry weight. These levels are within the range of concentrations detected in sediments near the discharges of other sewage treatment plants in the lower Great Lakes (Bennett and Metcalfe 2000). There are no data on the concentrations of other EDSs in Cootes Paradise. Feminization of male snapping turtles in Cootes Paradise, as indicated by altered development of the cloacal opening, was reported by de Solla et al. (1998). These researchers speculated that the high concentrations of persistent organic pollutants (e.g., polychlorinated biphenyls, organochlorine pesticides) observed in these turtles may have contributed to feminization, but the present study indicates that other EDSs of industrial or domestic origin may have had impacts upon the development of secondary sex characteristics.

There are no data on the levels of EDSs at the Bay of Quinte and Lake St. Clair sites where white perch were collected; however, contamination is suspected at these locations. For example, there are four major sites of industrial pollution and six sewage treatment plants that discharge into the Bay of Quinte (Poulton 1992). One of these, the Deseronto sewage treatment plant, discharges into the Bay of Quinte in the vicinity of the sample site. Similarly, white perch sampled in Lake St. Clair were collected at a site within several kilometers of a municipal sewage treatment plant.

Although much remains to be studied concerning the development and differentiation of the gonad in white perch, it appears that gonadal intersex is not a common condition in this fish species. Municipal sewage treatment plants or industries near the study sites may discharge xenoestrogens that affect the gonadal development of white perch at these locations. The presence of elevated plasma concentrations of VTG in male white perch collected from Cootes Paradise is consistent with this hypothesis. The present study contributes to a growing body of evidence that fish may be affected by exposure to EDSs originating from discharges of industrial and domestic wastewater.

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