Consequences of Xenoestrogen Exposure on Male Reproductive Function in Spottail Shiners (*Notropis hudsonius*)

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There is limited information on the physiological consequences associated with exposure to xenoestrogens under field conditions. The objectives of this study were to determine the presence of estrogenic chemicals in the St. Lawrence River and their effects on male reproduction in the spottail shiner (*Notropis hudsonius*). Hepatic vitellogenin (VTG) mRNA levels in immature shiners indicate extensive estrogenic contamination spanning almost 50 km both upstream and downstream from the island of Montreal. Stages of spermatogenesis were assessed in fish captured at sites having varying levels of estrogenic contamination. In control fish, 95% had testis of either stage IV (50%) or stage V (45%) of having varying levels of estrogenic contamination. In control fish, 95% had testis of either stage IV (50%) or stage V (45%) of spermatogenesis. At Îlet Dorval, where VTG mRNA levels are moderate, fish had testes of stage III (38%) and IV (45%) and only 15% of fish were at spermatogenic stage V. In contrast, at Îlet Vert and Île Beauregard, located in the sewage effluent plume from the City of Montreal and where hepatic VTG mRNA levels are high in fish, none of the fish were at stage V and 8% of fish at Îlet Vert were at stage II of development. Sperm concentration and various motility parameters were significantly lower in shiners from Îlet Vert as compared with those from Îles de la Paix (reference). Histological analyses of testes revealed that more than one-third of the fish captured at sites with the highest estrogenic contamination displayed intersex, a condition in which ovarian follicles were developing within the testis. These data indicate that there is significant estrogenic contamination in the St. Lawrence River that is associated with impaired reproductive function in male fish.

**Key Words:** xenoestrogen; toxicity; sperm motility; spermatogenesis; intersex; vitellogenin.

The potential adverse effects of xenoestrogens on the endocrine function of fish have been a growing concern. Feminization has been reported in male fish exposed to effluents from industrial and sewage treatment plants in the UK (Harries et al., 1994). Consequences of estrogenic contamination in the St. Lawrence River and their effects on male reproduction in the spottail shiner (*Notropis hudsonius*). Hepatic vitellogenin (VTG) mRNA levels in immature shiners indicate extensive estrogenic contamination spanning almost 50 km both upstream and downstream from the island of Montreal. Stages of spermatogenesis were assessed in fish captured at sites having varying levels of estrogenic contamination. In control fish, 95% had testis of either stage IV (50%) or stage V (45%) of spermatogenesis. At Île Dorval, where VTG mRNA levels are moderate, fish had testes of stage III (38%) and IV (45%) and only 15% of fish were at spermatogenic stage V. In contrast, at Îlet Vert and Île Beauregard, located in the sewage effluent plume from the City of Montreal and where hepatic VTG mRNA levels are high in fish, none of the fish were at stage V and 8% of fish at Îlet Vert were at stage II of development. Sperm concentration and various motility parameters were significantly lower in shiners from Îlet Vert as compared with those from Îles de la Paix (reference). Histological analyses of testes revealed that more than one-third of the fish captured at sites with the highest estrogenic contamination displayed intersex, a condition in which ovarian follicles were developing within the testis. These data indicate that there is significant estrogenic contamination in the St. Lawrence River that is associated with impaired reproductive function in male fish.

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The potential adverse effects of xenoestrogens on the endocrine function of fish have been a growing concern. Feminization has been reported in male fish exposed to effluents from industrial and sewage treatment plants in the UK (Harries et al., 1994; Jobling et al., 1996; Lye et al., 1997). These include effects on sperm counts, spermatogenesis, and atrophy of germ cells (Christiansen et al., 1998; Gimeno et al., 1998; Haubruge et al., 2000; Zaroogian et al., 2001). A three-month exposure to a sublethal dose of 4-tert-pentylphenol or 17β-estradiol causes progressive disappearance of spermatozoa and spermatogenic cysts, and reduces the seminiferous tubule diameter in mature male carp (Gimeno et al., 1998). Furthermore, it has been reported that that sperm motility may be decreased in male fish exposed to xenoestrogens, although other studies have failed to observe an effect of ethinyl estradiol exposure on sperm motility (Jobling et al.,...
In a study of a wide range of rivers throughout the British Isles, Jobling et al. (2002) demonstrated a high incidence of intersex, a condition in which female reproductive tissue is present in the testis, which was correlated with induced levels of VTG. Several studies have shown that intersex can be induced by exposing fish to estradiol (Koger et al., 2000; Krisfalusi and Nagler, 2000).

Municipal sewage effluent, industrial effluents, and agricultural runoff remain major sources of xenoestrogen pollution. The Montreal Urban Community (MUC) sewage treatment facility discharges all of its effluent at a single site near the eastern tip of the Island of Montreal, where it enters the St. Lawrence River. Thus, the sewage created by a population of approximately 1.8 million people enters the St. Lawrence at a single discharge point. Chemical analyses of sediments around the Island of Montreal suggest that contaminant levels are generally low, with the exception of zinc in sewage effluent and alkyl phenols, nonylphenol ethoxylates and octylphenol that are present downstream from the Montreal Urban Community (MUC) discharge point (Bennie et al., 1998; Gagnon and Saulnier 2003). Sabik et al. (2003) reported the presence of alkylphenol ethoxylates in the sediments of the St. Lawrence River at many sites around the Island of Montreal and found levels to be low at Île de la Paix, high at Île Dorval, and much higher at Île Vert and Île Beauregard (see Fig. 1). Ethinyl estradiol is also present in these effluents (MUC Treatment Plant, personal communication).

The objective of this study was to determine whether or not estrogenic substances were present in the St. Lawrence River in proximity of the Island of Montreal and to assess the effects of such contaminants on male reproductive function in the spottail shiner (Notropis hudsonius). This species was selected to examine the impact of sewage effluent on fish in the St. Lawrence because it was common at all the sites and could be readily studied for site-specific effects. Spottail shiners are minnows that usually live for approximately 5 years and mature by 1–2 years of age. In the St. Lawrence River, spawning occurs in June. It is an important foraging species for gamefish and is distributed widely throughout eastern North America (Jenkins and Burkhead, 1994).

MATERIALS AND METHODS

Study area. We established sampling sites upstream and downstream of the MUC municipal discharge area (Fig. 1). The downstream sites (Îlet Vert, Île Beauregard and Île St. Ours) and the upstream sites (Ottawa River, Îles de la Paix, Île Dorval, Îles de Boucherville) receive varying concentrations of effluents from different sources. Îlet Vert is located 4 km downstream from the sewage outfall, Île Beauregard 10 km, and Île St. Ours approximately 35 km. Two other sites (Bout de l’île and Île au Bois Blanc) are located downstream of Montreal but outside the city’s sewage effluents, and thus any effluents received are from other sources. With the exception of those fish captured in the Ottawa River, all fish were captured in the St Lawrence River near the city of Montreal (Québec, Canada).

Animal sampling. Spottail shiners were captured in June (1999–2002) from each site using a beach seine. Fish were placed in aerated river water and transported to the laboratory. Fish were euthanized using a solution of 0.1% tricaine methansulfonate (MS222, Boreal, Ontario, Canada) and tissue samples were collected. All protocols used in this study were done according to the guidelines of the Canadian Council for Animal Care Committee.
**Condition factor.** Fork length and body weight were recorded for each fish. Condition factors were calculated using the equation: $K = \frac{\text{body weight (g)}}{\text{fork length (cm)}}$.

**VTG mRNA levels.** Spottail shiners were captured at Îles de la Paix, Île Dorval, Îles de Boucherville, Îlet Vert, and Île St. Ours in June 1999 and at all nine sites in June 2000. Total RNA was extracted from livers of immature spottail shiners using the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). Following RNA isolation, RT-PCR was performed to determine VTG mRNA levels. The primers used for RT-PCR of VTG were 5'-AGCATCAACGACAAACGAC-3' (forward) and 3'-AAACCTGAGACACCACTGGTTAAG-5' (reverse). Primers specific for the 28S rRNA, 5'-TGCAGATGATGTGTTGTAAGTGCG-3' (forward) and 3'-AGAGCGCACTTCCATGTCGAAGTT-5' (reverse) were used as internal controls. A 2 µg aliquot of total RNA from immature shiners provided template for the reverse transcription reaction (RT). RT was performed in a 20 µl reaction volume with a final concentration of 1.5 mM 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl [pH 9.0], 3.5 mM MgCl$_2$, 1 mM dNTP, 1 U RNase inhibitor, 20 U reverse transcriptase, 0.75 mM of the 3'-VTG primer, and 0.75 mM 3'-28S primer at room temperature for 10 min and then at 42°C for 60 min. PCR was performed in a 50 µl reaction volume with a final concentration of 1.5 mM 10X PCR buffer, 0.5 mM MgCl$_2$, 0.2 mM dNTPs, 2.5 U Taq DNA polymerase, 0.5 µM forward, and 0.5 µM reverse primer. Amplification was carried out with 28 cycles (linear amplification) at 94°C for 5 min, 55°C for 1 min 30 s, and 72°C for 2 min for VTG and 11 cycles (linear amplification) at 94°C for 5 min, 55°C for 1 min 30 s, and 72°C for 2 min for the 28S. Aliquots of 10 µl of the reaction products were then analyzed on a 1.2% agarose gel containing ethidium bromide. Gels were scanned using a Bio-Rad Fluor Image analyzer and quantified using the integrated area under the curve for each band.

The PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen, Burlington, Ontario, Canada) and used to transfect *Escherichia coli*. Plasmids were amplified and purified from individual colonies using the Qiagen Plasmid Purification Kit (Qiagen, Mississauga, Ontario, Canada) according to the manufacturer’s instructions. The insert was digested from the plasmid with EcoRI restriction enzyme (Amersham Biosciences, Baie d’Urfe, Quebec, Canada) and sequenced using an automated sequencer (Sheldon Biotechnology Centre, McGill University, Montreal, Quebec, Canada).

**Spermatogenesis.** Male spottail shiners were collected with a beach seine at different sites in June 2002 along the St. Lawrence River: Îles de la Paix, the Centre, McGill University, Montreal, Quebec, Canada) and sequenced using an automated sequencer (Sheldon Biotechnology Centre, McGill University, Montreal, Quebec, Canada). The amplified cDNA product for spottail shiner VTG was 556 bp long (GenBank access number AY365131). The VTG transcript was 95% homologous with rainbow trout (*Oncorhynchus mykiss*) VTG. RNA from a mature female rainbow trout was used as a positive control in RT-PCR reactions (Fig. 2). VTG mRNA levels were standardized using a 28S RNA internal standard which resulted in the synthesis of a 524 bp amplicon, which was then used to standardize mRNA loading. The linearity of the PCR amplifications was determined experimentally and the number of cycles used for both VTG and the 28S rRNA was done within the linear range of the assay (Fig. 3).

**RESULTS**

**Size and Condition Factor**

External examination of the fishes showed no overt abnormalities such as fin erosion, gross malformation of the spine, external lesions, or visible tumors. The length, weight, and condition factor were not significantly different among sites (data not shown). Analyses of covariance indicate that there were no differences between the three variables for each of the collection sites. The fact that there was no decrease in the condition factor suggests that the fish were not overtly affected by exposure to xenoestrogens (at least with respect to somatic growth) and were not moribund.

**Hepatic VTG mRNA Levels**

The amplified cDNA product for spottail shiner VTG was 556 bp long (GenBank access number AY365131). The VTG transcript was 95% homologous with rainbow trout (*Oncorhynchus mykiss*) VTG. RNA from a mature female rainbow trout was used as a positive control in RT-PCR reactions (Fig. 2). VTG mRNA levels were standardized using a 28S RNA internal standard which resulted in the synthesis of a 524 bp amplicon, which was then used to standardize mRNA loading. The linearity of the PCR amplifications was determined experimentally and the number of cycles used for both VTG and the 28S rRNA was done within the linear range of the assay (Fig. 3).
In 1999, VTG mRNA levels were significantly higher at Île Dorval, Boucherville, Îlet Vert, and Île Saint-Ours as compared with the reference site at Îles de la Paix (Fig. 4A). This led us to hypothesize that xenoestrogens may be originating from the Ottawa River. Therefore we expanded our sampling effort in 2000 to encompass new sites in the St. Lawrence River and a site at the mouth of the Ottawa River (Fig. 1). In the year 2000 sampling campaign, the highest concentrations of hepatic VTG mRNA were observed in fish from Îlet Vert, Île Beauregard, and Île Saint-Ours, all of which are downstream sites of the MUC wastewater discharge (Fig. 4B). VTG mRNA concentrations were also significantly higher in fishes captured upstream of the MUC wastewater discharge, including the Ottawa River, as compared with the reference site at Îles de la Paix.

Effects on Spermatogenesis

Testes of spottail shiners consist of two dorsal elongated structures. Each testis contains a sperm duct that runs lengthwise. Examination of histological sections of the testis by light microscopy indicates that the testis is surrounded by a continuous layer of connective tissue and is composed of cysts containing germ cells with interstitial spaces containing myoid cells present at all the stages of the reproductive cycle. Representative stages of development are shown in Figure 5.

Stages II through IV represent the process of spermatogenesis, during which the cysts increase in size and the spaces between the cysts become narrow. The number of spermatogonia gradually decreases in stage II to IV due to their development into spermatocytes (Fig. 5A–C). Spermatocytes undergo meiosis to produce spermatids (stage III, Fig. 5B), and eventually spermatozoa (stage IV, Fig. 5C). Spermiation (stage V, Fig. 5D) is the release of sperm cells from the cysts into the tubules; the tubules are filled with spermatozoa and the inter-

FIG. 2. RT-PCR of hepatic VTG in a mature vitellogenic female spottail shiner and rainbow trout (positive control). Lane 1 contains the molecular ladder; Lane 2 contains the RT-PCR product obtained for spottail shiner VTG cDNA; and Lane 3 contains amplified rainbow trout VTG cDNA. The DNA was visualized by staining the gel with ethidium bromide (0.1% w/v).

FIG. 3. Linear PCR amplification of spottail shiner VTG (A) and 28S rRNA (B). RNA was isolated from the liver of mature female spottail shiners and reverse transcribed as described in the Material and Methods. Using specific primers, VTG or the 28S rRNA were amplified over a range of PCR cycles. PCR products were electrophoresed on an agarose gel and stained with ethidium bromide. The volume of the PCR products were determined by measuring the optical density of the DNA bands using a Bio-Rad Fluor Image Analyzer. VTG amplification was linear from 26 to 30 cycles while the 28S rRNA was linear from 10 to 13 cycles.
stitium becomes very narrow. Following stage V, the testes regress.

In order to determine if spermatogenesis was altered by exposure to estrogenic contaminants in the St. Lawrence River, stages of spermatogenesis were determined for spottail shiners captured at different sites along the St. Lawrence River (Fig. 6). Histological analyses of shiner testes from the reference site (Îles de la Paix) indicated that fish had testes of stage IV (50%) and stage V (45%) of spermatogenesis, indicating that spermatogenesis was either nearly completed or completed (only 5% of the fish were still at stage III of development). At Île Dorval, where VTG mRNA levels were moderate, fish had testes at stage III (38%) and stage IV (45%), and only 15% of testes were at stage V of development. In contrast, at Îlet Vert and Île Beauregard, where hepatic VTG mRNA levels were high, none of the testes were at stage V, and 8% of testes at Îlet Vert were still at stage II of development. The majority of fish testes from these sites were at stages III and IV of development.
These data indicate that spermatogenesis is markedly delayed at sites where shiners were exposed to xenoestrogens. Effect of Xenoestrogen Exposure on Fish Spermatozoa Motility

Milt from male spottail shiners at Îlet Vert were compared with fish captured at Îles de la Paix. Motility parameters were generated by computer assisted semen analysis. The spermatozoa appeared bright against a dark background and their movement was clearly visible. Sperm concentrations were significantly lower in shiners from Îlet Vert as compared with Îles de la Paix shiners (Fig. 7). Progressive motility was significantly reduced in sperm from fish captured at Îlet Vert (Fig. 8). Spermatozoa were classified according to their swimming velocity: static spermatozoa (<5 μm/s), slow spermatozoa (5–10 μm/s), medium motile spermatozoa (10–20 μm/s), and rapid spermatozoa (>20 μm/s). Results show that xenoestrogen exposure had a significant effect on certain sperm motility parameters. Analysis of data based on speed indicated a significant decrease in the percentage of rapid cells and a significant increase in the percentage of static cells in the milt of spottails from Îlet Vert (Fig. 9). Path velocity, progressive velocity, and curvilinear velocity were also lower in fish from Îlet Vert (Fig. 10). Beat cross frequency did not differ between shiners from the two sites. Average straightness and linearity was also significantly reduced in the milt from Îlet Vert (Fig. 11). These results indicate that in fish exposed to municipal effluent and which had induced VTG mRNA levels, sperm concentrations and most sperm motility parameters were significantly affected.

Intersex

The presence of intersex in male fish was determined for fish sampled at Îles de la Paix, Île Dorval, Îlet Vert, and Île Beauregard (Fig. 12). Intersex testes are comprised primarily of testicular tissue, but with one or more oocytes located randomly within the testicular tissue. At Îles de la Paix, our reference site, a single fish had an oocyte in its testis (2.6% of fish sampled; n = 38). At Île Dorval, 15% (n = 13) of male fish exhibited intersex, while 31% (n = 13) of fish at Îlet Vert and 27% (n = 11) of fish at Île Beauregard exhibited intersex (Fig. 12). There was a significant inverse correlation between the

**FIG. 7.** Sperm concentration (mean spermatozoa concentration in millions/μl) in spottail shiners captured at Îles de la Paix and Îlet Vert. Milt was obtained from isolated testes and diluted 100-fold with dilution buffer. Spermatozoa were then activated by a further 1:100 dilution in Tris-HCl (30 mM) alone. Sperm concentration was assessed using the IVOS semen analyzer. Data are expressed as the mean ± SEM. Asterisks indicate significant differences between groups (p < 0.05).

**FIG. 8.** Mean percentage sperm motility from spottail shiners captured at Îles de la Paix and Îlet Vert. Data are expressed as the mean ± SEM. Asterisks indicate significant differences between groups (p < 0.05).
presence of intersex testes and stage V of spermatogenesis. Together these data indicate that at sites where VTG mRNA were elevated, there was also an increase in the percentage of fish with intersex testes.

**DISCUSSION**

Experiments involving long-term exposure are required in order to fully understand the physiological consequences as-

![FIG. 9.](image9.png) Mean percentage of (a) rapid, (b) medium, (c) slow, and (d) static spermatozoal cells from spottail shiners captured at Îles de la Paix and Îlet Vert. Data are expressed as the mean ± SEM. Asterisks indicate significant differences between groups ($p < 0.05$).

![FIG. 10.](image10.png) Sperm motility parameters from spottail shiners captured at Îles de la Paix and Îlet Vert. Mean (a) Straightness (b) linearity was determined using the IVOS semen analyzer. Data are expressed as the mean ± SEM. Asterisks indicate significant differences between groups ($p < 0.05$).

![FIG. 11.](image11.png) Sperm motility parameters from spottail shiners captured at Îles de la Paix and Îlet Vert. Mean (a) path velocity, $V_{AP}$; (b) progressive velocity, $V_{SL}$; (c) curvilinear velocity, $V_{CL}$; (d) beat frequency, $BCF$, were determined using the IVOS semen analyzer. Data are expressed as the mean ± SEM. Asterisks indicate significant differences between groups ($p < 0.05$).

![FIG. 12.](image12.png) Intersex in male spottail shiners captured at different sites along the St. Lawrence River. Testes were fixed in Bouin’s solution and mounted in paraffin. Sections (5 μm section) were mounted on glass slides and stained with hematoxylin and eosin. Sections were examined under the light microscope. Results are expressed as percentage of fish exhibiting intersex.
sociated with chronic, life-long environmental exposure to xenoestrogens. Our results indicate that water from within the municipal effluent plume from the city of Montreal contains estrogenic compounds, which can be monitored using VTG mRNA levels in immature spottail shiners. Unexpectedly, the results indicate that fish sampled upstream from the Montreal sewage discharge point also had induced VTG mRNA levels, for example at Île Dorval. While this contamination may be due to other industries and sources, sewage overflow outlets are present along the Island of Montreal and these can release effluent into the St. Lawrence River upstream from the main discharge point when the system is saturated or following heavy rainfall. Therefore, we cannot rule out the possibility that sewage effluents along the Island of Montreal are responsible for the sizeable estrogenic contamination of the St. Lawrence River. Furthermore, the Ottawa River also appears to contribute xenoestrogens to the St. Lawrence River as indicated by significantly higher VTG mRNA levels in fish sampled at the mouth of the Ottawa River.

There is evidence that environmental estrogens can alter testicular function in fish. Our results indicated a marked delay in the stages of spermatogenesis in shiners from sites containing significant levels of xenoestrogens. In fact, at sites where VTG mRNA levels are high, none of the spottail shiner testes were at stage V of spermatogenesis, compared with 45% of the testis in fish from our reference site. These findings are supported by a study on the effects of estrogen on spermatogenesis in male trout. Estrogen treatment via diet resulted in a marked decrease in spermatogenesis in rainbow trout (Billard et al., 1981) compared with fish exposed to natural estrogens. On the other hand, another study on the effects of alkylphenol on spermatogenesis in fully mature trout reported no effect on spermatogenesis (Jobling et al., 1996). This may be explained by the fact that the period of spermatogenesis, in which germ cells develop, is more sensitive to estrogenic treatment and may be associated with failure of Sertoli cells to develop (Billard et al., 1982). One of the differences between laboratory and field studies is that fish in the wild are likely exposed to estrogenic contaminants over the entire reproductive cycle, a situation which can be difficult to reproduce under laboratory conditions with seasonal spawners.

Estradiol receptors have been identified in fish testis. Wu et al. (2001) reported that the α- and β-isoforms of the estradiol receptor are localized in secondary spermatocytes and spermatids of channel catfish. Furthermore, Bouma and Nagler (2001) reported the localization of the estrogen receptor-α in precursors of Leydig cells in the testis of rainbow trout during the early stages of the reproductive cycle, and in fully differentiated Leydig cells. Whether or not the effects of xenoestrogens present in the St. Lawrence River on spermatogenesis in spottail shiners is the result of a direct effect on developing germ cells, inhibition of androgen synthesis or action via the hypothalamo-pituitary-gonadal axis (Christiansen et al., 1998) remains to be established.

While other environmental factors such as temperature are known to alter spermatogenesis, the water temperature in the present varied by less than 2°C between collection sites and all fish were collected within 4 days of each other. Furthermore, other factors such as dissolved oxygen levels did not vary between sites in the St. Lawrence, a large river system whose water is well oxygenated. While in a field study such as this, it is impossible to limit all variables, the weight of evidence strongly suggests that the effects observed on spermatogenesis are the result of effects related to the presence of xenoestrogens.

The sperm motility data of the fish from sites exposed to xenoestrogens were compared with those obtained from a nonestrogenic reference site using CASA. This approach has been used to monitor the effects of heavy metals on sperm quality (Rurangwa et al., 1995), to improve the efficiency of cryopreservation and storage (McNiven et al., 1993; Rurangwa et al., 2001), and to optimize conditions for fertilization (Dulinsky, 1982; McMaster et al., 1992; Morisawa et al., 1983; Toth et al., 1995). Sperm concentrations in fish from Îlet Vert were significantly lower than those in fish from Îles de la Paix (reference site).

A recent study of roach (Rutilus rutilus) in the UK, indicated that sperm concentrations ranged from 6,000,000–8,000,000/μl (Jobling et al., 2002). While sperm concentrations or sperm density vary among species of fish, the higher sperm concentration observed in shiners is likely attributable to the different method of collection (manual stripping after administration of pituitary extract versus the removal of testis and extracting sperm). Differences in the time of sampling or age of the fish may also contribute to interspecies differences.

This decreased gamete concentration in shiners from Îlet Vert was well correlated with the high VTG mRNA levels, the high incidences of intersex fish, and the observed delay in spermatogenesis. Studies have shown that exposure to estrogens or xenoestrogens which induce VTG in fish (Christiansen et al., 1998; Hill and Janz, 2003) have inhibitory effects on fish spermatogenesis (Hassannin et al., 2002; Jobling et al., 2002), sperm production (Haubruege et al., 2000; Jobling et al., 2002), and sperm motility (Jobling et al., 2002; McMaster et al., 1992).

In this study, shiners from Îlet Vert exhibited a lower percentage of progressively motile sperm when compared to our reference site. To further evaluate sperm motility, sperm were classified into four different classes according to speed (rapid, medium, slow, or static). Our observations suggest that different subpopulations of spermatozoa based on sperm velocity coexist within the milt. Distribution of sperm among the different categories of velocities varied between fish from Îles de la Paix and Îlet Vert. There was a significant increase in the number of static spermatozoa and a concomitant decrease in the number of rapid spermatozoa in shiners from Îlet Vert. The results from the present study demonstrate that sperm concentration and percentage of motile spermatozoa may not always
tell the whole story, especially if effects cause abnormal sperm motion. Spottail shiner spermatozoa displayed a linear motility pattern, unlike trout spermatozoa, which exhibit distinct temporal phases of swimming starting with a circular path, lasting up to 5 s, followed by a linear path (up to 60 s; Boitano and Omoto, 1992). Velocity parameters (VAP, VSL, VCL) were decreased in spermatozoa of shiners from Îlet Vert. These parameters directly express sperm motion (swimming speed) and decreased low velocities may reduce the probability of the spermatozoa to reach the micropyle. Investigations have revealed that teleost spermatozoa must swim actively into the micropylar channel for successful fertilization (Hart, 1990; Iwamatsu et al., 1993).

Spermatozoal motility in teleost fish is activated when they come in contact with water and the motility lasts for only a few minutes. Although good fertilization rates were obtained from Atlantic salmon and rainbow trout spermatozoa that showed little or no motility (Erdahl and Graham, 1987; Levanduski and Cloud, 1988), these studies are confounded by the limitations of the traditional subjective scoring methodology in defining sperm motility and the low sensitivity of fertilization tests with excessive spermatozoa. A relationship between motility and the capacity for fertilization for teleost spermatozoa has been confirmed by several other authors (Billard and Cosson, 1992; Ohta et al., 1995). Though the efficiency of fertilization was not determined in this study, it stands to reason from the motility studies that spermatozoa in shiners from Îlet Vert will be at a disadvantage, considering the shorter distance of displacement due to its decreased velocity; thereby reducing the probability of spermatozoa meeting with the micropyle. Also given that the sperm activation conditions in the present study were maintained between fish, and sperm motility parameters were examined immediately after activation, the results from this study indicate that spermatozoa from fish captured at Îlet Vert have an initial “poor” capacity to move.

Histological examination of spottail shiner testes revealed that fish with induced VTG mRNA levels had a high incidence of intersex. While intersex male fish were found at all sites, the incidence of intersex ranged from 2.6% for the reference site to 31% at Îlet Vert where VTG mRNA levels were high (Fig. 12). This finding is similar to a recently published field study of intersex in the roach (Jobling et al., 2002). In these experiments, fish were sampled both upstream and downstream of sewage treatment works in the UK. They reported that intersex was also found at all sites and that the incidence of intersex ranged from 4% (at two reference sites) and increased to as much as 100% in fish downstream of the sewage treatment (Jobling et al., 1998). The low proportion of intersexuality at the reference site seen our studies is comparable with the reported level of intersexuality in roach (Jobling et al., 1998) and carp of 5% (Billard et al., 1981). Interestingly, in the roach, gamete production, milt release, and sperm motility were all reduced in fish living in aquatic habitats receiving input from sewage effluent (Jobling et al., 2002). These results are similar to observations from the present study on spottail shiners in the St. Lawrence River. Further studies will be needed to determine potential effects on fecundity and development of the F-1 progeny, both of which were reported to be altered in the roach.

In conclusion, exposure of immature and male spottail shiners to xenoestrogens in the St. Lawrence River was widespread, as indicated by the induced levels of VTG mRNA. Furthermore, this exposure was shown to have marked effects on their reproductive function in males. Exposure to xenoestrogens was linked to delayed spermatogenesis, reduced spermatozoal production, decreased sperm motility, and high incidence of intersexuality. This is among the first studies which demonstrate that exposure of wild fish to xenoestrogens is correlated with a reduction in male reproductive function, and suggests that fish populations may be affected in the St. Lawrence River as a result of altered reproductive functions.

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