2013

Reproductive health of yellow perch Perca flavescens in selected tributaries of the Chesapeake Bay

Vicki S. Blazer
U.S. Geological Survey

Alfred E. Pinkney
U.S. Fish and Wildlife Service

Jill A. Jenkins
U.S. Geological Survey

Luke R. Iwanowicz
U.S. Geological Survey, liwanowicz@usgs.gov

Steven Minkkinen
U.S. Fish and Wildlife Service

See next page for additional authors

Science of the Total Environment 447 (2013) 198–209

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Reproductive health of yellow perch *Perca flavescens* in selected tributaries of the Chesapeake Bay

Vicki S. Blazer [a,*], Alfred E. Pinkney [b], Jill A. Jenkins [c], Luke R. Iwanowicz [a], Steven Minkkinen [b], Rassa O. Draugelis-Dale [c], James H. Uphoff [d]

[a] U.S. Geological Survey, National Fish Health Research Laboratory, Leetown Science Center, 11649 Leetown Road, Kearneysville, WV 25430, USA
[b] U.S. Fish and Wildlife Service, Chesapeake Bay Field Office, 177 Admiral Cochrane Drive, Annapolis, MD 21401, USA
[c] U.S. Geological Survey, National Wetlands Research Center, 700 Cajundome Blvd., Lafayette, LA 70506, USA
[d] Maryland Department of Natural Resources, Fisheries Service, Cooperative Oxford Laboratory, 904 South Morris Street, Oxford, MD 21654, USA

HIGHLIGHTS

- Reduced recruitment of yellow perch has occurred in urban tributaries of Chesapeake Bay.
- We compared reproductive health biomarkers in perch from two urban, one developing, two less developed watersheds.
- Lack of final maturation, abnormal yolk and zona pellucida were noted in females.
- Leydig cell proliferation was noted in males.

ARTICLE INFO

Article history:
Received 3 July 2012
Received in revised form 21 December 2012
Accepted 21 December 2012
Available online 4 February 2013

Keywords:
Yellow perch
Chesapeake Bay
Reproductive health
Oocyte maturation
Leydig cell proliferation

ABSTRACT

Reduced recruitment of yellow perch has been noted for a number of years in certain urbanized watersheds (South and Severn Rivers) of the Chesapeake Bay. Other rapidly developing watersheds such as Mattawoman Creek are more recently showing evidence of reduced recruitment of anadromous fishes. In this study, we used a battery of biomarkers to better document the reproductive health of adult yellow perch collected during spring spawning in 2007–2009. Perch were collected in the South and Severn Rivers, Mattawoman Creek and the less developed Choptank and Allen's Fresh watersheds for comparison. Gonadosomatic indices, plasma reproductive hormone concentrations, plasma vitellogenin concentrations and gonad histology were evaluated in mature perch of both sexes. In addition, sperm quantity (cell counts) and quality (total and progressive motility, spermatogenic stage and DNA integrity), were measured in male perch. Many of these biomarkers varied annually and spatially, with some interesting statistical results and trends. Male perch from the Choptank and Allen's Fresh had generally higher sperm counts. In 2008 counts were significantly lower in the perch from the Severn when compared to other sites. The major microscopic gonadal abnormality in males was the proliferation of putative Leydig cells, observed in testes from Severn and less commonly, Mattawoman Creek perch. Observations that could significantly impact egg viability were an apparent lack of final maturation, abnormal yolk and thin, irregular zona pellucida. These were observed primarily in ovaries from Severn, South and less commonly Mattawoman Creek perch. The potential association of these observations with urbanization, impervious surface and chemical contaminants is discussed.
Published by Elsevier B.V.

1. Introduction

The native range of yellow perch (YP) *Perca flavescens* includes much of Canada, from central Canada east and southeast through the Great Lakes–St. Lawrence and the upper Mississippi basins and on the Atlantic slope from Maine to Georgia (Grzybowski et al., 2010). Historically, YP populations in Chesapeake Bay and the Great Lakes supported major recreational and commercial fisheries (Piavis, 1991; Wells, 1977). However, at selected sites or tributaries in both geographic regions, major population declines and a lack of recruitment have occurred (Yellow Perch Work Group, 2002; Clapp and Dettmers, 2004; Wilberg et al., 2005).

In the Chesapeake Bay (Fig. 1), commercial YP harvest began to decline during the mid-1960s and reached its nadir during 1976–1982 (Yellow Perch Work Group, 2002). Declines in YP recreational fishing participation in the urbanizing subestuaries within the Baltimore–Washington corridor became evident in the 1980s (O'Dell 1987) and

* Corresponding author at: U.S. Geological Survey, National Fish Health Research Laboratory, 11649 Leetown Road, Kearneysville, WV 25430, USA. Tel.: +1 304 724 4434; fax: +1 304 724 4435.
E-mail addresses: Vblazer@usgs.gov (V.S. Blazer), Fred_Pinkeny@fws.gov (A.E. Pinkney), jenkinsj@usgs.gov (J.A. Jenkins), Liwanowicz@usgs.gov (L.R. Iwanowicz), steve_minkkinen@fws.gov (S. Minkkinen), daler@usgs.gov (R.O. Draugelis-Dale), juphoff@dnr.state.md.us (J.H. Uphoff).

0048-9697/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.scitotenv.2012.12.088

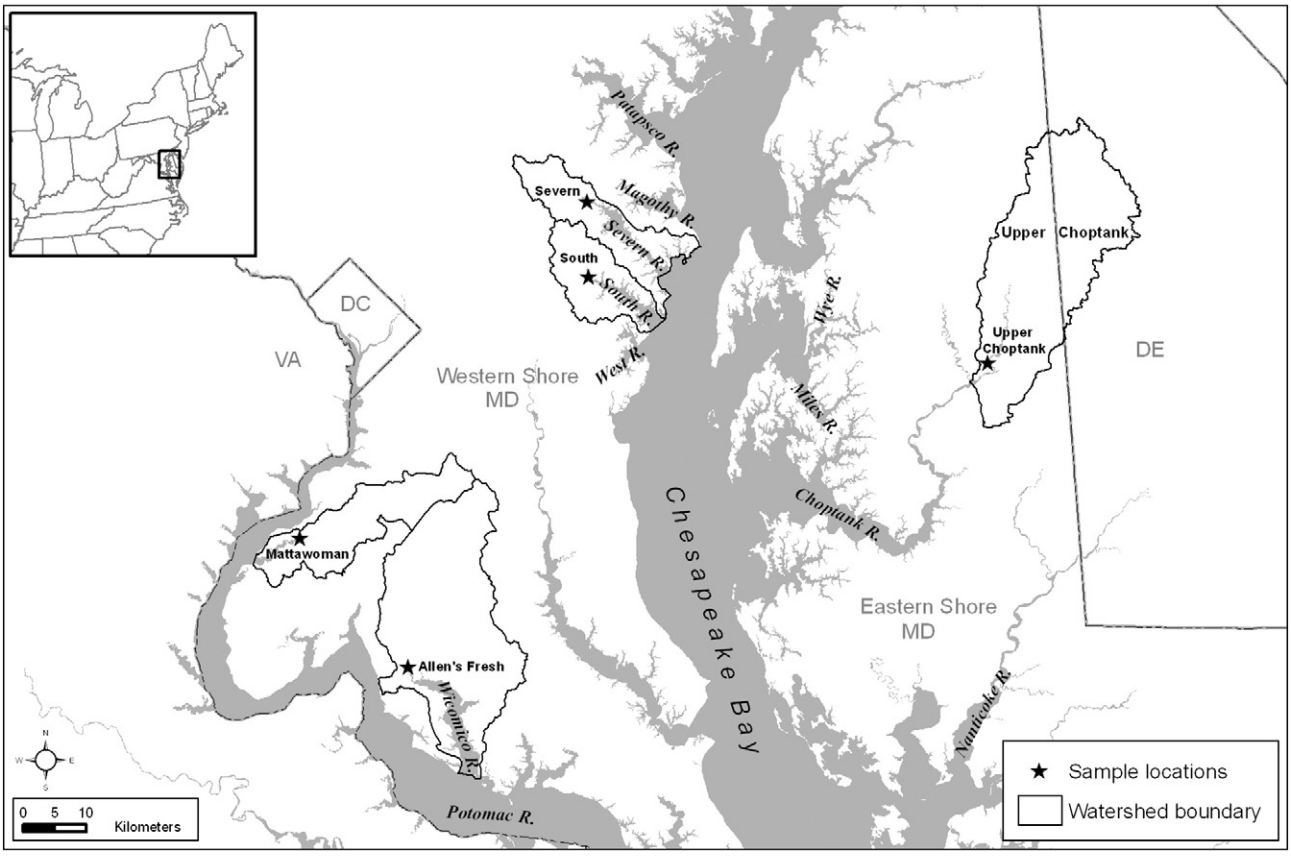

Fig. 1. Watersheds and sampling locations for collections of yellow perch in Chesapeake Bay tributaries 2007 through 2009.

these declines were attributed to habitat degradation by urban/suburban development (Yellow Perch Work Group, 2002; Uphoff et al., 2005). Subestuaries, including the Severn and South Rivers, closed to YP fishing for nearly 20 years, were reopened for recreational fishing in 2009. The rationale (for the Severn, South and West Rivers) being that these watersheds are extremely degraded habitat, reproductive output is low, while the adult population appears stable and therefore recreational harvest would not impact the total reproductive capacity (Maryland Fisheries Service, 2012).

Yellow perch are semi-anadromous in the Chesapeake Bay watershed and adults remain in their natal tributaries. As a result, they are exposed to chemical mixtures and stressors typical of their respective tributaries. Upstream spawning migration of adults and the subsequent downstream dispersal of juveniles are their primary movements (Muncy, 1962; Piavis, 1991). The adults migrate from the lower parts of the tributaries to the upper regions in search of suitable spawning habitat in late February–early March. Males tend to reach the freshwater spawning areas first and do not migrate downstream until the females leave. In the 1950s studies in the Severn River indicated YP first appeared when water temperatures were 3.9–6.7 °C and peak spawning occurred at water temperatures of 5.6–7.2 °C (Mansueti, 1964). Later studies indicated peak spawning occurs when water temperatures reach sustained levels of 8.5 to 11 °C (Hardy, 1978). Peak spawn is usually fewer than five days and in some years two to three days (S. Minkkinen, personal observation).

During spawning females extrude distinctive, long, accordion-like strands of eggs, which have a thick gelatinous egg membrane (Mansueti, 1964). Two indicators that have been used to assess reproductive success and year class strength in selected tributaries are egg mass surveys and larval abundance. The Coastal Conservation Association of Maryland conducts egg mass surveys in YP spawning reaches. These surveys are performed by volunteers making counts or semi-quantitative estimated counts (i.e. >100 or >300 egg masses per 50 m of shoreline) by walking along the shore or on the water by kayak or other watercrafts. These data are not consistently collected annually for all tributaries; however, they provide useful comparative observations suggestive of differential spawning success. For instance, counts from survey data in 2005–2009 were consistently high in the Choptank (>200) and Mattawoman (>100 except 82 in 2008). Counts in Allen's Fresh (a tributary of the Wicomico River on the western shore of the Chesapeake Bay) varied annually from 350 in 2006 to 40 in 2009. Counts have been consistently low (<100 and often <50) in the South and Severn with none observed in the South in 2009 and only seven in the Severn in 2006 (Coastal Conservation Association Maryland, Ken Hastings, personal communication).

Larval presence, L_p, is defined as the proportion of 0.5 m plankton tows with larvae during the peak weeks from late March through early May, and is used as an indicator of year class strength. The L_p index integrates egg production, egg hatching success, and survival of first-feeding larvae. Brackish systems with small watersheds and high levels of development (South, Severn and Magothy Rivers) have exhibited a persistent depression in L_p, below a reference minimum since 2002. Regression analyses indicated that development (percent of impervious surface in the watershed, i.e. pavement, rooftops and compacted soils) was negatively related to L_p. Other systems may exhibit wide variation in larval presence, but the low levels similar to those seen in these urbanized subestuaries are not common. However, since 2008 spawning site loss for anadromous fishes is evident in the developing Mattawoman Creek (Uphoff et al., 2010, 2011).

Neither excessive adult mortality nor reduced growth has been observed in Severn River YP. Additionally, Severn River broodstock induced to spawn in the hatchery produced visually abnormal egg chains and too few viable eggs to support hatchery production. Hence, the depressed egg and larval survival are hypothesized to be critical factors suppressing resident YP populations in western shore

subestuaries subject to high levels of development (Uphoff et al., 2005). However, neither the biological basis for the depressed egg and larval survival, nor specific chemicals or other stressors leading to these effects has been documented. Hence, the goal of the current study was to compare the reproductive health of spawning adult YP from historically important spawning reaches in the Chesapeake Bay watershed to better understand the biological effects leading to reduced reproduction. Yellow perch from two developed tributaries (South and Severn) with evidence of population declines were compared to those from Mattawoman Creek with intermediate but increasing development and the less developed Choptank River and Allen's Fresh. A broad suite of indicators including gonadal histology, plasma vitellogenin (Vtg), sex steroid hormone concentrations and sperm quality parameters was assessed in multiple years.

2. Materials and methods

2.1. Site descriptions

Yellow perch were collected during spawning runs in the Choptank River (CH) at Mill Creek near Route 16; South River (SO) near Defense Highway; Severn River (SE) at Severn Run; Mattawoman Creek (MA) at Mason's Branch and Allen's Fresh (AF), Zekiah Swamp above Route 234. Watersheds for the areas sampled (Fig. 1) were delineated using 12-digit level hydrologic units developed as part of the National Watershed Boundary Database (USGS and USDA, 2011) except the Choptank River, where only the upper portion (as delineated by Maryland DNR) was used. Salinities ranged from 0.2 to 11.0 ppt for MA and CH, 0.7–3.6 ppt for AF, 0.1–6.9 ppt for SO, and 8.2–10.2 ppt for SE. Within each watershed, general land use proportions (developed, agriculture, forest, water, other) and impervious surface (Table 1) characteristics were estimated for the year 2010 using the Phase 5.3.2 Land Use Dataset (Claggett et al., in press).

To aid in the interpretation of the biological data, river discharge data were obtained from the nearest U.S. Geological Survey (USGS) gage data (AF: gage 01660920; CH: gage 01491000; MA: gage 01658000; SE: gage 01589795; http://md.water.usgs.gov/data/sw_active.html). The average discharge for the two months prior to spawning (January and February) in each year was calculated as a percentage of 8–10 year average (2000 through 2009 for AF, CH and SE; 2002 through 2009 for MA). No data were available for SO (Table 1). Water temperatures were recorded over eight days prior to sampling (Table 2).

Table 1
Land use, impervious surface and average discharge for watersheds containing yellow perch collection sites 2007–2009.

Parameters	Choptank	Mattawoman	Allen's Fresh	South	Severn
Watershed size					
# Hectares	132,253	25,059	64,218	17,091	22,253
Landuse[a]					
% Developed	8.2	44.7	22.1	81.2	69.3
% Agriculture	58.5	5.1	19.7	0.3	1.0
% Forest	29.3	43.0	46.2	2.0	9.1
% Water	1.6	2.9	7.6	13.3	18.8
% Other	2.4	4.3	4.4	3.2	1.8
Impervious surface					
% Impervious	1.7	10.2	4.8	24.6	20.7
Average discharge[b]					
2007	104.9	116.2	131.1	ND[c]	87.0
2008	50.4	85.7	43.6	ND	87.2
2009	44.0	46.3	44.3	ND	77.0

[a] Landuse categories and impervious surface estimated for 2010 using the Phase 5.3.2 Land Use Dataset (Claggett et al., in press).
[b] Average discharge (cubic feet/second) for the two months prior to spawning is presented as a percentage of the 8–10 year average (2000 through 2009).
[c] ND indicates no data.

Table 2
Average water temperatures (°C) recorded 8 days prior to yellow perch sampling in tributaries of the Chesapeake Bay.

Site	Year sampled		
	2007[a]	2008	2009
Choptank	7.1	6.1; 9.0[c]	5.1
Mattawoman	10.7	10.1	4.7
Allen's Fresh	NS[b]	10.1	6.9
South	11.6	5.2:5.7[c]	NS
Severn	9.0	5.1	5.1

[a] Temperatures in 2007 were recorded on the sampling date, only.
[b] Site was not sampled.
[c] Fish were collected on two different dates for the Choptank River (03/07 and 03/12) and the South River (03/05 and 03/07) in 2008.

2.2. Field procedures and sampling handling

Adult YP were collected during spawning runs in 2007 (3/6–3/15), 2008 (3/5–3/12) and 2009 (3/09–3/11). Fish were captured in fyke nets, transported in aerated river water from the respective collection site to the FWS Chesapeake Bay Field Office and necropsied on the same day. Fish were euthanized with an overdose of MS-222 (Finquel; Argent Chemical Co., Redmond, WA) and each fish was weighed (g) and measured for total length (mm). Fish were bled from the caudal vessels using heparinized 3 cm^3 syringes with 23 gage needles. Blood was placed in heparinized Vacutainer tubes containing 62 units sodium heparin (Fisher Scientific, Pittsburgh, PA) and stored on wet ice until returned to the laboratory. Blood was centrifuged for 10 min at 1000 $\times g$ at 4 °C for plasma separation within 4 h of collection. Plasma was aliquoted into separate cryovials for sex steroid hormone and vitellogenin (Vtg) analyses and stored at −80 °C. Liver and gonads were removed and weighed (mg). Gonadosomatic index (GSI; gonad weight/body weight × 100) was calculated. Pieces of liver, spleen, kidney, gill, ovary and one lobe of the testes were removed and placed in Z-Fix™ fixative (Anatech LTD, Battle Creek, MI) for histological examination. The other testis lobe was placed into a conical tube completely filled with calcium and magnesium free Hank's balanced salt solution (HBSS, pH 7.5, 311 mOsm, Glenn, 1998) and shipped overnight on wet ice to Veterinary Clinical Sciences, Louisiana State University School of Veterinary Medicine in Baton Rouge for sperm motility analyses. Other sperm quality analyses were performed at the National Wetlands Research Center, Lafayette, LA. Scales and/or otoliths were removed for estimating age of each fish collected.

2.3. Laboratory methods

2.3.1. Age determination

Age was calculated based primarily on otoliths, although scales were used to estimate age when otoliths were not available. Whole otoliths were placed in black porcelain trays and examined under reflected light using a dissecting microscope at 10× magnification (Maciena and Betsill, 1987). Annuli were distinguished as opaque zones separated by clear areas. Scales were mounted on glass slides and viewed with a microfiche reader. Annuli were identified using this method as described by Pearson (1929). While otoliths provide the more precise estimate of age, studies in the Chesapeake watershed (Casey et al., 1988), as well as in other geographic regions (Vandergoot et al., 2008; Niewinski and Ferreri, 1999), indicate that scales can be used to adequately describe the age structure, particularly in YP less than six years of age.

2.3.2. Plasma analyses

Yellow perch Vtg standards were prepared using YP originating from the CH and concentrations of plasma Vtg were determined by direct enzyme-link immunosorbent assay (ELISA) using the monoclonal

antibody (mAb) 3G2 (purified from striped bass plasma, Cayman Chemical Co., Ann Arbor, MI) (see Supplemental methods for more information). Plasma hormone concentrations, 17β-estradiol and testosterone were measured using radioimmunoassay according to Sower and Schreck (1982) (see Supplemental methods for more information).

2.3.3. Gonad histology

Pieces of tissue fixed for histology were routinely processed, embedded into paraffin and sectioned at 6 μm (Luna, 1992). At least five cross sections along the length of each gonad were sectioned and examined. Observations from gonad histology were used to confirm sex, determine reproductive stage, and to detect the presence of abnormalities as described by Blazer (2002) and Dietrich and Krieger (2009). Microscopic abnormalities were rated on a scale of 0–4; 0 not observed, 1 minimal, 2 mild, 3 moderate and 4 severe. Histologic slides were read blind (without knowledge of the site of origin).

2.3.4. Motility of spermatozoa

Aliquots of milt were diluted in HBSS for motility assessed by computer assisted motion analysis (CASA) using 2 μl of activated milt in a chamber slide (Leja 20 SC20-010040-B, Leja Products, Nieuw-Vennep, The Netherlands) and viewing with phase microscopy (Olympus BX41, Olympus America, Inc., Center Valley, PA) at 200× magnification. Due to the short-lived motility, only one visual field or 500 cells per sample was electronically captured and analyzed (SpermVision, Version 3.0, Minitube of America, Verona, WI). Software settings included: area of cell identification 8 to 35 μm^2; immotile at average orientation of head (μm) (AOC) + distance straight line (μm) (DSL) < 25 and DSL < 1; locally motile at DSL < 5; hyperactive as velocity curved line (VCL; μm/s) > 80 and linearity (LIN) (as VSL/velocity average path) < 0.65 and amplitude of lateral head displacement (μm) (ALH) > 6.5; linear at straightness (STR) (as VSL/VCL) > 0.7 and LIN > 0.5; non-linear STR < 0.9 and LIN < 0.5; and, curvilinear at DAP/radius ≥ 3 and LIN < 0.5. Total motility is a measure of all types of motion detected, including vibratory movement, circular, nonprogressive and progressive motion.

2.3.5. Testicular cell counts

In 2007 and 2008, a piece of testis was cut and weighed, minced for 1 min in HBSS, and fixed in 10% buffered formalin. Testicular cell counts were generated based on tissue mass after cell resuspension and filtering through 30 μm nylon mesh (Small Parts, Miami Lakes, FL) (Jenkins and Draugelis-Dale, 2006). In 2009, milt was fixed in 4% paraformaldehyde and sperm counts were based on volume of milt. Cells were enumerated by flow cytometry (FCM) using a Bacteria Counting Kit (Molecular Probes, Eugene, Oregon) and analyzed with a FACSCalibur® (Becton Dickinson Immunocytometry Systems, San Jose, CA) with 20 K events collected in duplicate (Jenkins et al., 2011).

2.3.6. Spermatogenic staging

To assess the relative numbers of cells in early or late spermatogenic stages of maturation (Kaufman and Nagler, 1987) in 2008 and 2009, fixed testicular cells were diluted to 2×10^6/ml, filtered with 30 μm nylon mesh, stained with propidium iodide, incubated at 37 °C for 1 min and 24 °C for an additional 14 min. Cells (10 K events per sample) were then analyzed by FCM (Jenkins and Draugelis-Dale, 2006; Jenkins, 2011). The percentage of haploid nuclei out of the total haploid plus diploid events was determined.

2.3.7. DNA integrity

The procedure was developed from several reports on fixed or fresh mammalian sperm stained for the measurement of fragmented DNA (Evenson et al., 1985; Ballachey et al., 1988). In 2008 and 2009, milt stored 1:1 by volume in 4% paraformaldehyde was pelleted. Cells were resuspended to 2×10^6 cells/ml in HBSS, added to an acid denaturation solution (0.08 N HCl, 0.15 M NaCl, 0.1% Triton X-100) or control HBSS (200 μl) in a 37 °C waterbath and incubated in the dark for 1 min. Samples were then stained at 4 °C for 30 min in the dark with propidium iodide (25 μg/ml), RNase A (1 μg/ml), and 0.1% (v/v) Triton X-100 in sodium citrate (Sigma-Aldrich) (Crissman and Steinkamp, 1973) to 800 μl total volume. Negative and positive controls included exposure to HBSS or acid/detergent solution at 100 °C for 1 min prior to incubation on ice, respectively. Stained nuclei were filtered through 30-μm nylon mesh prior to analysis by FCM. Instrument calibration was performed by using DNA QC Particles (Becton Dickinson) and nuclei were analyzed at 1×10^6/ml at ≥300 per second, and 10,000 events per sample in duplicate were collected by using a 1024-channel FL2 parameter measured at 585 nm. Cytograms were generated with CellQuest (Becton Dickinson) upon linear analysis of nuclei in forward scatter (FSC-H) (size) versus side scatter (SSC). Aggregates were gated out from analysis using doublet discrimination mode with FL-2 width (FL2W) versus FL2 area (FL2A). Histograms and dot plots were analyzed by CellQuest and FlowJo (FlowJo Flow Cytometry Analysis Software, Ashland, OR), respectively. Nuclei outside the main peaks or the main population were indicative of DNA fragmentation (Jenkins, 2011; Jenkins et al., 2011).

2.4. Data analysis and interpretation

For continuous response data (e.g., GSI, hormone concentrations, Vtg concentrations, sperm quality parameters), statistical comparisons among locations were made using analysis of variance procedures for each sex separately (Sokal and Rohlf, 1981). Log or arcsine (square root) data transformation were applied to induce normality and homogenize variances of counts and proportions, respectively. Tukey's multiple comparison tests were used to detect differences among means. When parametric assumptions were not satisfied, comparisons of data as ranks were analyzed using the Kruskal–Wallis rank-sum test, followed by Dunn's comparison method. The use of temperature as a covariate for certain responses was investigated for 2008 and 2009. For prevalence data (histopathological observations), comparisons were made using categorical modeling (PROC CATMOD) in SAS. The level of significance was $\alpha = 0.05$. Statistical analyses were performed using SAS (1999) or GraphPad Instat 3.0 (GraphPad Software Inc., La Jolla, CA).

3. Results

3.1. Site comparisons

Landuse categories (estimated for 2010) indicated the CH (8.2%) and AF (22.1%) had fairly low percentages of developed land, while MA (44.7%) was intermediate and SE (69.3%) and SO (81.2%) were high. The percent of impervious surface showed the same pattern with CH (1.7%) and AF (4.8%) low, MA (10.2%) intermediate and SO (24.6%) and SE (20.7%) high. The SO and SE also have low percentages of forested land, 2.0 and 9.1% respectively (Table 1).

3.2. Morphometric observations

Yellow perch were collected from four rivers in 2007, five rivers in 2008 and four rivers in 2009. Unfortunately, AF was sampled late in the spawning run during 2008 and only eight females and one male (disregarded in analyses) were collected. A total of 18 YP were collected at this site during 2009. While there was some variation among sites as well as annually in morphometric characteristics, all fish appeared in good condition and were sexually mature individuals. Irrespective of the structure (otolith, scale) used for aging, ages ranged from 2 to 8 with the majority being 3–5 years olds (Supplemental Tables 1 and 2).

3.3. Reproductive endpoints — male

3.3.1. Gonadosomatic index

In 2007 and 2008, sites were different ($P<0.0001$) with the highest GSI in SE YP. In 2009, there were also differences among sites ($P=0.0329$) with GSI highest in SE, MA and AF and lowest in CH (Fig. 2). Annual variation in male GSI was also observed in YP collected in CH ($P<0.0001$), SO ($P=0.0161$), and MA ($P<0.0001$), but not in SE ($P=0.3718$). The GSI for YP collected in the CH and MA were lower in 2008 than in both 2007 and 2009, while the GSI of YP from the SO were higher in 2008 than 2007.

3.3.2. Sperm motilities

No differences were observed in total motilities, but progressive motilities were different ($P=0.0159$) and SO ≥ SE ≥ MA in 2007. Data were not available from CH in 2007. In 2008, sperm from CH exhibited higher total ($P=0.0025$) and progressive ($P=0.0032$) motilities, where CH ≥ SE ≥ SO ≥ MA. In 2009, motilities were different among sites ($P<0.0001$), where total was AF = CH > MA > SE, and progressive was AF = CH > MA = SE (Table 3). Total and progressive motility were not correlated with temperature in 2007 and 2008 but were correlated in 2009 ($P=0.0004$ and <0.0001, respectively). For both total and progressive motilities, the arcsine(sqrt) transformation was used.

3.3.3. Sperm counts

No site differences were noted in sperm counts in 2007 and temperature was not correlated. In 2008, site differences were observed ($P=0.0007$), where SE > SO = CH = MA and there was a negative correlation with temperature ($P=0.0021$). In 2009, site differences were not significant ($P=0.0503$); a trend was observed where the order from high to low was CH, AF, MA and SE (Table 4) and counts were not correlated with temperature. The log transformation was applied to sperm count data.

3.3.4. Spermatogenic staging

Individual site differences in percent of haploid nuclei were not detected in either year (2008, 2009) analyses were conducted. In 2008 the percentage of haploid cells was 88.5% at MA, 85.5% at CH, 84.7% at SE and 83.5% at SO. In 2009 the percentage of haploid cells was 85.0% at CH, 83.1% at AF, 82.7% at MA and 78.4% at SE. The percentage of testicular cells in the haploid stage was correlated with temperature ($r=0.3423$; $P=0.0008$; $n=93$). The arcsine(sqrt) transformation was used for these data.

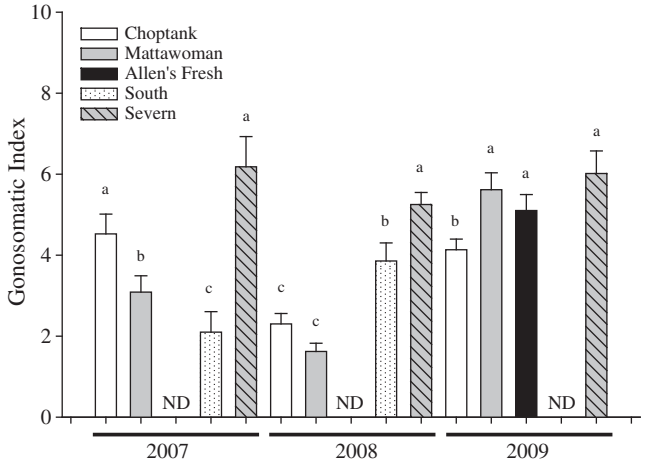

Fig. 2. Gonadosomatic indices of male yellow perch collected during the spawning runs 2007–2009. Within each year bars with different letters are significantly different ($P<0.05$).

3.3.5. DNA integrity

The arcsine(sqrt) transformation was applied to the percentage of fragmented nuclear DNA data. In 2008, there were no site differences ($P=0.0851$); however, a trend of greatest to least fragmentation was SE (14.5%), SO (12.2%), MA (9.1%) and CH (7.4%). Again in 2009, no differences were noted ($P=0.6548$) and the observed trend from greatest to least fragmentation was SE (8.0%), AF (5.4%), CH (4.9%) and MA (4.8%).

3.3.6. Male reproductive hormones

There were no significant differences in plasma testosterone or estrogen of male YP among sites in any of the years. Nor were there any annual differences in the plasma concentrations of either hormone at any of the sites (Supplemental Fig. 1). There was considerable individual variation. Plasma testosterone ranged from 1.011 to 35.774 ng/ml in 2007, from 1.282 to 37.146 ng/ml in 2008 and 2.256 to 34.649 ng/ml in 2009. Plasma estrogen of male YP ranged from 0.061 to 0.274 ng/ml in 2007, from 0.060 to 1.220 ng/ml in 2008 and 0.062 to 0.377 ng/ml in 2009.

3.3.7. Male plasma vitellogenin

Circulating Vtg was detected in only eight of 127 male YP collected throughout the three year study. In 2007, one male from the CH showed a low concentration (0.006 mg/ml), while none from the other sites had measurable amounts. In 2008, at least one male from each site, except the CH, had measurable Vtg. The SO had one positive male (0.023 mg/ml), SE had two (0.014, 0.016 mg/ml) and MA had three (0.005, 0.011, 0.090 mg/ml). Only one male was captured in AF in 2008 and had a measurable Vtg concentration (0.016 mg/ml). In 2009, no male YP were observed to have measurable plasma vitellogenin concentrations.

3.3.8. Testicular histology

The male testes collected were all mature. Testes full of sperm with thin epithelium surrounding the lobules were classified as stage 4 (Fig. 3A). Testes with less sperm and the lobule epithelium slightly thickened with lining cells that were hypertrophied and hyperplastic (Fig. 3B) appeared to have partially or totally spawned and for comparison purposes these were rated as stage 5. Often the hypertrophied lining cells, identified as putative Sertoli cells, contained phagocytized sperm (Fig. 3C and D). There was variation from site to site and among years in the proportion of testes in each of the two stages. In 2007, all testes examined from the CH and SE were stage 4 while 50% or more of the testes from MA and SO were stage 5. In 2008, all of MA testes were stage 5 while all of SE were stage 4. In 2009 all tributaries had a mixture of the two stages and ranged from 20 to 40% stage 5 (Fig. 4).

Macrophage aggregates or ceroid/lipofuscin deposits were noted in most testes, although the mean severity varied annually and spatially and no significant differences were noted among sites (Supplemental Table 3). Sperm clumping and necrosis were less commonly observed and also varied annually and spatially among sites. While there was no significant difference among sites in the percentage of samples showing this change, testes collected in 2008 had a higher percentage than those collected in 2007 and 2009 (Supplemental Table 3). Helminth parasites and associated inflammation were only occasionally observed (40% and 10% of testes from CH in 2007 and 2009; 10% of testes from SE in 2009; all others were negative). In testes from SE (40% in 2007; 30% in 2008; 10% in 2009) and MA (20% in 2009 only), foci of hypertrophied, vacuolated cells, putative Leydig cells were observed. These cells were enlarged, most often with a centrally-located nuclei and a vesicular cytoplasm (Fig. 5A and B). Neoplasia was observed in one perch from SE. Accumulations of Leydig cells formed tubule-like

Table 3
Total and progressive sperm motilities of yellow perch collected in 2007–2009 from tributaries of Chesapeake Bay.

River	2007			2008			2009		
	n[a]	Total[b]	Progressive	n	Total	Progressive	n	Total	Progressive
Choptank		ND[c]	ND	12	76.5 (4.4)^{A}	73.4 (4.2)^{A}	15	63.2(4.5)^{A}	54.5 (4.9)^{A}
Mattawoman	13	54.3 (6.4)	37.8 (8.6)	8	49.9 (2.4)^{C}	48.1 (2.8)^{C}	17	39.2 (4.6)^{B}	27.1 (4.7)^{B}
Allen's Fresh		ND	ND		ND	ND	9	75.4 (6.0)^{A}	70.4 (6.6)^{A}
South	16	64.7 (6.5)	57.3 (5.2)	10	60.6 (5.2)^{BC}	60.6 (5.2)^{BC}		ND	ND
Severn	14	54.7 (8.0)	44.5 (8.1)	13	67.0 (3.5)^{AB}	67.0 (3.5)^{AB}	11	21.9 (6.8)^{c}	19.1 (6.5)^{B}

Values followed by the same capital letter within a column are not significantly different.
[a] n = sample size.
[b] Mean (standard error).
[c] ND = no data.

structures (Fig. 5C), sometimes containing components resembling zona pellucida and yolk proteins (Fig. 5D).

3.4. Female reproductive endpoints

3.4.1. Female gonadosomatic index

In 2007, GSI among the sites were different ($P = 0.0130$) in female YP, with the highest GSI in SO (SO > MA = SE > CH). In 2008, only site, AF was significantly lower ($P = 0.0003$) than the other sites. Histological examination indicated that the AF females had already spawned. In 2009, the sites were different ($P = 0.0183$), with SE YP having the highest GSI, CH the lowest and MA and AF intermediate (Table 5).

3.4.2. Female plasma vitellogenin

In 2007, one female plasma sample from MA and one from SE had no measurable Vtg. There were no difference ($P = 0.465$) among sites in 2007. However, in both 2008 ($P = 0.0007$) and 2009 ($P = 0.0003$) differences among sites were noted. Females from CH, AF and SE had the lowest concentrations, MA the highest and SO intermediate in 2008. In 2009, CH and SE again had the lowest, MA the highest and AF was intermediate (Table 5).

3.4.3. Female reproductive hormones

There were no significant differences among the sites for either estradiol or testosterone in female plasma (Supplemental Fig. 2). Estradiol concentrations ranged from 0.643 to 3.511 ng/ml in 2007, from 0.086 to 3.270 ng/ml in 2008 and from 0.824 to 3.482 ng/ml in 2009. Plasma testosterone concentrations ranged from 2.201 to 36.767 ng/ml in 2007, 1.706 to 41.590 ng/ml in 2008, and 1.321 to 40.523 ng/ml in 2009.

3.4.4. Ovarian histology

Ovaries from all female YP contained vitellogenic oocytes. For comparative purposes, ovaries containing oocytes with yolk globules and a centrally or slightly eccentric germinal vesicle (Fig. 6A) were classified as stage 3, those with oocytes containing fused yolk and a peripheral or no germinal vesicle (Fig. 6B) as stage 4 and ovaries containing primarily post-ovulatory follicle complexes (Fig. 6D) as stage 5. Ovaries with some areas of postovulatory follicle complexes and some areas still containing oocytes (Fig. 6C) were staged as 4.5. The proportion of ovaries at each stage of development varied among sites and years. Interestingly, neither stage 4.5 (partial spawn) or stage 5 (post spawn) ovaries were observed in YP from the SE in any year (Fig. 7).

The most common abnormalities were oocytes with a thin and irregular zona pellucida and abnormal yolk fusion (Fig. 6E and F). Atretic eggs were only occasionally observed. In 2007, ovaries from the MA (60%), SO (82%) and SE (20%) had oocytes with abnormal zona pellucida. In 2008 (25%) and 2009 (30%), this abnormality was only documented in the SE (Table 6). Ovaries containing oocytes assigned a mild to severe abnormality rating for abnormal yolk were observed in YP collected from all sites in at least one year of the sampling period. The highest percentages of such abnormalities was observed in ovaries from the SO and SE which were significantly higher than MA, CH and AF (Table 6).

4. Discussion

Reports of reproductive endocrine disruption/modulation of aquatic organisms are increasing worldwide. Chemical disruption or modulation of the hypothalamic–pituitary–gonadal axis can result in numerous adverse effects. The most commonly reported effects are those resulting from exposure to estrogenic/antiandrogenic chemicals. Feminization of male fishes as evidenced by testicular oocytes or intersex (Jobling et al., 1998; Kavanagh et al., 2004; Dietrich and Krieger, 2009; Blazer et al., 2012) and production of Vtg (Denslow et al., 1999; Jones et al., 2000; Dang et al., 2011; Vajda et al., 2011) has been observed in numerous fish species. In a few cases, population effects are reported (Kidd et al., 2007; An et al., 2009). The results of this study (a very low prevalence of male YP with measurable plasma Vtg or testicular oocytes) indicate aberrations in or disruption of estrogen receptor signaling are unlikely the cause of reduced reproductive success of YP populations in the urban rivers of interest. However, other effects were demonstrated

Table 4
Cell counts from yellow perch testes collected 2007–2009 from tributaries of Chesapeake Bay.

River	2007		2008		2009	
	n[a]	Cell counts[b]	n	Cell counts	n	Cell counts
Choptank		ND[c]	24	7.90×10^{10} (6.21×10^{9})^{A}	15	9.00×10^{10} (1.85×10^{10})
Mattawoman	16	4.73×10^{10} (2.42×10^{9})	12	1.09×10^{11} (5.25×10^{9})^{A}	16	3.87×10^{10} (3.88×10^{9})
Allen's Fresh		ND		ND	8	5.05×10^{10} (5.65×10^{9})
South	13	5.06×10^{10} (2.27×10^{9})	12	1.55×10^{11} (7.81×10^{9})^{A}		ND
Severn	10	4.90×10^{10} (2.98×10^{9})	12	3.49×10^{11} (2.39×10^{10})^{B}	11	2.36×10^{10} (3.84×10^{9})

Values followed by the same capital letter within a column are not significantly different.
[a] n = sample size.
[b] Means (standard error).
[c] ND = no data.

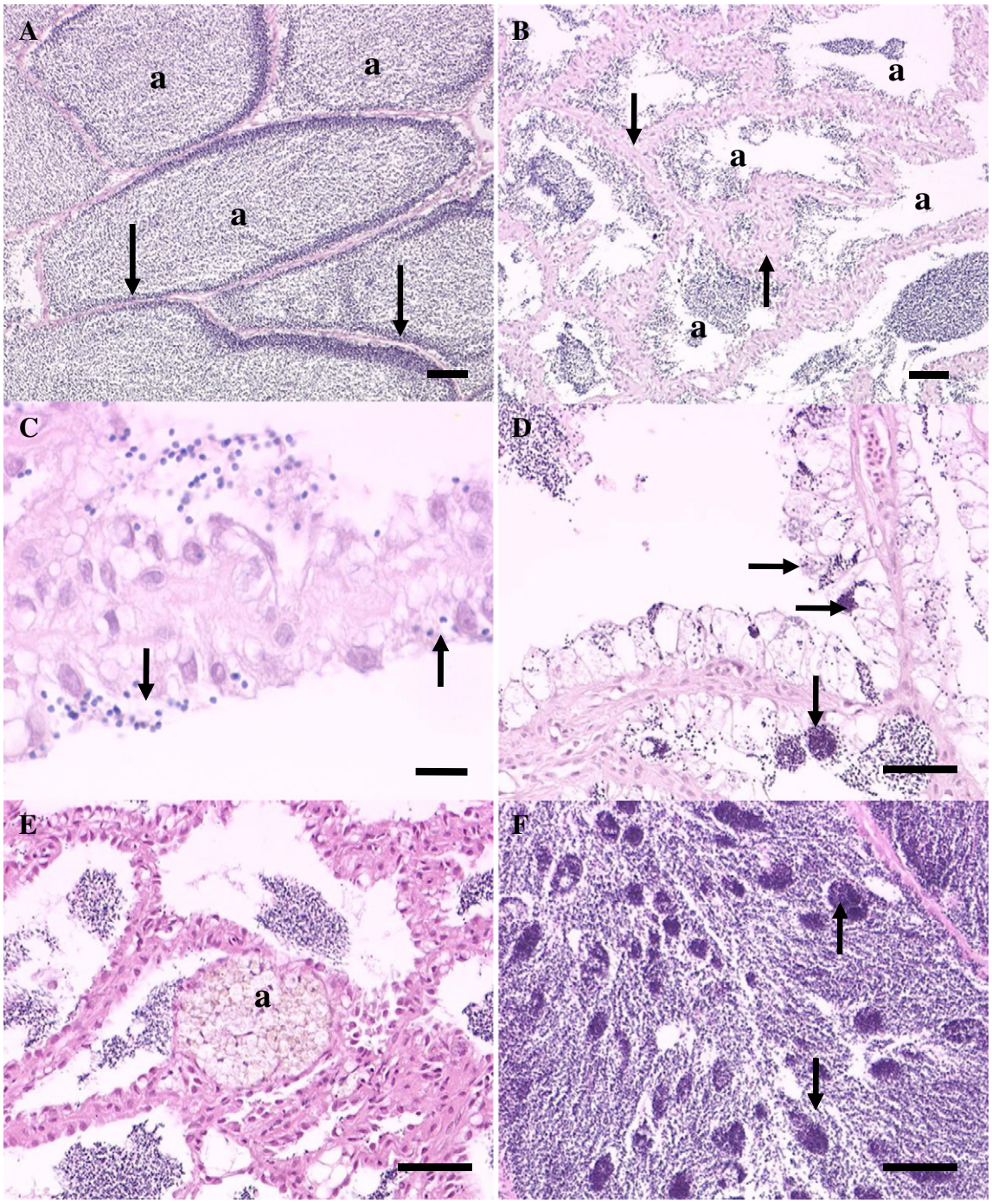

Fig. 3. Spermatogenic stages and microscopic observations in yellow perch testes collected 2007–2009 in tributaries of the Chesapeake Bay. A. Stage 4 testes in which lobules are packed with sperm (a) and lobular epithelium (arrows) is thin. Scale bar = 50 μm. B. Stage 5 testes in which lobules (a) contain few sperm and the epithelium (arrows) is thickened. Scale bar = 50 μm. C. Higher magnification of the stage 5 lobular epithelium illustrating lining cells which are hypertrophic and often contain intracellular sperm (arrows). Scale bar = 10 μm. D. Hyperplastic and hypertrophic Sertoli cells containing large numbers of sperm (arrows). Scale bar = 50 μm. E. Macrophage aggregates within the containing lipofuscin/ceroid (a). Scale bar = 50 μm. F. Sperm clumping (arrows). Scale bar = 50 μm. H and E stain.

that could affect reproductive success in YP from the highly urbanized rivers. Yellow perch from the SE and SO had the highest percentage of DNA fragmentation of sperm in 2008, highest number of ovaries with abnormal yolk and an apparent lack of final maturation in all years and, in 2008 and 2009, the SE was the only site at which abnormal zona pellucida were observed. In 2007, ovaries from SO, SE and MA contained oocytes with abnormal zona pellucida. Leydig cell hypertrophy and hyperplasia were only observed in testes from the SE in all years except 2009 when this response was also observed in two YP from the MA.

The ovarian cycle or egg production of teleost fishes can be separated into six stages: 1) formation of primordial germ cells; 2) transformation of primordial germ cells into oogonia (sex differentiation); 3) transformation of oogonia into oocytes (onset of meiosis); 4) growth of oocytes while under meiotic arrest through yolk deposition (vitellogenesis); 5) resumption of meiosis (maturation); and 6) ovulation (Patiño and Sullivan, 2002; Grier et al., 2009). Yellow perch exhibit a group synchronized maturation cycle during which the growth and maturation of a group of oocytes develop in unison. As a result the developing cells within the ovarian lamellae are of similar size

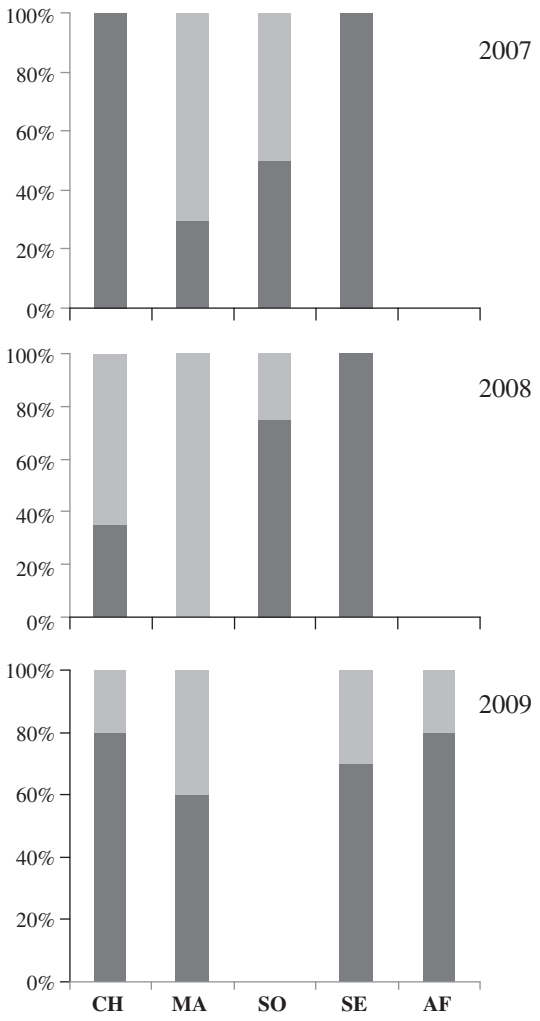

Fig. 4. Proportion of testes in stage 4 (dark bars) and stage 5 (light bars) of development (see Fig. 3A and B), in yellow perch from the Choptank River (CH), Mattawoman Creek (MA), South River (SO), Severn River (SE) and Allen's Fresh (AF) 2007–2009.

and stage (Wallace and Selman, 1981). Oocytes from all sites had reached stage 4 and were fully grown suggesting the first four stages were not adversely affected.

The termination of vitellogenesis and progression to meiosis resumption or oocyte maturation is accompanied by an increase in plasma luteinizing hormone (LH), increased expression of LH receptors and the LH-driven transition from estrogen production to the synthesis of maturation-inducing steroids (Lubzens et al., 2010). Maturation-inducing steroids are species-specific derivatives of progesterone. In YP these include 17,20 β-dihydroxy-4-pregnen-3-one (Goetz and Theofan, 1979) and 17,20β,21-trihydroxy-4-pregnen-3-one (Rinchard et al., 2002). Unfortunately quantitative assays or specific antibodies for these steroids are not commercially available and consequently measurement of these hormones was not possible in this study. Dopamine, a catecholamine neurotransmitter, is the primary inhibitor of LH secretion from the pituitary which is necessary for maturation (Nagahama and Yamashita, 2008). For instance, treatment of goldfish (*Carassius auratus*) with dopamine agonists produced reductions of plasma LH concentrations of up to 43% (Popesku et al., 2010). The inhibitory effect of dopamine on LH release is primarily mediated through activation of the pituitary dopamine 2 (D2) receptor (Vacher et al., 2000; Aizen et al., 2005). Hence, exposure to chemicals that are dopamine agonists or that interfere with LH or synthesis of maturation-inducing steroids may cause the observed effects on egg quality. Dopamine agonists have also been associated with Leydig cell hyperplasia and neoplasia in rats (Dirami et al., 1996; Cook et al., 1999). Luteinizing hormones are believed to play a role in the regulation and production of male reproductive hormones (Schulz et al., 2001). Exposure to the pharmaceutical ketoconazole, used as an experimental prototype for the imidazole and triazole fungicides, resulted in reduced egg production and Leydig cell proliferation in fathead minnows. It was demonstrated that ketoconazole exposure altered steridogenesis and the proliferation of Leydig cells was a compensatory mechanism (Ankley et al., 2007) indicating a variety of pathways could be affected.

The sublethal effects of "contaminants of emerging concern" are increasingly evident. These contaminants include chemicals in personal care products, plastics, flame retardants and current use herbicides/pesticides, as well as animal and human pharmaceuticals. The mechanisms of effects for many of these chemicals are through the endocrine system and hence, importance of the endocrine-modulating effects of legacy compounds such as PCBs and organochlorine pesticides has also been recognized (Colburn and Thayer, 2000; Guillette, 2006). The potential for adverse effects to aquatic resources of neurotransmitter receptor agonist/antagonist exposure from human pharmaceutical use exists (Villenuve et al., 2010a,b; Schultz et al., 2011), particularly since the presence of these pharmaceuticals has been demonstrated in fish brain tissue (Brooks et al., 2005; Schultz et al., 2010). Exposure of zebrafish to a mixture of pharmaceuticals (carbamazepine, fenofibrate, propranolol hydrochloride, sulfamethoxazole and trimethoprim) at environmentally relevant concentrations resulted in ovaries with less mature oocytes (Madureira et al., 2011).

Dopamine agonists and other chemicals, such as methoxychlor, carbamazepine, and o,p′-DDD, shown to inhibit oocyte maturation in fishes, have been shown to induce Leydig cell hyperplasia and adenomas in mammalian species (reviewed by Clegg et al., 1997). Although not extensively studied in aquatic organisms, the dopaminergic system appears to be a major target of environmental neurotoxicants including pesticides (paraquat, organochlorine pesticides, rotenone), heavy metals (lead, manganese) and other endocrine disruptors (reviewed by Jones and Miller, 2008). A few previous studies have implicated reduced reproductive success of fish to environmental exposure of neurotransmitter receptor agonists/antagonists. For instance, impairment of reproduction by exposure to pulp mill effluents has been known for many years. Recently, it has been suggested that this impairment may be at least partially explained by effects on the neurotransmitter system. Extracts from both primary and secondary-treated effluent activated D2 receptors (Basu et al., 2009). A variety of environmental contaminants such as hepatochlor (Miller et al., 1999), dieldrin (Richardson et al., 2006), certain PCBs, organochlorine pesticides, metals and bisphenol A (Jones and Miller, 2008) have been shown to influence dopamine homeostasis in mammals and the hypothalamic–pituitary–gonadal axis in fishes (Khan and Thomas, 2006; Martyniuk et al., 2010; Page et al., 2011). Long-term dietary exposure of zebrafish to PCB mixtures mimicking environmental situations resulted in a decrease of maturing follicles and a reduction of fertilization rate (Daouk et al., 2011). In zebrafish, exposure to pentachlorophenol and methoxychlor inhibited oocyte maturation (Tokumoto et al., 2005). The observation of Leydig cell hyperplasia plus effects on oocyte maturation may indicate effects of exposure to a variety of these compounds in urban environments which are not yet fully understood.

One factor associated with lack of YP recruitment in selected Chesapeake Bay tributaries is increased development and urbanization (Uphoff et al., 2005). Impervious surface (IS; paved surfaces, buildings, and compacted soils) has been used as an indicator of development and is a critical input variable in many water quality and quantity models (Arnold and Gibbons, 1996; Brabec et al., 2010). Impervious surface increases runoff volume and intensity in streams, leading to increased physical instability, erosion, sedimentation, thermal pollution, contaminant loads, and nutrients. Measurable adverse physical and chemical changes (increased salinity, upland erosion, increased

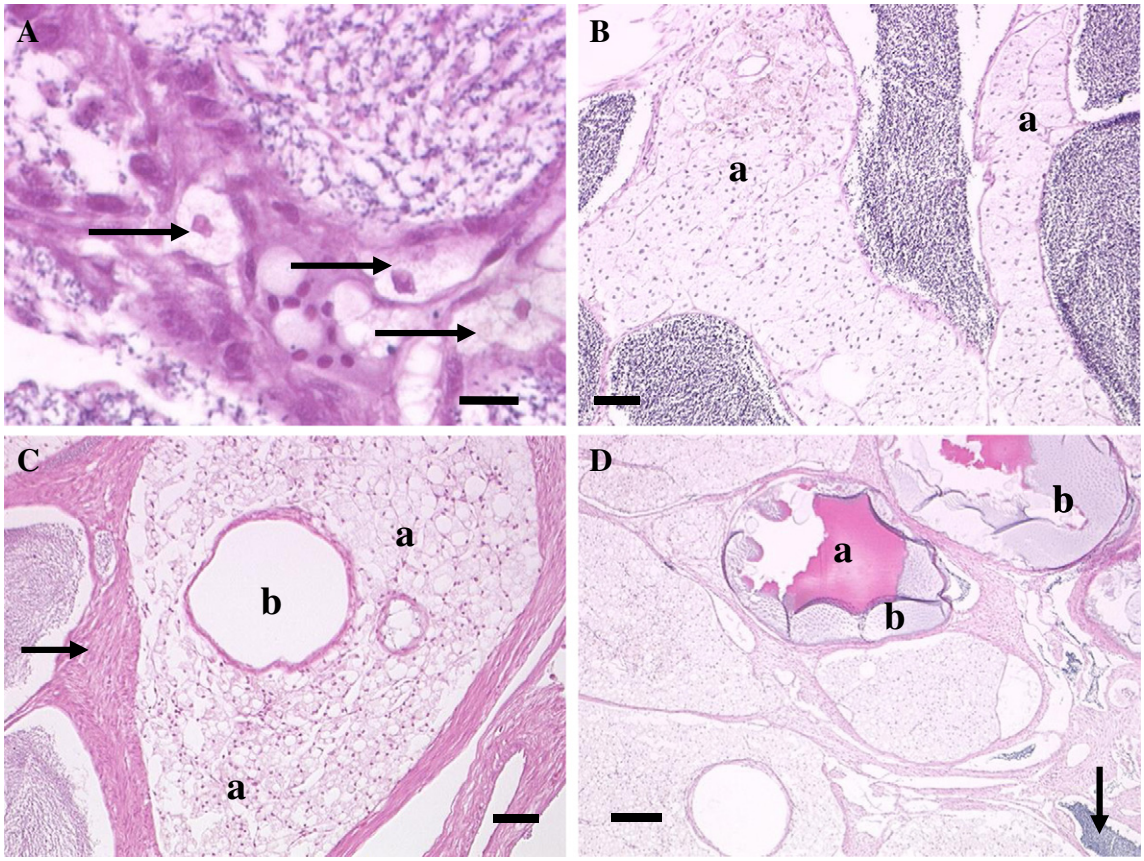

Fig. 5. Microscopic pathology observed in testes of yellow perch from the Severn River. A. Hypertrophied cells with a vacuolated cytoplasm and centrally-located nuclei (arrows), believed to be Leydig cells were observed in the interstitium. Scale bar = 10 μm. B. In some fish, both hypertrophy and hyperplasia (a) of these cells were observed. Scale bar = 50 μm. C. In one fish these cells (a) appeared to form tubular structures around a lumen (b). In some areas these structures were surrounded by fibrous tissue (arrow). Scale bar = 50 μm. D. Within the lumen of some of the structures formed by the hyperplastic cells were materials that resembled vitellogenin (a) and zona pellucida (b). Some sperms (arrow) were also apparent. Scale bar = 100 μm. H and E stain.

sediment, increased chemical contaminants) were noted in tidal creek ecosystems in South Carolina when IS exceeded 10–20% (Holland et al., 2004). Arnold and Gibbons (1996) synthesizing studies conducted through the mid-90s indicated that stream health degradation first occurs at 10%, impacted health is observed between 10–30% and above 30% IS within a watershed there is degradation. A more recent review recognizes that thresholds will vary depending on whether biotic or abiotic measures are used and the specific metric measured. Thresholds for fish population health ranged from 3.6 to 12%, however many factors such as IS type, locations of the IS within the watershed, other factors affecting stream health and the surrounding landuse can all influence the impact (Brabec et al., 2010).

Table 5
Gonadosomatic index and plasma vitellogenin concentrations in female yellow perch collected from Chesapeake Bay tributaries 2007–2009.

River	Sample size	Gonadosomatic index[a,b]	Vitellogenin mg/ml
2007 Choptank	11	14.9 (1.6)^{A}	4.666 (0.722)^{A}
Mattawoman	10	21.3 (0.9)^{AB}	5.814 (1.098)^{A}
Allen's Fresh	ND[c]	ND	ND
South	11	22.7 (2.5)^{B}	4.894 (0.764)^{A}
Severn	11	15.8 (3.3)^{AB}	3.859 (0.827)^{A}
2008 Choptank	13	22.3 (1.1)^{A}	4.231 (0.456)^{A}
Mattawoman	10	17.7 (4.1)^{A}	10.887 (1.979)^{B}
Allen Fresh	8	4.8 (1.9)^{B}	4.715 (0.333)^{A}
South	12	24.0 (0.7)^{A}	8.438(1.260)^{AB}
Severn	12	26.0 (1.1)^{A}	4.935 (0.666)^{A}
2009 Choptank	11	25.2 (0.9)^{A}	5.426 (1.534)^{A}
Mattawoman	10	27.6 (1.0)^{AB}	21.598 (3.834)^{B}
Allen Fresh	10	27.1 (0.8)^{AB}	9.176 (2.250)^{AB}
South	ND	–	ND
Severn	10	29.1 (0.8)^{B}	5.154 (1.826)^{A}

Values followed by the same capital letter within a column for each year are not significantly different.
[a] Gonadosomatic index calculated as gonad weight (g)/total body weight (g) × 100.
[b] Mean (standard error).
[c] ND = no data.

The SE and SO watersheds have the highest percentage of developed land (69.3 and 81.2%) and IS (20.7 and 24.6%) of watersheds studied and are within the 10–30% at which stream health is reported to be impacted. The MA Creek watershed has an intermediate percentage of developed and (44.7%) and IS (10.2%), while the CH and AF were below 5% IS. While hypoxia and increased salinity, as a result of increased IS, have been identified as contributing factors to mortality of eggs and larvae (Uphoff et al., 2005), they do not fully explain the lack of reproductive success. The abnormal eggs chains and lack of viable eggs from the SE when induced to spawn in a hatchery (Uphoff et al., 2005), together with the results presented in this study indicate factors other than hypoxia and increased salinity are contributing to the observed reproductive failure.

While much research has been directed toward assessing endocrine-disrupting and reproductive impacts of point sources such as WWTP, nonpoint sources such as urban runoff are also important. For example, studies in Lake Champlain (Phillips and Chalmers, 2009) and New Orleans (Boyd et al., 2004) demonstrated that urban stormwater runoff contributes significant amounts of organic wastewater compounds (OWC) to lakes and rivers. In some cases, OWC that are effectively removed during

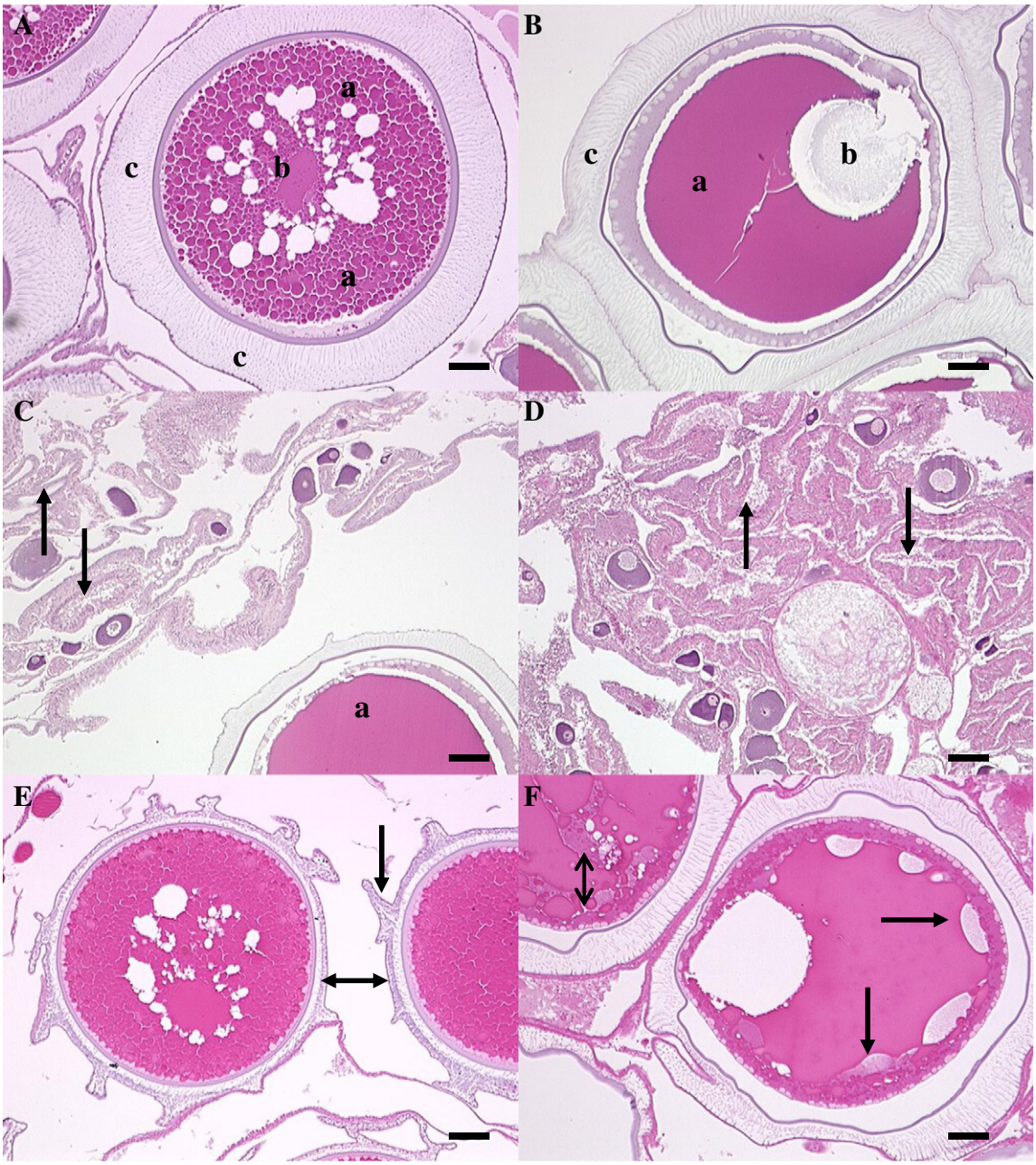

Fig. 6. Microscopic observations in ovaries of yellow perch collected from tributaries of the Chesapeake Bay. A. Stage 3 oocytes with yolk globules (a), nucleus (b) and zona pellucida (c). B. Stage 4 oocyte in which the nucleus or germinal vesicle (a) has migrated to the periphery of the cell and the yolk (b) has fused. C. Ovaries staged as 4.5 had portions of mature oocytes (a) and areas of postovulatory follicles (arrows). D. Ovaries staged as 5 contained primarily postovulatory follicles (arrows). E. Oocytes from a yellow perch ovary collected in the South River illustrating a thin and irregular zona pellucida (arrows). F. Ovary from a yellow perch collected in the Severn River illustrating abnormal-appearing yolk (arrows). Bar equals 100 μm. H and E stain.

wastewater treatment were found at concentrations similar to or greater than WWTP effluent in the urban stormwater runoff (Phillips and Chalmers, 2009) and increased concentrations in stormwater canals occur with rainfall events (Boyd et al., 2004). Pharmaceuticals, hormones and personal care products have also been found in groundwater affected by residential septic systems (Swartz et al., 2006; Barnes et al., 2008). In one study of surface water ecosystems fed by groundwater where septic systems predominated, water contamination of OWC was demonstrated, with progesterone, carbamazepine, sulfamethoxazole and trimethoprim among the most commonly detected compounds (Standley et al., 2008). The annual differences in prevalence and severity of some of the observed effects may be explained by different annual flow regimes and hence varying runoff and/or groundwater contributions. Unfortunately, to our knowledge, monitoring data for chemicals of emerging concern such as pharmaceuticals, personal care products and current use pesticides is not available for the watersheds of this study.

In conclusion, a number of biological effects, including a lack of final maturation, abnormal yolk and zona pellucida in the females and Leydig cell proliferation in the males, were observed in YP from urbanized watersheds. These effects could contribute to poor reproductive success. However, it was difficult to obtain wild YP at exactly the same stage of reproduction. Hence, further studies including additional biological endpoints, such as other plasma and tissue hormones, as well as water and/or tissues contaminant analyses are necessary to both

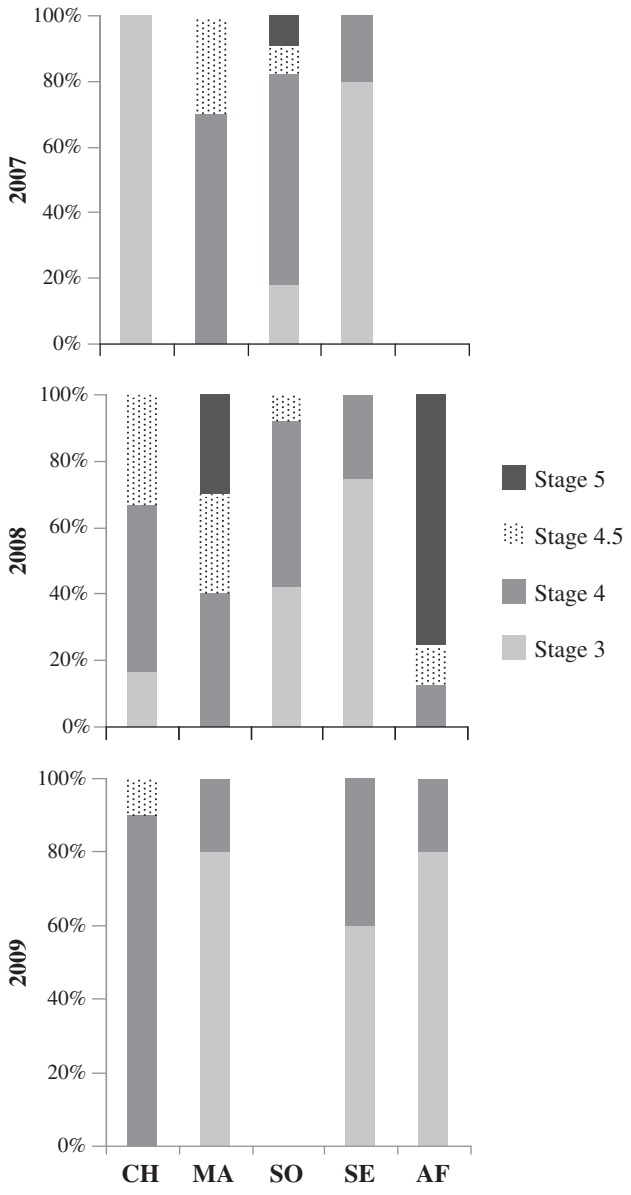

Fig. 7. Proportion of developmental stages (as illustrated in Fig. 6) of yellow perch ovaries collected from the Choptank River (CH), Mattawoman Creek (MA), South River (SO), Severn River (SE) and Allen's Fresh (AF) 2007–2009.

elucidate the mechanisms for the observed effects and identify potential chemical compounds and their sources.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.12.088.

Acknowledgments

Funding for this project was provided by the U.S. Fish and Wildlife Service, Chesapeake Bay Field Office, Mirant Corporation and the U.S. Geological Survey's Chesapeake Bay Priority Ecosystems, Fisheries and Toxic Substances Hydrology Programs. The authors thank Deb Iwanowicz and Emily Chambers for assistance with perch necropsies; Kathryn Spring and Darlene Bowling (USGS National Fish Health Research Laboratory) for histological slide preparation; E. Theall and H. Olivier (USGS National Wetlands Research Center) and B. Eilts (Louisiana State University) for CASA, and Peter Claggett, USGS for land use/land cover information. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government.

Table 6
Percentage of ovaries collected from yellow perch in selected tributaries of the Chesapeake Bay with selected microscopic abnormalities.

Year	Site	Abnormal yolk[a]	Abnormal envelope
2007	Choptank	0^{A}	0
	Mattawoman	10^{A}	60
	South	36^{B}	82
	Severn	70^{B}	20
2008	Choptank	17^{A}	0
	Mattawoman	0^{A}	0
	Allen's Fresh	13^{A}	0
	South	50^{B}	0
	Severn	58^{B}	25
2009	Choptank	20^{A}	0
	Mattawoman	0^{A}	0
	Allen's Fresh	30^{A}	0
	Severn	70^{B}	30

Values followed by the same capital letter within a column for each year are not significantly different.

[a] Percentage of ovaries containing oocytes with mild to severe ratings of abnormal-appearing yolk.

References

Aizen J, Meiri I, Tzchori I, Levavi-Sivan B, Rosenfeld H. Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. Gen Comp Endocrinol 2005;142:212–21.

An W, Hu J, Giesy JP, Yang M. Extinction risk of exploited wild roach (*Rutilus rutilus*) populations due to chemical feminization. Environ Sci Technol 2009;43:7895–901.

Ankley GT, Jensen KM, Kahl MD, Makynen EA, Blake LS, Greene KJ, et al. Ketoconazole in the fathead minnow (*Pimephales promelas*): reproductive toxicity and biological compensation. Environ Toxicol Chem 2007;26:1214–23.

Arnold Jr CL, Gibbons CJ. Impervious surface coverage: emergence of a key environmental factor. J Am Plan Assoc 1996;62:243–58.

Ballachey BE, Evenson DP, Saake RG. The sperm chromatin structure assay relationship with alternate tests of semen quality and heterospermic performance of bulls. J Androl 1988;9:109–15.

Barnes KK, Koplin DW, Furlong ET, Zaugg SD, Meyer MT, Barber LB. A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States — 1) groundwater. Sci Total Environ 2008;402:192–200.

Basu N, Ta CA, Waye A, Mao J, Hewitt M, Arnason JT, et al. Pulp and paper mill effluents contain neuroactive substances that potentially disrupt neuroendocrine control of fish reproduction. Environ Sci Technol 2009;43:1635–41.

Blazer VS. Histopathological assessment of gonadal tissue in wild fishes. Fish Physiol Biochem 2002;26:85-101.

Blazer VS, Iwanowicz LR, Henderson H, Mazik PM, Jenkins JA, Alvarez DA, et al. Reproductive endocrine disruption in smallmouth bass (*Micropterus dolomieu*) in the Potomac River basin: spatial and temporal comparisons of biological effects. Environ Monit Assess 2012;184:4309–34.

Boyd GR, Palmeri JM, Zhang S, Grimm DA. Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA. Sci Total Environ 2004;333:137–48.

Brabec E, Schulte S, Richards PL. Impervious surfaces and water quality: a review of current literature and its implications for watershed planning. J Planning Lit 2010;16:499–514.

Brooks BW, Chambliss CK, Stanley JK, Ramirez A, Banks KE, Johnson RD, et al. Determination of select antidepressants in fish from an effluent-dominated stream. Environ Toxicol Chem 2005;24:464–9.

Casey JF, Minkkinen SP, Soldo JB. Characterization of Choptank River populations of white (*Morone americana*) and yellow (*Perca flavescens*) perch. Final Report FY 1986. Annapolis, MD: Maryland Department of Natural Resources; 1988.

Claggett PR, Irani FM, Thompson RL. Estimating the extent of impervious surfaces and turf grass across large regions. J Amer Water Res Assoc in press.

Clapp DE, Dettmers JM. Yellow perch research and management in Lake Michigan: evaluating progress in a cooperative effort, 1997–2001. Fisheries 2004;29:11–9.

Clegg ED, Cook JC, Chapin RE, Foster PMD, Daston GP. Leydig cell hyperplasia and adenoma formation: mechanisms and relevance to humans. Reprod Toxicol 1997;11:107–21.

Colburn T, Thayer K. Aquatic ecosystems: harbingers of endocrine disruption. Ecol Appl 2000;10:949–57.

Cook JC, Klinefelter GR, Hardisty JF, Sharpe RM, Foster PMD. Rodent Lyedig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. Crit Rev Toxicol 1999;29:169–261.

Crissman HA, Steinkamp JA. Rapid simultaneous measurement of DNA, protein and cell volume in single cells from large mammalian cell populations. J Cell Biol 1973;59:766–71.

Dang ZC, Li K, Yin H, Hakkert B, Vermeire T. Endpoint sensitivity in fish endocrine disruption assays: regulatory implications. Toxicol Lett 2011;202:36–46.

Daouk T, Larcher T, Roupsard F, Lyphout L, Rigaud C, Ledevin M, et al. Long-term food-exposure of zebrafish to PCB mixtures mimicking some environmental situations induces ovary pathology and impairs reproductive ability. Aquat Toxicol 2011;105:270–8.

Denslow ND, Chow M, Kroll KJ, Green L. Vitellogenin as a biomarker of exposure to estrogen or estrogen mimics. Ecotoxicology 1999;8:385–98.

Dietrich DR, Krieger HO. Histological analysis of endocrine disruptive effects in small laboratory fish. Hoboken, NJ: John Wiley and Sons, Inc.; 2009.
Dirami G, Teerds KJ, Cooke BA. Effect of a dopamine agonist on the development of Leydig cell hyperplasia in Sprague–Dawley rats. Toxicol Appl Pharmacol 1996;141:169–77.
Evenson DP, Higgins PH, Grueneberg D, Ballachey BE. Flow cytometric analysis of mouse spermatogenic function following exposure to ethylnitrosourea. Cytometry 1985;6:238–53.
Glenn III DW. Effect of osmolality, extender and temperature on gamete storage of koi carp (*Cyprinus carpio*). Master's thesis. Louisiana State University, 1998.
Goetz FW, Theofan G. In vitro stimulation of germinal vesicle breakdown and ovulation of yellow perch (*Perca flavescens*) oocytes. Effects of 17α-hydroxy-20β-dihydroprogesterone and prostaglandins. Gen Comp Endocrinol 1979;37:273–85.
Grier HJ, Uribe Aranzábal MC, Patiño R. The ovary, folliculogenesis, and oogenesis in teleosts. In: Jamieson BGM, editor. Reproductive biology and phylogeny of fishes, vol. 8A. 2009. p. 25–84.
Grzybowski M, Sepulveda-Villet OJ, Stepien CA, Rosauer D, Binkowski F, Klaper R, et al. Genetic variation of 17 wild perch populations from the Midwest and East Coast analyzed via microsatellites. Trans Am Fish Soc 2010;139:270–87.
Guillette Jr LJ. Endocrine disrupting contaminants — beyond the dogma. Environ Health Perspect 2006;114(S1):9-12.
Hardy Jr JD. Development of fishes of the mid-Atlantic bight. Vol. III. Aphredoderidae through Rachycentridae. U.S. Fish and Wildlife Service; 1978. FWS/OBS-78/12.
Holland AF, Sanger DM, Gawle CP, Lerberg SB, Santiago MS, Riekerk GHM, et al. Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. J Exp Mar Biol Ecol 2004;298:151–78.
Jenkins JA. Male germplasm in relation to natural resource condition: synoptic focus on DNA. In: Tiersch TR, editor. Cryopreservation in aquatic species. 2nd ed. Baton Rouge, LA: World Aquaculture Society; 2011.
Jenkins JA, Draugelis-Dale R. Bioindicators from mosquitofish (*Gambusia affinis*) sampled from the Imperial Valley in southern California. U.S. Geological Survey Open File Report 2006-1307; 2006 (48 pp. http://pubs.usgs.gov/of/2006/1307).
Jenkins JA, Eilts BE, Guitreau AM, Figiel CR, Draugelis-Dale RO, Tiersch TR. Sperm quality assessments for endangered razorback suckers *Xyrauchen texanus*. Reproduction 2011;141:55–65.
Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. Widespread sexual disruption in wild fish. Environ Sci Technol 1998;32:2498–506.
Jones PD, De Coen WM, Tremblay L, Giesy JP. Vitellogenin as a biomarker for environmental estrogens. Water Sci Technol 2000;42:1-14.
Jones DC, Miller GW. The effects of environmental neurotoxicants on the dopaminergic system: a Possible role in drug addiction. Biochem Pharmacol 2008;76:569–81.
Kaufman DG, Nagler HM. Aspiration flow cytometry of the testes in the evaluation of spermatogenesis in the infertile male. Fertil Steril 1987;48:287–91.
Kavanagh RJ, Balach GC, Kiparissis Y, Niimi AJ, Sherry J, Tinson C, et al. Endocrine disruption and altered gonadal development in white perch (*Morone americana*) from the lower Great Lakes region. Environ Health Perspect 2004;112:898–902.
Khan IA, Thomas P. PCB congener-specific disruption of reproductive neuroendocrine function in Atlantic croaker. Mar Environ Res 2006;62:S25–8.
Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JL, et al. Collapse of a fish population after exposure to a synthetic estrogen. Proc Natl Acad Sci 2007;21:8897–901.
Lubzens E, Young G, Bobe J, Cerdà J. Oogenesis in teleosts: how fish eggs are formed. Gen Comp Endocrinol 2010;165:367–89.
Luna L. Histopathologic methods and color atlas of special stains and tissue artifacts. Gaitheresburg, MD: American Histolabs, Inc.; 1992.
Maciena MJ, Betsill RK. Verification and use of whole otoliths to age white crappie. Age and growth of fish; 1987. p. 267–78.
Madureira TV, Rocha MJ, Cruzeiro C, Galante MH, Monteiro RAF, Rocha E. The toxicity potential of pharmaceuticals found in the Douro River estuary (Portugal): assessing impacts on gonadal maturation with a histopathological and stereological study of zebrafish ovary and testis after sub-acute exposures. Aquat Toxicol 2011;105:292–9.
Mansueti AJ. Early development of the yellow perch, *Perca flavescens*. Chesap Sci 1964;5:46–66.
Martyniuk CJ, Feswick A, Spade DJ, Kroll KJ, Barber DS, Denslow ND. Effects of acute dieldrin exposure on neurotransmitters and global gene transcription in largemouth bass (Micropterus salmoides) hypothalamus. Neurotoxicol 2010;31:356–66.
Maryland Fisheries Service. Recreational yellow perch management options: opening currently closed seasons. Viewable online at www.dnr.state.md.us/fisheries/management/yperch/mgntrecoptions.pdf.
Miller GW, Kirby ML, Levey AI, Bloomquist JR. Heptachlor alters expression and function of dopamine transporters. Neurotoxicology 1999;20:631–7.
Muncy RJ. Life history of the yellow perch, *Perca flavescens*, in estuarine waters of Severn River, a tributary of Chesapeake Bay, Maryland. Chesap Sci 1962;3:143–59.
O'Dell CJ. Status of yellow perch (*Perca flavescens*) in Maryland, 1987: a situation paper on the biology, distribution, stocks, and fisheries of yellow perch with suggested study and management actions. Annapolis, MD: Maryland Department of Natural Resources; 1987.
Nagahama Y, Yamashita M. Regulation of oocyte maturation in fish. Dev Growth Differ 2008;50:S195–219.
Niewinski BC, Ferreri CP. A comparison of three structures for estimating the age of yellow perch. North Am J Fish Manag 1999;19:872–7.
Page YL, Vosges M, Servili A, Brion F, Kah O. Neuroendocrine effects of endocrine disruptors in teleost fish. J Toxicol Environ Health, Part B 2011;14:370–86.
Patiño R, Sullivan CV. Ovarian follicle growth, maturation, and ovulation in teleost fish. Fish Physiol Biochem 2002;26:57–70.
Pearson JC. Natural history and conservation of redfish and other sciaenids on the Texas coast. Bull U S Bur Fish 1929;44:129–214.
Phillips P, Chalmers A. Wastewater effluent, combined sewer overflows, and other sources of organic compounds to Lake Champlain. J Am Water Res Assoc 2009;45:45–57.
Piavis P. Yellow perch *Perca flavescens*. In: Funderburk SL, Jordan SJ, Mihursky JA, Riley D, editors. Habitat requirements for Chesapeake Bay living resources. 2nd edition. Solomons, MD: Chesapeake Research Consortium; 1991. p. 14.1-14.15.
Popesku JT, Martyniuk CJ, Denslow ND, Trudeau VL. Rapid dopaminergic modulation of the fish hypothalamic transcriptome and proteome. PLoS One 2010;5(8):e12338. http://dx.doi.org/10.1371/journal.pone.0012338.
Richardson JR, Caudle WM, Wang M, Dean ED, Pennell KD, Miller GW. Developmental exposure to the pesticide dieldrin alters the dopamine system and increases neurotoxicity in an animal model of Parkinson's disease. Fed Amer Soc Exp Biol J 2006;20:E976–85.
Rinchard J, Dabrowski K, Ottobre J. Changes in plasma steroid concentrations associated with spontaneous or induced ovulation in yellow perch, *Perca flavescens*. Fish Physiol Biochem 2002;26:239–48.
S.A.S. Institute. SAS/STAT user's guide, version 8. Cary, NC: SAS Institute Inc.; 1999.
Schulz RW, Vischer HF, Cavaco JEB, Santos EM, Tyler CR, Goos HJT, et al. Gonadotropins, their receptors, and the regulation of testicular functions in fish. Comp Biochem Physiol B. Biochem Mol Biol 2001;129:407–17.
Schultz MM, Furlong ET, Koplin DW, Werner SL, Schoenfuss HL, Barber LB, et al. Antidepressant pharmaceuticals in two U.S. effluent-dominated streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue. Environ Sci Technol 2010;44:1918–25.
Schultz MM, Painter MM, Bartell SE, Logue A, Furlong ET, Werner SL, et al. Selective uptake and biological consequences of environmentally relevant antidepressant pharmaceutical exposures on male fathead minnows. Aquat Toxicol 2011;104:38–47.
Sokal RR, Rohlf FJ. Biometry. 2nd edition. New York, NY: Freeman; 1981.
Sower SA, Schreck CB. Steroid and thyroid hormones during sexual maturation of coho salmon (*Oncorhynchus kisutch*) in seawater or fresh water. Gen Comp Endocrinol 1982;47:42–53.
Standley LJ, Rudel RA, Swartz CH, Attfield KR, Christian J, Erickson M, et al. Wastewater-contaminated groundwater as a source of endogenous hormones and pharmaceuticals to surface water ecosystems. Environ Toxicol Chem 2008;27:2457–68.
Swartz CH, Reddy S, Benotti MJ, Yin H, Barber LB, Brownawell BJ, et al. Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. Environ Sci Technol 2006;40:4894–902.
Tokumoto T, Tokumoto M, Nagahama Y. Induction and inhibition of oocyte maturation by EDCs in zebrafish. Reprod Biol Endocrinol 2005;3:69–77.
Uphoff J, McGinty M, Lukacovic R, Mowrer J, Pyle B. Development of habitat-based reference points for Chesapeake Bay fishes of special concern: impervious surface as a test case. Chesapeake Bay finfish/habitat investigations 2009, project 3. Annapolis, MD: Maryland Department of Natural Resources; 2010.
Uphoff J, McGinty M, Lukacovic R, Pyle B, Topolski M, Bornhoeft S. Job 1: development of habitat-based reference points for recreationally important Chesapeake Bay fishes of special concern: development targets and thresholds. Performance Report for Federal Aid Grant F-63-R, Segment 1 2010, Marine and Estuarine Finfish Ecological and Habitat Investigations. Annapolis, MD: Maryland Department of Natural Resources; 2011.
Uphoff J, McGinty M, Richardson B, Piavis P, Speir H, Topolski MF. Interim assessment of yellow perch *Perca flavescens* habitat and population dynamics in Severn River, a suburbanized Chesapeake Bay sub-estuary. Fisheries tech. rep. series, Number 46. Stevensville, MD: Maryland Department of Natural Resources, Fisheries Service; 2005.
U.S. Geological Survey and U.S. Department of Agriculture, Natural Resources Conservation Service (USGS and USDA). Federal standards and procedures for the National Watershed Boundary Dataset (WBD). U.S. Geological Survey Techniques and Methods 11-A32nd edition. 2011 (62 pp. Viewable online at http://pubs.usgs.gov/tm/tm11a3/).
Vacher C, Mañanos E, Breton B, Marmignon MH, Saligaut C. Modulation of pituitary dopamine D1 or D2 receptors and secretion of follicle stimulating hormone and lutenizing hormone during the annual reproductive cycle of female rainbow trout. J Neuroendocrinol 2000;12:1219–26.
Vajda AM, Barber LB, Gray JL, Lopez EM, Bolden AM, Schoenfuss HL, et al. Demasculinization of male fish by wastewater treatment plant effluent. Aquat Toxicol 2011;103:213–21.
Vandergoot CS, Bur MT, Powell KA. Lake Erie yellow perch age estimation based on three structures: precision, processing times and management implications. N Am J Fish Manag 2008;28:563–71.
Villenuve DL, Garcia-Reyero N, Martinović D, Mueller ND, Cavallin JE, Durhan EJ, et al. I. Effects of a dopamine receptor antagonist on fathead minnow, *Pimephales promelas*, reproduction. Ecotoxicol Environ Saf 2010a;73:472–7.
Villenuve DL, Garcia-Reyero N, Martinović D, Mueller ND, Cavallin JE, Durhan EJ, et al. Effects of a dopamine receptor antagonist on fathead minnow dominance behavior and ovarian gene expression in the fathead minnow and zebrafish. Ecotoxicol Environ Saf 2010b;73:478–85.
Wallace RA, Selman K. Cellular and dynamic aspects of oocyte growth in teleosts. Am Zool 1981;21:325–43.
Wells L. Changes in yellow perch (*Perca flavescens*) populations of Lake Michigan, 1954–75. J Fish Res Board Can 1977;34:1821–9.
Wilberg MJ, Bence JR, Eggold BT, Makauskas D, Clapp DF. Yellow perch dynamics in southwestern Lake Michigan during 1986–2002. North Am J Fish Manag 2005;25:1130–52.
Yellow Perch Work Group. Maryland tidewater yellow perch fishery management plan. Annapolis, MD: Maryland Department of Natural Resources; 2002 (Viewable online at www.dnr.state.md.us/fisheries/management/yperch/ypfmp.pdf).