Indicators of Reproductive Endocrine Disruption in Fish in the Chesapeake Bay Watershed
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INTRODUCTION

As the largest and most productive estuary in North America, Chesapeake Bay is a vital ecological and economic resource. The bay and its tributaries have been degraded in recent decades, however, by excessive inputs of nutrients and sediment and by the impacts of toxic contaminants on the health of fish and wildlife. Although Total Maximum Daily Loads (TMDLs) have been established to reduce nutrient and sediment inputs to the bay (U.S. Environmental Protection Agency, 2011), scientists need to improve the current understanding of the extent and severity of the effects of toxic contaminants and other factors on the health of fish and wildlife.

USGS Studies of Fish Health in the Chesapeake Bay Watershed

In 2002, the U.S. Geological Survey (USGS) began comprehensive fish health assessments to determine the cause(s) of fish mortality in the South Branch Potomac River. During these investigations, a high prevalence of intersex was observed in smallmouth bass (*Micropterus dolomieu*). Intersex is the presence of characteristics of both sexes in an organism that normally exhibits the characteristics of only one sex throughout its life. In the case of smallmouth bass, the intersex condition is present in the male fish, which have oocytes (immature eggs) in the testes (fig. 1). Additional monitoring indicated that the prevalence of testicular oocytes ranged from 100 percent at some sites in the Shenandoah River to low or background levels (10—14 percent) at selected out-of-basin sites such as the Gauley and Cheat Rivers. Prevalence in the South Branch Potomac River in West Virginia was intermediate, with 50 to 75 percent of the male bass affected (Blazer and others, 2007). Since the initial observation, intersex has been found throughout the Potomac River watershed (fig. 2).

Natural estrogens, as well as synthetic chemicals with estrogenic activity, are endocrine-disrupting chemicals that affect the hormonal system of fishes when present at sufficiently high concentrations. The presence of testicular oocytes in fish is one indicator of exposure to these chemicals; another is the presence of vitellogenin in the circulating blood of male fishes. Vitellogenin is a precursor of egg yolk that normally is found only in the blood of egg-laying female fish.

Aquatic organisms in the Chesapeake Bay watershed are exposed to complex mixtures of chemicals that can have additive, synergistic, or antagonistic effects. The USGS, working with the U.S. Fish and Wildlife Service and State partners, is documenting the biological effects of these chemicals on fish, conducting chemical monitoring to determine the extent and potential causes of reproductive endocrine disruption throughout the Potomac River and nearby watersheds, and identifying potential sources (point and nonpoint) of relevant chemicals.

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This Science Summary is one in a series that is designed to facilitate the understanding and application of results of relevant USGS studies by Chesapeake Bay resource managers and policy makers. It provides a brief overview of the most recent published work by the USGS and collaborators on indicators of reproductive endocrine disruption in fish in the Chesapeake Bay watershed, an understanding of how this information can be used to develop effective management policies and practices, and a list of references for additional information.

The **KEY FINDINGS** and the **IMPLICATIONS FOR MANAGEMENT POLICIES AND PRACTICES AND NEXT STEPS** listed below are from Blazer and others (2007; 2010; 2011), Alvarez and others (2009), and Iwanowicz and others (2009).

KEY FINDINGS

•The most sensitive stage for induction of testicular oocytes is during sexual differentiation or within the first 2 to 3 weeks after hatching. Exposures at these early life stages can lead to a greater sensitivity to estrogenic exposure later in life. Hence, it is important to document both the prevalence and the severity of testicular oocytes. Severity (0-4) is based on the relative number and distribution of immature oocytes within the testes (fig. 1). In contrast, the presence of plasma vitellogenin in immature and male fishes is indicative of more recent exposure. Simultaneous examination of both indicators maximizes the information that can be learned about exposure regimes and effects in wild fish populations.

•In the Potomac River watershed, largemouth bass show signs of feminization (testicular oocytes and vitellogenin in males) but appear to be less sensitive than smallmouth bass to the effects of estrogenic compounds. Therefore, prevalence of intersex is greater in smallmouth bass than in largemouth bass. In addition, a high prevalence of skin lesions and spring mortality of adults in smallmouth bass in the Potomac and James Rivers indicates that they may be a sensitive indicator of environmental health in the Chesapeake Bay watershed. Black bass species, both smallmouth and largemouth (*M. salmoides*) bass, in other geographic areas have also been shown to be sensitive to reproductive endocrine disruption. Surveys conducted in nine U.S. river basins during 1995—2004 found only one other species, channel catfish, that exhibited testicular oocytes, although other species exhibited plasma vitellogenin in males (Hinck and others, 2009).

•Initial surveys in the Potomac River watershed indicated the likelihood of a gradient of intersex associated with human population and agricultural land use (table 1). Although feminization of male fishes has most commonly been associated with exposure to human wastewater-treatment-plant effluent or other point sources, the prevalence of male smallmouth bass with intersex characteristics is not consistently higher downstream from these point sources than upstream in the areas of the Potomac River watershed that were studied (fig. 3). However, some additional biomarkers, such as gonadosomatic index (ratio of gonad weight to body weight) and plasma vitellogenin concentrations in female bass, do appear to be

adversely affected by the presence of wastewater-treatment plants upstream from the study site (table 2).

• The prevalence of male smallmouth bass with intersex characteristics varies across the watershed and with time. The percentage of agricultural land use and animal density within the watershed were significantly associated with the prevalence of testicular oocytes in smallmouth bass in the Potomac River watershed, whereas other factors, including wastewater-treatment-plant effluent flow, number of animal feeding operations, and number of poultry houses in addition to the percentage of agricultural land use and animal density were associated with an increase in intersex severity (table 3).

•No evidence of reduced reproductive success of Potomac River smallmouth bass is currently (2011) observed, although bass collected from the South Branch Potomac River (where both prevalence and severity are moderate to high) had fewer sperm with lower motility than bass collected from the out-of-basin Gauley River (where both prevalence and severity are low). Therefore, an increase in prevalence and (or) severity at some sites could potentially lead to reduced reproductive success. In other species such as roach (*Rutilus rutilus*), a high prevalence of intersex males with a severity of 2 or more has been associated with population effects (Harris and others, 2011). In a shorter lived species, the fathead minnow (*Pimephales promelas*), exposure to 4 to 6 nanograms per liter of the synthetic estrogen ethinylestradiol in an experimental lake resulted in a population collapse within 3 years (Kidd and others, 2007).

IMPLICATIONS FOR MANAGEMENT POLICIES AND PRACTICES AND NEXT STEPS

- The USGS will be working with the Chesapeake Bay Program (CBP) partners to use these results and other information to summarize the extent and severity of the impact of toxic contaminants on the bay and its watershed. The CBP partners will use the findings to develop toxic contaminant reduction outcomes by 2013, and strategies to accomplish those outcomes by 2015.

- Management actions to reduce the prevalence of intersex in smallmouth bass in the Potomac River watershed likely will be most effective if the sources of chemicals can be reduced, particularly at times when the bass are undergoing sexual differentiation or during the first 2 to 3 weeks after hatching.

- The sources of the chemicals associated with intersex conditions appear to be effluent from wastewater-treatment plants as well as runoff from agricultural land, animal feeding operations, and urban/suburban land use.

- The USGS is working with its CBP partners to more accurately identify the chemicals that have the greatest adverse effects on smallmouth bass and other aquatic organisms and to better define the sources of these chemicals so that resource managers can develop practices that could reduce their presence in aquatic systems.

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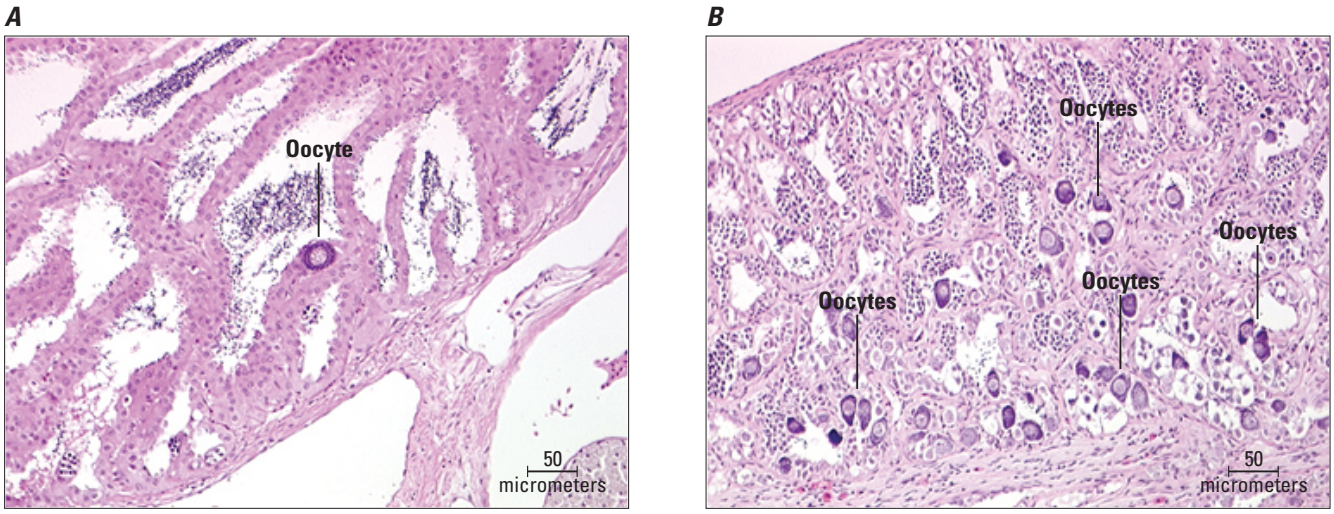

Figure 1. Microscopic appearance of smallmouth bass testes containing *(A)* a single oocyte (severity rating 1); and *(B)* multiple oocytes, commonly in clusters (severity rating 4) (modified from Blazer and others, 2007).

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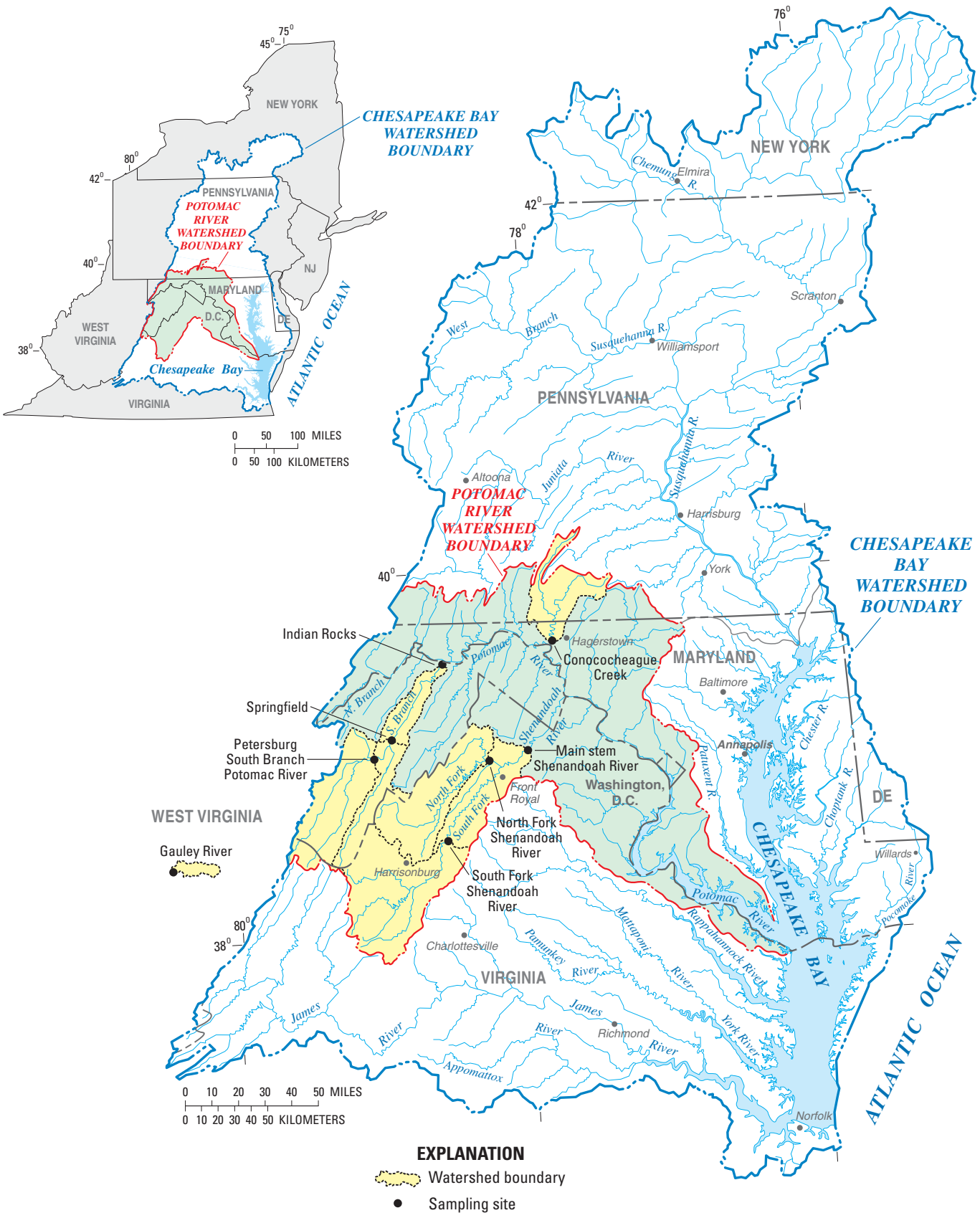

Figure 2. Potomac River watershed, selected watersheds in which intersex has been studied by the U.S. Geological Survey, and generalized locations of sampling sites (modified from Blazer and others, 2011).

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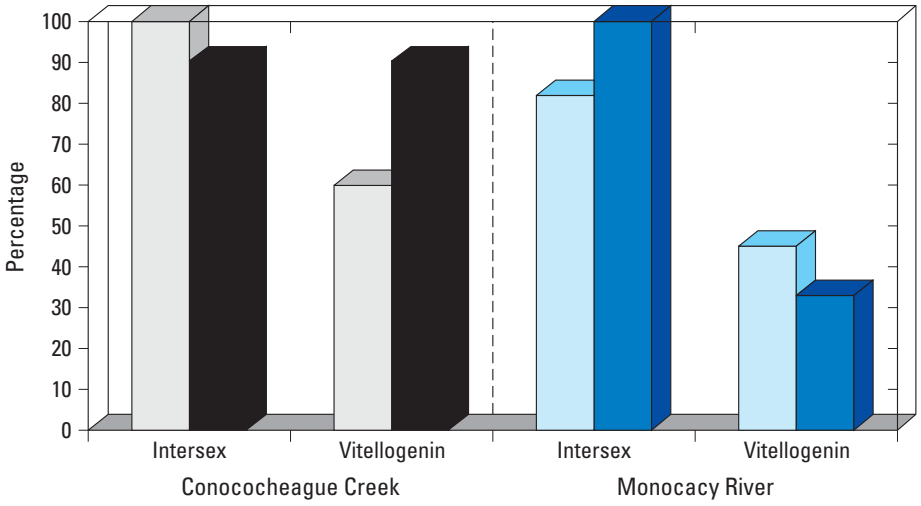

Figure 3. Percentage of male smallmouth bass exhibiting testicular oocytes (intersex) or plasma vitellogenin at sites upstream and downstream from wastewater-treatment plants on Conococheague Creek and the Monocacy River, Maryland, fall 2005 (data from Iwanowicz and others, 2009).

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Table 1. Human population and agricultural land use in counties containing sampling sites for study of smallmouth bass with testicular oocytes (modified from Blazer and others, 2007)[1].

River	County	Percentage (and number) of samples exhibiting testicular oocytes[2]	Human population density (number per square kilometer)[3]	Percentage of area occupied by agricultural land[4]
		Out-of-basin sites		
Tygart	Randolph	14 (7)	10	15.2
Gauley	Webster	17 (6)	7	3.2
Elk	Webster	36 (11)	7	3.2
Greenbrier	Pocahontas	22 (18)	4	20.5
	Greenbrier	75 (12)	13	29.5
		Potomac River sites		
South Branch Potomac	Pendleton	54 (24)	5	38.2
	Hardy	65 (35)	8	34.4
	Grant	47 (74)	9	35.2
	Hampshire	77 (35)	12	33.8
Shenandoah	Page	100 (8)	28	32.2
	Shenandoah	80 (10)	26	40.6
	Warren	100 (13)	57	35.8

[1] Based on all smallmouth bass collected during 2004–05.

[2] n = total sample size of male bass.

[3] Based on data from U.S. Census Bureau (2002).

[4] Based on data from U.S. Department of Agriculture (2004).

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Table 2. Biological indicators of smallmouth bass collected upstream and downstream from wastewater-treatment plants on tributaries of the Potomac River (modified from Iwanowicz and others, 2009).

[Data are presented as mean ± standard deviation. Upstream and downstream values for the same river that are followed by different letters indicate significant difference ($p < 0.05$) between upstream and downstream sites]

River	Female gonadosomatic index	Female vitellogenin (milligrams per milliliter)	Male gonadosomatic index	Severity of testicular oocytes
Conococheague				
Upstream	1.26 ± 0.35[a]	1.25 ± 0.49[a]	0.39 ± 0.06[a]	2.1 ± 0.3
Downstream	0.63 ± 0.06[b]	0.12 ± 0.11[b]	0.13 ± 0.06[b]	1.8 ± 0.4
Monocacy				
Upstream	0.94 ± 0.11	1.89 ± 1.01	0.30 ± 0.04	1.2 ± 0.3
Downstream	0.82 ± 0.09	0.40 ± 0.12	0.30 ± 0.15	1.9 ± 0.3

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Table 3. Pearson correlation coefficients (r^2) for the relation of intersex prevalence and severity with land-use characteristics at sites in the South Branch Potomac River (West Virginia), Shenandoah River (Virginia), Conococheague Creek and Monocacy River (Maryland), and Gauley River (West Virginia) watersheds (modified from Blazer and others, 2011).

[Values in **bold** are considered to be significant; WWTP, wastewater-treatment plant]

Land-use characteristic	Intersex prevalence		Intersex severity	
	r^2	*p*	r^2	*p*
Human population density	0.39	0.10	0.42	0.08
Number of WWTPs	0.22	0.24	0.34	0.13
WWTP flow	0.32	0.15	**0.63**	**0.02**
Percent agricultural land use	**0.63**	**0.02**	**0.50**	**0.05**
Number of animal feeding operations	0.28	0.17	**0.56**	**0.03**
Number of poultry houses	0.27	0.18	**0.50**	**0.05**
Total number of animals	0.27	0.18	0.48	0.06
Animal density	**0.49**	**0.05**	**0.58**	**0.03**

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