Estrogen in Our Rivers and Fishery Problem References

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Striped Bass Feminization

www.sfestuary.org/estuarynewsletter.html

In the 1920's California's significant western population of striped bass had about a 50 percent ratio of females to males. Today, females represent only about 10% of the much smaller population. UC Riverside concluded that we are now learning that there is a world-wide epidemic of fish feminizing activity.

Present indications are that steroidal estrogens, pesticides, and insecticides such as the pyrethroids may be part of the problem that can result in reduced sperm counts and reproductive abnormalities. In some cases, such chemicals have been found to disrupt neurological and immunological functions. Urban drain waters have also been found to be generally toxic.

The telltale marker vitellogenin has been found in open-sea and deep water fish. The feminization of flatfish off the Southern California Coast has been documented. One British study found this estrogen in fish resident below waste water treatment plants. A Canadian study added a minute amount of the ethinyl estradiol steroid to a lake and the fathead minnows were destroyed. UC Berkeley scientists found 16 sites in the Sacramento-San Joaquin watersheds that have the potential for salmonid feminization.

The December issue of the Cal-Fed sponsored "Estuary Magazine" are by Daniel Schlenk, US   Riverside (daniel.schlenk@ucr.edu) summarizes some of these Problems and also has a pesticide article. These conditions are serious:

http://www.danblanton.com/viewmessage.php?id=114063

For unknown reasons, at present San Francisco Bay Delta Anglers and Dept of Fish and Game Trawls in the same area have not yet found problems with the male/female striped bass take. This is not the case in other areas/fish and with species such as Maine Lobsters.

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http://www.sciencedaily.com/releases/2008/09/080918170628.htm

ScienceDaily (Sep. 21, 2008) — The Montreal water treatment plant dumps 90 times the critical amount of certain estrogen products into the river.

It only takes one nanogram (ng) of steroids per liter of water to disrupt the endocrinal system of fish and decrease their fertility.

These are the findings of Liza Viglino, postdoctoral student at the Université de Montréal's Department of Chemistry, at the NSERC Industrial Research Chair in Drinking Water Treatment and Distribution, who is under the supervision of Professors Sébastien Sauvé and Michèle Prévost.

The presence and effects of estrogen residues on aquatic wildlife are well documented. However, this research is unique because it didn't only consider natural hormones and those used in oral contraceptives – it also included products used in hormone therapy that is prescribed to menopausal women. Data indicates that 128 million contraceptive pills and 107 million doses of hormone therapy are consumed every year in Quebec.

According to Professor Sauvé, ozone treatments could eliminate these hormonal compounds. He also stresses that 80 to 90 percent of antidepressants remain in the water after treatment. These molecules can have a variety of effects on aquatic wildlife. Again, ozone treatment could destroy these molecules

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http://www.sciencedaily.com/releases/2007/04/070417114749.htm
ScienceDaily (Apr. 17, 2007) —

A new study from the University of Pittsburgh Cancer Institute's Center for Environmental Oncology suggests that fish caught in Pittsburgh rivers contain substances that mimic the actions of estrogen, the female hormone. Since fish are sentinels of the environment, and can concentrate chemicals from their habitat within their bodies, these results suggest that feminizing chemicals may be making their way into the region's waterways.

The study, abstract number 3458, was presented at the annual meeting of the American Association for Cancer Research. The study demonstrated that the chemicals extracted from the local fish can cause growth of estrogen-sensitive breast cancer cells cultured in the laboratory.

"We decided to look at pisciverous fish, those that eat other fish, for this project because we know that they bioaccumulate contaminants from water and their prey, which may include toxic metals, farm and industrial runoff and wastes from aging municipal sewer systems," said Conrad D. Volz, Dr.P.H., M.P.H., principal investigator, department of environmental and occupational health, University of Pittsburgh Graduate School of Public Health.

The experiments to determine if estrogenic substances were present in the fish were performed in the laboratory of Patricia K. Eagon, Ph.D., co-principal investigator of the study with the Veterans Affairs Medical Center and the University of Pittsburgh School of Medicine. Dr. Eagon found that extracts from the fish acted like estrogen, a female hormone, by binding to estrogen receptors -- the proteins within cells that render the cells sensitive to estrogen.

Of six bass extracts tested for estrogenic activity, four displayed a strong or moderate ability to bind with the estrogen receptors. Of 21 catfish extracts tested, nine displayed a similar ability to bind with the estrogen receptors. The researchers also examined whether the fish extracts could result in growth of breast cancer cells cultured in the laboratory, and they found that two bass extracts produced strong-to-moderate cell growth, as did five catfish extracts. Additionally, the consumption of river-caught fish, especially by semi-subsistence anglers, may increase the risk for endocrine-mediated health endpoints like some cancers and developmental problems."

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http://www.gulfofmaine.org/times/winter2005/quanda.html

Dr. Karen Kidd, University of New Brunswick
Drugs leaking into waterways bad news for fish

Kidd and her colleagues have started research on the Saint John River, and expect some early results next spring or summer. Prior to that she was a research scientist with the federal government's Fisheries and Oceans Canada.

She received her Ph.D. from the University of Alberta in 1996, and began her career investigating pollution in fisheries in the Yukon, Eastern Arctic and East Africa. This work focused on pesticides and other persistent pollutants that concentrate and get into food webs. She also is conducting several studies in Atlantic Canada on the accumulation of mercury in freshwater food webs.

Kidd is concerned that these kinds of pollutants are getting into fish and accumulating to high levels that may cause health effects for humans or fish-eating wildlife. In addition, she also studies the impact of pollution, including pharmaceuticals in sewage outfall, on fish.

The reason why we got into that work is that in Britain they were seeing that a lot of the male fish downstream of sewage treatment plants were becoming feminized. These fish were producing egg proteins, and they were developing eggs in the more
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serious cases, because they were being exposed to estrogens in the water.

That work was done in the early to mid-1990s, but there have been a number of studies since then in the United States and Canada that show that estrogens are having similar impacts on fish here. A number of small-bodied fish species, like the fathead minnow, respond quite dramatically to estrogens.

Seventy-five percent or more of these estrogens can be broken down or degraded in the sewage treatment plant process. But there are still enough of these natural and synthetic estrogens getting into some rivers for male fish to become feminized.

In the United States there's been a fair amount of work done in a number of states including Colorado, Florida, Texas and Minnesota looking for feminized male fish and for the presence of estrogens in rivers. The closer one is to the outfall of the sewage treatment plant, the higher the concentrations are. And concentrations will change over a season or over a year depending on how much rainfall there has been and on how much sewage is in the river flow. The synthetic estrogen that's used in birth control pills is very potent and can impact males at levels below one nanogram per liter, or one part per trillion. And the fish don't have to be exposed for very long.

Sometimes a few weeks is enough to impact whether an egg develops into a female or a male, to cause a male fish to start producing egg proteins or to impact the survival of young fish or their sexual development. In mature male fish it may take longer, sometimes months, for them to start developing eggs. The hormones we use to reproduce are very similar.

Every fish species reacts differently to chemicals. We tend to see more impacts in the fish that live in a small area. They may live just downstream of the sewage outfall and be exposed to more of these estrogens than a fish that would move up and down the river and in and out of the sewage outfall. But every fish will be affected by estrogen if they are exposed to enough of it. Some of the stationary fish include the freshwater fathead minnow and slimy sculpin and estuarine species like mummichogs.

Researchers at Baylor University in the United States have found that the active ingredient in Prozac, fluoxetine, is accumulating in fish muscle. They've also shown in lab studies that fluoxetine impacts fish reproduction and fish behavior. To date, we have found more than 50 different drugs in sewage effluents and in surface waters, and as technology improves, we're going to find more drugs in the environment. Some, like the painkiller ibuprofen, are effectively degraded by sewage treatment processes. Others like fluoxetine are more resistant to degradation, so they're going to get into the environment.

There are chemicals used in detergents that can interfere with reproduction with fish as well, but they tend to be much less potent than something like an estrogen, because fish naturally use estrogens to control their reproduction.
For more information contact

Dr. Karen Kidd at kiddk@unbsj.ca or visit the Canadian Rivers Institute Web site at www.unb.ca/cri/

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http://www.setacjournals.org/perlserv/?request=get-abstract&doi=10.1897%2F03-41&ct=1

SEDIMENTS ARE MAJOR SINKS OF STEROIDAL ESTROGENS IN TWO UNITED KINGDOM RIVERS

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The occurrence of intersex fish in a number of European rivers has been attributed to exposure to estrogenic chemicals present in sewage treatment work (STW)
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effluents. To further understand the environmental fate of these contaminants, the estrogenic activity of effluents, water, and sediments were investigated both upstream and downstream of the major STW discharge in two United Kingdom rivers. Estrogenic activity, determined using the yeast estrogen-receptor transcription screen, of the major STW effluents on both rivers was similar, ranging from 1.4 to 2.9 ng 17?-estradiol equivalents (EEQ)/L.

Estrogenic activities of surface waters 1 km upstream and downstream of both STW inputs were less than the limits of detection (0.04 ng/L); however, levels of estrogenic activity in sediments were between 21.3 and 29.9 ng EEQ/kg and were similar at both upstream and downstream sites. Effluent and sediment extracts were fractionated by reverse phase–high-performance liquid chromatography, and estrogenic active fractions were further analyzed by gas chromatography–mass spectrometry. The major active chemicals in the two effluents and in the sediments were estrone with lesser amounts of 17?-estradiol; however, at one site, a number of other unidentified estrogenic fractions were detected in the sediments. These results suggest that riverine sediments are a major sink and a potential source of persistent estrogenic contaminants
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http://www.enhs.umn.edu/current/5200/estrogen/gov.html

EPA identified endocrine disruption as one of it top 6 research priorities and created the Endocrine Disruptors Research Initiative. As a result of this the EPA has funded many studies further investigating the effect of environmental estrogens on the health of humans and wildlife, as well as the true concentrations of these chemicals in the environment. In addition the EPA is working on better ways to accurately screen for small amounts of these chemicals in the environment. Finally, the EPA has sought to increase awareness of environmental estrogens, and provides links to several awareness organizations, both public and private on their website

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Science Links Japan

http://sciencelinks.jp/j-east/article/200517/000020051705A0615044.php

Estrogenic activity of the river waters was 0.8g/L-E2equivalent for mean. E1, E2 and NP mainly contributed to estrogenic activity of the river waters that were observed in high estrogenic activity.

The intersex condition of wild male carp in major Japanese rivers was better than that of Roach in the UK, but a quarter of them had plasma vitellogenin suggesting biomarker of feminization. Assuming that estrogenic activity of river waters should be lower than 1ng/L E2-equivqlent that is equal to Environmental

Quality Standard proposed by UK Environmental Agency, a quarter of river waters could not comply with this standard in Japan. A positive relationship was found between a population on each river watershed and estrogenic activity of each river water.
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http://archive.southcoasttoday.com/daily/01-07/01-14-07/01perspective.htm

When lobsters started disappearing from Buzzards Bay, UMass-Dartmouth professor Yuegang Zuo thought he might know why. He talked to area lobstermen, who suspected pollution, then collected water samples from the Acushnet River Estuary and near the Fairhaven Bridge and hurricane barrier.
Back in the lab, he found a possible culprit.

Using sophisticated equipment that separates the molecules of different contaminants in the water, Dr. Zuo identified several types of natural and synthetic estrogen hormones – most likely passed through human waste and released into the river and bay from the nearby Fairhaven and New Bedford wastewater treatment plants.

Dr. Zuo and other scientists suspect that the naturally-occurring and synthetic
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female estrogen in birth control pills and hormone replacement therapy drugs could be hindering larval lobster development, as well as shell growth and reproduction in adult lobsters.

Estrogen, which mimics lobsters' own molting hormone, may interfere with their molting process and make them more susceptible to the bacteria that causes shell disease.

While estrogen is not the only endocrine disruptor that researchers suspect may contribute to shell disease and other marine life abnormalities, the potency of its synthetic forms make it particularly worrisome.

Both male and female hormones are part of a broad, disparate group of chemicals, including ingredients common in pesticides, cosmetics, detergents and other pharmaceuticals, known as endocrine disruptors, so called because they interfere with the endocrine system's ability to regulate growth, development and reproduction in humans, fish and wildlife.

Research has implicated endocrine disruptors in a wide range of health problems, including cancer, reproductive defects, reduced sperm count and immune system disorders.

One of the most startling recent examples is the Potomac River in Washington, D.C. Federal and state researchers found that at least 80 percent of male bass surveyed in major tributaries that feed the Potomac were growing immature eggs. In the Potomac itself, roughly half of a smaller sample of bass showed signs of so-called feminization. Research elsewhere in the United States, Canada and Europe has exposed similar phenomena
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http://www.ce.berkeley.edu/~sedlak/CALFEDwebsite.htm

The primary objective of the project is to determine if endocrine-disrupting chemical contaminants are present in the Sacramento-San Joaquin river systems at concentrations capable of feminizing Chinook salmon or other fish species. A secondary objective is to develop and test a biomarker to assess the prevalence of feminized male fish in this river system.
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http://articles.latimes.com/2003/dec/11/nation/na-cattle11

A group of scientists from five U.S. institutions, led by the University of Florida in Gainesville, reported “significant alterations in the reproductive biology” of fish immediately downstream from a large Nebraska feedlot.

The male fish had about one-third less testosterone and testes about half as big as unexposed fish upstream, according to the study, which was published last week in the online version of the scientific journal Environmental Health Perspectives. The female fish had about 20% less estrogen and 45% more testosterone than females from the uncontaminated section of stream, the study found.

In addition, lab tests confirmed that feedlot effluent contained a complex and potent mix of androgens, the male sex hormones, and estrogens, the female hormones, said Edward Orlando, the study’s lead author. He is now at St. Mary’s College of Maryland in St. Mary’s City.

The ability of dozens of contaminants, mostly pesticides and pharmaceuticals, to mimic hormones has been a growing concern since the early 1990s, when scientists began finding mixed-up sexual characteristics in wild animals, including alligators and polar bears. In human beings, some studies have linked contaminants to lower sperm counts and premature puberty.
The European Union has banned U.S. hormone-treated beef. Because nearly all the study’s funding came from the European Commission, the beef groups said the researchers might have had an agenda.

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http://www.praguepost.com/articles/2007/04/25/pollutants-leaving-the-vltava-river.ph
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Some of the pressure on the country to clean up its act came from surrounding countries. For example, the Vltava turns into the Moldau in Germany, which forged an agreement with the Czech Republic to keep its waters clean.

PCBs are estrogenlike compounds that disrupt natural hormone production and may be responsible for heightened levels of breast and testicular cancer in the Czech Republic. Unlike the estrogens used in contraceptives and the natural estrogen that women produce, PCBs are persistent organic chemicals, meaning they do not degrade easily.
Instead, PCBs and similar long-lasting chemical compounds such as brominated flame retardants (BFRs), which are used in everything from couches to computers, settle into the sediment underneath rivers. After they mix with the sediment, they cannot be easily removed.

Fish are particularly sensitive to these contaminants and are a good biomarker of the health of the river. Downstream of wastewater treatment plants, altered estrogen levels can even cause male fish to develop female sex organs, creating hermaphrodite fish.

On the other hand, the amounts of natural estrogen produced by women and those found in contraceptives, are a negligible part of all the types of estrogen (and estrogenlike chemicals) in the country’s water supplies, according to Hajšlová.
Tomáš Pa?es of the Czech Geological Survey agrees. His studies show that, although concentrations of natural estrogen and estrogen from oral contraceptives are four times higher around Prague than at other points of the Vltava, the amounts are barely detectable. And those types of estrogens do not accumulate in significant amounts in fish, Hajšlová said.

Though Jakub Kašpar of the Environment Ministry said drinking-water treatment plants cannot eliminate all steroids (such as estrogens) and pharmaceuticals, water treatment methods ensure that very little actually makes its way into drinking water, according to Hajšlová.

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http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TG8-4MKKCN5-9&_user=10&_r
doc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_u
serid=10&md5=bd584a04ea41f5fbdc433a29fa33ae2c

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Estrogens in Swiss Rivers and Effluents

http://www.ingentaconnect.com/content/scs/chimia/2008/00000062/00000005/art00014

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Estrogens in Colorado Waters

http://www.msnbc.msn.com/id/6436617/

Government researchers recently found natural estrogens and estrogen mimickers in 80 percent of the streams they tested in 30 states.
"We would be ingesting those chemicals, would absorb them, and they would add to whatever natural hormones we already have in the body," says Dr. Norris. No one is certain what the impact is on humans. But since finding evidence that estrogen may be turning male fish into female fish, scientists are now looking at what it means

for the nation's drinking water.
This fish has characteristics of both male and female," says Dr. David O. Norris of the University of Colorado, Boulder. And scientists have found lots of them in three Colorado rivers, all of them downstream from sewage treatment plants. In the Boulder Creek, female white suckers outnumbered males five to one and 50 percent of the males also had female sex tissue. Researchers say the cause is too much estrogen in the water, a natural female hormone that is found in every sewer system. But also, they say, certain chemical compounds in detergents and soaps can mimic estrogen. Barbara Biggs, of Denver's largest sewage plant, says most of the nation's sewage plants simply can't remove all the estrogen in the water. "We're concerned about the effect on aquatic life, but we're also concerned about our ability to actually treat for these estrogens and estrogen mimickers," says Biggs. Estrogen mimickers are believed to be caused by chemicals called nonylphenols, found in everything from paints and rubber to cosmetics and plastics. They are considered a possible cause of kidney, eye, liver and reproductive problems.   They’ve been banned in much of Europe and are under review in Canada, but are still common in America, where they are flowing out of sewage plants and into clean water flowing into America's rivers.

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http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1874167

Predicted Exposures to Steroid Estrogens in U.K. Rivers Correlate with Widespread Sexual Disruption in Wild Fish Populations

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Steroidal estrogens, originating principally from human excretion, are likely to play a major role in causing widespread endocrine disruption in wild populations of the roach (Rutilus rutilus), a common cyprinid fish, in rivers contaminated by treated sewage effluents. Given the extent of this problem, risk assessment models are needed to predict the location and severity of endocrine disruption in river catchments and to identify areas where regulation of sewage discharges to remove these contaminants is necessary. In this study we attempted to correlate the extent of endocrine disruption in roach in British rivers, with their predicted exposure to steroid estrogens derived from the human population. The predictions of steroid estrogen exposure at each river site were determined by combining the modeled concentrations of the individual steroid estrogens [17? -estradiol (E2), estrone (E1), and 17? -ethinylestradiol (EE2)] in each sewage effluent with their predicted dilution in the immediate receiving water. This model was applied to 45 sites on 39
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rivers throughout the United Kingdom. Each site studied was then categorized as either high, medium, or low “risk” on the basis of the assumed additive potency of the three steroid estrogens calculated from data derived from published studies in various cyprinid fish species. We sampled 1,438 wild roach from the predicted high-, medium-, and low-risk river sites and examined them for evidence and severity of endocrine disruption. Both the incidence and the severity of intersex in wild roach were significantly correlated with the predicted concentrations of the natural estrogens (E1 and E2) and the synthetic contraceptive pill estrogen (EE2) present. Predicted steroid estrogen exposure was, however, less well correlated with the plasma vitellogenin concentration measured in the same fish. Moreover, we found no correlation between any of the end points measured in the roach and the proportion of industrial effluents entering the rivers we studied. Overall, our results provide further and substantive evidence to support the hypothesis that steroidal estrogens play a major role in causing intersex in wild freshwater fish in rivers in the United Kingdom and clearly show that the location and severity of these endocrine-disrupting effects can be predicted.
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Increasing evidence shows that wild populations of both estuarine and freshwater fish are being exposed to endocrine-disrupting chemicals (EDCs) in concentrations sufficient to cause disruption of their reproductive physiology [reviewed by Jobling and Tyler (2003)]. In particular, the occurrence of intersex fish in a number of countries has been associated with the proximity of these fish to point source sewage effluent discharges

(Allen et al. 1999a, 1999b; Aravindakshan et al. 2004; Folmar et al. 1996, 2001; Gercken and Sordyl 2002; Harshbarger et al. 2000; Hassanin et al. 2002; Hecker et al. 2002; Jobling et al. 1998; Kavanagh et al. 2004; Minier et al. 2000; van Aerle et al. 2001, Vethaak et al. 2002; Vigano et al. 2001). Intersex fish can have feminized reproductive ducts and/or developing oocytes within their testes (Nolan et al. 2001). They can also have abnormal concentrations of sex steroid hormones (Jobling et al. 2002a) and (often) elevated concentrations of the estrogen-dependent blood protein vitellogenin (VTG) in their blood (Jobling et al. 1998). In severely feminized fish, fertility is reduced (Jobling et al. 2002b); hence, the contribution of these fish to the growth rate of the population is likely to be reduced. Studies published to date strongly suggest that the concentration of sewage effluent in a river is a major cause of intersexuality in wild roach (Jobling et al. 1998).

Furthermore, the association between the degree of intersexuality in fish and their plasma VTG concentration suggests that the two effects have a common cause and, therefore, the estrogenic constituents of sewage effluents are likely to be responsible for the occurrence of intersexuality in wild fish populations (Jobling et al. 1998).

Toxicity, identification, and evaluation procedures employing in vitro estrogen screening assays have identified the steroid estrogens as being the most significant estrogenic compounds present in most sewage effluents (e.g. Desbrow et al. 1998; Korner et al. 2001; Matsui et al. 2000;). Because of their human origin, steroid estrogens are regularly detected in domestic sewage effluents throughout Europe, Japan, and the United States, with concentrations ranging from < 1 to 48 ng/L for 17? -estradiol (E2), from 1 to 76 ng/L for estrone (E1), and from < 1 to 7 ng/L for 17? -ethinylestradiol (EE2) (e.g., Desbrow et al. 1998; Johnson et al. 2000; Komori et al. 2004; Niven et al. 2001). The concentrations of these estrogens in river water and their impact on fish are dictated in the first instance by the available dilution in the receiving water. Over time (and with increasing distance from the discharge point), biodegradation and sorption (Jurgens et al. 2002; Williams et al. 1999) further lower these concentrations, as has been illustrated clearly in the river Nene in the United Kingdom (Williams et al. 2003). Although the concentrations of these estrogenic substances in effluents are extremely low (in the tens of nanograms per liter range), when replicated in laboratory experiments, they are high enough to induce VTG synthesis and intersex in some fish species (e.g., Balch et al. 2004; Lange et al. 2001; Metcalfe et al. 2001; Nash et al. 2004; Orn et al. 2003; Palace et al. 2002; Thorpe et al. 2003a; Van den Belt et al. 2004). Furthermore, laboratory studies have shown that the combined effects of steroid estrogens can be
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additive (Brian et al. 2005; Silva et al. 2002; Thorpe et al. 2003b).

Hence, it is now an acknowledged possibility that even where the water concentration of one of these steroid estrogens is below the lowest observable effect concentration (LOEC), the combined mixture of steroid estrogens present could still cause an effect.

Association does not always imply causation, however, and there is still no overwhelming evidence that steroidal estrogens are the only cause of endocrine disruption in wild freshwater fish. Xenobiotic endocrine disruptors with estrogenic properties, such as 4-t-nonylphenol, are also present in some effluents at high enough concentrations alone to cause significant effects on reproductive development and function (Blackburn and Waldock 1995; Harries et al. 1996; Sheahan et al. 2002; Sole et al. 2000). The parent alkylphenol polyethoxylates may be discharged into the sewers from both domestic cleaning products (Bennie 1999) and from more specialized industrial users.

Undoubtedly, the most important issue from a regulatory perspective is to determine whether steroidal estrogens cause the majority of the disruptive reproductive effects seen in wild fish populations. One approach to testing this hypothesis is to search for relationships between the spatial distribution and severity of endocrine disruption, the size of the local human population, and the available dilution in the river water. The human excretion of steroid estrogens is predictable (Johnson and Williams 2004; Johnson et al. 2000) and should be linked to the human population (at least outside significant animal husbandry areas). Conversely, the discharge of different xenobiotic EDCs is likely to be far more random (Blackburn and Waldock 1995).

If a convincing correlation were to be found between sexual disruption in wild fish and the predicted concentrations of steroid estrogens within the river water in which they reside, it would provide confidence to regulators for using such steroid estrogen models to predict the location and severity of endocrine disruption in river catchments, and consequently, where regulatory controls might need to be implemented.

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Estimation of the relative potencies of E1, E2, and EE2. Steroid estrogens cause a wide range of feminizing effects in fish, including the induction of intersex and the synthesis of VTG in male fish. Moreover, it is increasingly acknowledged that the combined effects of steroid estrogens on any of these end points are likely to be additive (Brian et al. 2005; Silva et al. 2002; Thorpe et al. 2003b). Hence, they are better predicted using a toxic equivalency approach.

The relative potencies of each steroid also depend on the end points measured, the fish species used, and the timing and duration of the exposure. With these factors in mind, we estimated predicted equivalent concentrations of E2 separately for induction of VTG and for induction of intersex, using data from studies on cyprinid fish only. We used this procedure to try to avoid false positives or false negatives caused by the possible differences in sensitivity to the three estrogens among fish species. Wherever possible, data to assess relative potencies were used only from studies in which the actual concentrations of estrogen dosed had been analytically verified.

The E2 equivalent concentrations calculated using the previously described methods were compared with the no observed effect concentrations (NOECs) and LOECs of E2 required to induce feminization (VTG induction or intersex) in cyprinid fish. We then placed each sample site into high-risk (> LOEC), medium-risk (between the NOEC and the LOEC), or low-risk (< NOEC) categories for each end point separately, using the values discussed below as boundaries.

VITELLOGENIN INDUCTION. We found three studies in the literature on the relative potencies of the steroid estrogens in inducing VTG in cyprinid fish in vivo. Van den Belt et al. (2004) reported relative potencies of 1E2:0.8E1:30.6EE2 in adult female zebrafish (Danio rerio) in a 21-day study, while Brian et al. (2005) reported 1E2:40EE2 (E1 was not tested) in adult male fathead minnow (Pimephales promelas) during an identical exposure period. Thorpe et al. (2001) reported 1E2:0.36E1:25EE2 in female fathead minnow after 14 days of exposure. Studies on the potency of E1 in male cyprinid fish (Panter et al. 1998; Routledge et al. 1998) indicate that its
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potency is about 1.5 times lower than that of E2 (Brion et al. 2004). On the basis of this information, we used the relative potencies of 1E2:0.75E1:40EE2 to calculate the equivalent concentrations of E2 for the VTG end point.

The LOECs and NOECs selected for setting of the high-, medium-, and low-risk categories were from in vivo studies on adult male cyprinid fish over a 3-week exposure period,
Predicted individual concentrations of E1, E2, and EE2 in the rivers where roach were captured ranged from 0.07 to 13.78 ng/L for E1, from 0.01 to 1.7 ng/L for E2, and from zero to 0.37 ng/L for EE2. Individually, with the exception of estrone, these concentrations generally were not thought to be sufficient to cause feminizing effects in fish. However, when we combined all three steroids using E2 equivalent concentrations, many more locations were predicted to cause endocrine-disrupting effects in fish (based on data from studies on small laboratory cyprinid fish species). When we calculated equivalent concentrations from E2equiv/intersex = 1E2 + 5EE2 + 1E1, they ranged from 0.060 to 17.34 ng/L. When we calculated equivalent concentrations using VTG as the end point, however (using E2equiv/VTG =1E2 + 40EE2 + 0.75E1), they ranged from 0.48 ng E2/L to 26.85 ng E2/L. Both methods for calculating the E2 equivalent concentrations yielded concentrations of E2 that were above the chosen NOECs for intersex (1 ng/L) and VTG (5 ng/L) for all sites. At some sites, the predicted E2 equivalent concentrations were higher than the chosen LOECs (25 ng/L for VTG and 10 ng/L for intersex). It should be noted that to ensure comparability, the LOECs and NOECs for VTG were from the limited data available on the induction of this end point in short-term studies. Very recent long-term (lifetime) studies with medaka indicate that exposure to estradiol can induce VTG at concentrations ? 8.66 ng E2/L (Seki et al. 2005), thus lowering the threshold for the induction of this response under these conditions.

The results of the biological analyses revealed that intersex fish were present at many of the sites sampled (Figure 1A). When fish collected from all of the sites in each of the high-, medium-, and low-risk categories were pooled, clear differences could be discerned between predicted high-, medium-, and low-risk groups for intersex incidence and severity (Figures 1B, 2, 3, and 4). The pooled VTG analyses, however, revealed no clear dose-dependent relationship between VTG concentration and E2 equivalent in the male and intersex fish ($r2 = 0.003$, $p = 0.184$; Figure 5). This result was thought to be because the fish were sampled at different times throughout the year. Hence, seasonal variations in endogenous and/or exogenous estrogen (and, therefore, plasma VTG) concentrations in female, male, and intersex fish may have complicated the interpretation of the data (Figure 5). It is also possible that long-term (lifelong) exposure to steroid estrogens might attenuate the expected response and clarify the relationships between exposure and effect that are difficult to discern. Nash et al. (2004) reported a strong down-regulation of the VTG response in the F1 generation of zebrafish after multigenerational long term exposure of zebrafish to EE2. Seki et al. (2005) also reported this phenomenon in F1 medaka. Therefore, it is likely that some of the variation in VTG concentrations in exposed male and female fish in the wild is caused by differences in the timing and duration of their exposure to estrogenic compounds.

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