Available online at www.sciencedirect.com

SCIENCE DIRECT®

Science of the Total Environment 343 (2005) 1–34

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

www.elsevier.com/locate/scitotenv

Review

# Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations?

Lesley J. Mills[a,b,*], Clinton Chichester[b]

[a]*U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI 02882, United States*
[b]*Department of Biomedical Sciences, University of Rhode Island, Kingston, RI 02881, United States*

Received 9 June 2004; accepted 10 December 2004

**Abstract**

In this paper, evidence from the current literature is presented that addresses either of two questions: 1) do EDCs in the aquatic environment have the potential to impact the reproductive health and survival of various fish species, and 2) are EDCs in the aquatic environment actually impacting the reproductive health and sustainability of indigenous populations of fish? Overall, data from laboratory experiments support the hypothesis that EDCs in the aquatic environment can impact the reproductive health of various fish species, but evidence that EDCs in the aquatic environment are actually impacting the reproductive health and sustainability of indigenous fish populations is less convincing. The scarcity of evidence linking impacts of environmental EDCs with changes in reproductive success of indigenous fish populations may reflect a critical need for a dependable method or indicator to assess reproduction of fish in situ. In addition, more studies that investigate whether fish populations routinely exposed to EDCs in situ are experiencing changes in population structure are needed. Linking endocrine disruption and reproductive impairment with an ecologically relevant impact on the sustainability of real fish populations remains, with few exceptions, an open challenge.


*Keywords:* Endocrine disruption; EDC; Fish; Fish population; Fish reproduction

**Contents**



* Corresponding author. U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, 27 Tarzwell Drive, Narragansett, RI 02882, United States. Tel.: +1 401 782 3050.
*E-mail address:* mills.lesley@epa.gov (L.J. Mills).

0048-9697/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2004.12.070

## 1. Introduction

An endocrine disrupter has been defined as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or subpopulations" (see Vos et al., 2000). Endocrine-disrupting chemicals (EDCs) exert their effects by mimicking endogenous hormones, antagonizing normal hormones, altering the natural pattern of hormone synthesis or metabolism, or modifying hormone receptor levels (Sonnenschein and Soto, 1998). Because of these actions, EDCs have the potential to interfere with normal reproduction and development, which are controlled by an array of hormonal signals. In 1996, the issue of EDCs came to the forefront of public attention when Dr. Theo Colburn and colleagues published their book "Our Stolen Future" (Colburn et al., 1996). The book was given intense political attention, partly because its foreword was written by a sitting vice-president of the United States, Al Gore. Since the publication of this book, the scientific literature on the effects of EDCs has increased dramatically.

The aquatic environment has been termed "the ultimate sink" for natural and man-made chemicals (Sumpter, 1998), and EDCs have been found in freshwater, estuarine, and marine environments, raising the possibility that EDCs impact organisms living in these aquatic environments. Others have reviewed the evidence for occurrence of endocrine disruption in wildlife in general (Tyler et al., 1998; Taylor and Harrison, 1999; Vos et al., 2000) and specifically in marine and estuarine organisms (Oberdorster and Cheek, 2000). The intent of this review is to examine evidence from the scientific literature that addresses possible impacts of EDCs on the reproductive health and sustainability of fish populations. From an economic viewpoint, fish form the basis of a large commercial fishery and aquaculture industry, as well as having widespread recreational value. If EDCs in the aquatic environment are affecting reproductive success of fish, thus threatening population sustainability over time, commercial and sport fisheries could be impacted.

There are a number of classes of chemicals that show endocrine-disrupting properties. In vitro assays have been widely used to determine if chemicals have endocrine-disrupting activity (Zacharewski, 1997). These assays include cell proliferation assays such as the E-SCREEN assay (Soto et al., 1995), competitive binding assays that assess binding of a chemical with hormone receptors (Bolger et al., 1998), and reporter gene assays which use yeast or cell lines to measure transcriptional activity of a "reporter" gene upstream of a hormone responsive gene (Balaguer et al., 1999). Among EDCs found in the aquatic environment are the steroidal estrogens such as 17β-estradiol, estrone, estriol and the xenoestrogen 17α-ethynylestradiol, all of which have been found in sewage effluent in low ng/L concentrations (Desbrow et al., 1998; Snyder et al., 1999; Baronti et al., 2000). These estrogens bind with estrogen receptors in exposed organisms with an affinity identical or similar to the endogenous estrogen hormone, 17β-estradiol, and have the potential to exert effects at extremely low concentrations.

Biodegradation products of alkyl polyethoxylate detergents, such as nonylphenol and octylphenol, are also found in sewage effluent and wastewater from septic systems, but at high ng/L to low μg/L concentrations (Lye et al., 1999; Rudel et al., 1998). Some alkylphenols have been shown to bind with the estrogen receptor and stimulate a biological response similar to 17β-estradiol, although concentrations at least 1000-fold more are required (Jobling and Sumpter, 1993; White et al., 1994; Korner et al., 2000). Bisphenol A, which is used in epoxy resins, polycarbonate plastics and dental sealants (Sonnenschein and Soto, 1998), exhibits estrogenic activity in both an in vitro cell proliferation assay with human breast cancer cells (Brotons et al., 1995) and an estrogen-receptor yeast-based assay (Metcalfe et al., 2001). Bisphenol A has been detected in sewage treatment effluent (Korner et al., 2000) and septic system wastewater (Rudel et al., 1998) in low μg/L concentrations. Some persistent environmental contaminants, such as polychlorinated biphenyls (PCBs) and pesticides (DDT and metabolites, chlordecone, methoxychlor) are also considered endocrine-disrupting, based largely on their ability to bind with estrogen receptors. For example, *o*,*p*′-DDT, *o*,*p*′-DDE, chlordecone, nonylphenol, methoxychlor and hydroxylated PCBs have been shown to compete with 17β-estradiol for binding to estrogen receptors from two fish species (Loomis and Thomas, 1999; Nimrod and Benson, 1997). Several polynuclear aromatic hydrocarbons (PAHs), which are released into the environment by fossil fuel combustion, oil spills and industrial processes, have shown anti-estrogenic activity in a yeast-based estrogen receptor binding assay (Tran et al., 1996). Some EDCs interact with androgen, rather than estrogen, receptors. Sohoni and Sumpter (1998) observed that the therapeutic agents hydroxytamoxifen and flutamide, the fungicide vinclozolin, and the DDT metabolite *p*,*p*′-DDE were anti-androgens in their in vitro yeast-based assay, which measures binding with a human androgen receptor. These studies indicated that the 'estrogenic' chemicals bisphenol A, butyl benzyl phthalate and *o*,*p*′-DDT were also anti-androgens, demonstrating that an EDC can have multiple hormonal activities. Indeed, steroid hormone receptors have been termed 'promiscuous' in regard to ligand binding because many EDCs bind with more than one type of steroid receptor (Cooper and Kavlok, 1997). Finally, a few EDCs are known or suspected to act through mechanisms other than interaction with hormone receptors. An example is tributyltin (TBT), which is an organotin that has been widely used as an anti-foulant in paints applied to ships and marine structures. TBT received attention as an EDC when it was discovered to be responsible for the induction of masculinized females ("imposex") in marine gastropod mollusks. Evidence indicates that this EDC exerts its effects by interfering with normal steroid hormone biosynthesis rather than interacting with steroid hormone receptors (see Matthiessen and Gibbs, 1998 for review).

Endocrine disruption occurs when exogenous chemicals interact with internal endocrine signaling pathways in an organism (Cheek et al., 1998). EDCs may affect the development or reproduction of organisms by interfering with normal synthesis, storage, release, transport, metabolism, binding, action or elimination of endogenous hormones (Kavlock et al., 1996). In general, the reproductive physiology of vertebrates, both mammalian and non-mammalian, is similar, with the broad structure and function of the reproductive axis involving the hypothalamus, pituitary and gonads conserved. In all vertebrates, the release of a decapeptide gonadotrophin-releasing hormone (GnRH) from the hypothalamus stimulates the pituitary to secrete gonadotrophic hormones (GTH) that signal the gonads to synthesize steroid hormones. The basic biosynthetic pathways for steroid hormones and the active steroid hormones themselves are also well-conserved in both mammalian and non-mammalian vertebrates. However, fish have some aspects of reproductive physiology that may cause them to respond differently to EDCs than mammals. For example, in fish, secretion of GnRH normally changes in response to environmental cues (Dawson, 1998). Some fish species are viviparous (bearing live young), while others are oviparous (egg-laying). Sex determination in fish can be entirely genetic, or it can be phenotypic depending on temperature or other environmental cues. Fish species may be dioecious (separate sexes) or hermaphroditic (male and female gonads in a single individual). Hermaphrodites may be simultaneous or sequential. Sequential hermaphrodism can consist of a male changing into a female (protandry) or a female changing into a male

(protogyny). In oviparous fish species, the production of vitellogenin, an egg yolk protein precursor, is a critical step for successful reproduction in females. Normally, only mature females produce enough endogenous estrogen to induce vitellogenesis in fish, but exposure to estrogenic chemicals in the external environment can trigger this response in male and juvenile fish as well.

In the aquatic environment, EDCs are easily bioavailable to fish through a variety of routes, including aquatic respiration, osmoregulation and maternal transfer of contaminants in lipid reserves of eggs (Van Der Kraack et al., 2001). Dermal contact with contaminated sediments or ingestion of contaminated food are additional exposure routes. EDCs that are apt to cause endocrine modulation in vivo have one of three characteristics: they are present in the environment at high concentrations, they are persistent and bioaccumulative, or they are constantly entering the environment (Tyler et al., 1998). EDCs that are resistant to biodegradation and are lipophilic may bioaccumulate in exposed organisms. Such EDCs include organochlorine pesticides, polychlorinated biphenyls (PCBs) and alkylphenols (Tyler et al., 1998), all of which are found in aquatic environments. Other EDCs, such as bisphenol A and 17β-estradiol, do not bioaccumulate to any extent (Tyler et al., 1998) but are constantly entering the aquatic environment through operations such as effluent from sewage treatment works and run-off from concentrated animal feeding operations.

The focus of this paper is to try to provide an assessment of the effects of EDCs on fish populations by reviewing the evidence from the current literature that addresses either of two questions. The first question is whether EDCs in the aquatic environment have the potential to impact the reproductive health and survival of various fish species, and concentrates on results from controlled laboratory studies. Laboratory studies have identified EDCs that affect reproductive success in vivo. The second question is whether EDCs in the aquatic environment are actually impacting the reproductive health and survival of indigenous populations of fish, and is based on the results of field studies. To ascertain that exposure to EDCs is affecting a fish population, evidence should exist that there are impacts on the reproductive success of that fish species, or on the size or age structure of a fish population.

## 2. Review of evidence

EDCs, by interfering with the sensitive hormone pathways that regulate reproductive functions, may lead to reduced egg production or fertility in fish (Arcand-Hoy and Benson, 1998). If reproductive success in a fish population decreases, adult survival (or recruitment of fish from adjoining areas) would have to increase in order to maintain sufficient stock of reproducing adults to ensure that the number of individuals in a population would be maintained. Historically, researchers have used various indicators to imply reproductive effects. Examples of some of these indicators are an increase in intersexuality in reproductive adults, presence of the female phospholipoprotein vitellogenin in male fish and the ability of a chemical to bind to hormone receptors in in vitro assays. To be ecologically relevant, these types of indicators must be linked with measures of survival or reproductive success in fish populations. For example, vitellogenin production by male or juvenile fish is an indicator that has been used extensively to indicate exposure to estrogenic EDCs, but the ecological relevance of the male vitellogenic response for predicting reproductive success or population effects is unclear. Fish species vary widely both in their sensitivity to induction of vitellogenin upon exposure to EDCs and in the magnitude of their response (Routledge et al., 1998; Purdom et al., 1994; Jobling et al., 2003; Thompson et al., 2000). Other factors may also contribute to a variable vitellogenic response, including water temperature (Purdom et al., 1994), migratory behavior (Kirby et al., 2004), prior history of EDC exposure (Pait and Nelson, 2003) and type of EDC exposure (Panter et al., 2002). One type of EDC exposure, to pulp mill effluent, does not induce vitellogenin production in fish (Munkittrick et al., 1998). In addition, vitellogenin concentrations that may impact survival or reproduction in one species may not affect these parameters in another. For example, ethynylestradiol added to an experimental lake in Canada increased vitellogenin levels 9000-fold in indigenous male fathead minnows (Palace et al., 2002) and the fathead minnow

population experienced a dramatic decline (Pelley, 2003). However, another of the lake's fish species, pearl dace (*Semotilus margarita*), has not shown any definitive effects on population structure (Pelley, 2003), although male vitellogenin levels are 1000- to 15 000-fold that of control males (Palace et al., 2003). Finally, although measurements of vitellogenin have been widely used to infer reproductive impairment in males, there is little evidence to support this assumption. In laboratory experiments, vitellogenin induction was not correlated with inhibition of male sexual behavior in male fathead minnows (Kramer et al., 1998), nor with percentage of fertile eggs or presence of motile sperm in male cunner (*Tautogolabrus adspersus*) (Mills et al., 2003). These researchers found that elevated male vitellogenin was inversely related to female egg production in both fathead minnows and cunner exposed to estrogens, but the male vitellogenin concentrations were much higher than those generally reported in male fish from the environment (Kramer et al., 1998; Mills et al., 2003). In short, elevated male plasma vitellogenin, which is an endocrine-specific endpoint (Hutchinson and Pickford, 2002), can serve as a warning sign that exposure to estrogenic EDCs is occurring in a fish population, but the value of the male vitellogenic response for predicting reproductive or population impacts is not supported by the current evidence. Thus, literature reporting only elevated vitellogenin in male fish, with no reproductive information, is not included in this review.

### 2.1. Laboratory studies with a single EDC

Results of laboratory studies with single EDCs indicate which EDCs have the potential to impact reproduction in fish. Numerous researchers have explored the effects of EDCs on fish reproduction in laboratory studies, although these studies have been conducted with a limited number of fish species. For the most part, research has been on chemicals that interfere with or mimic the actions of endogenous estrogens. In this section, results from laboratory studies are grouped according to the species of fish used in the studies, so that effects of EDCs on the different fish species can be compared. Results from laboratory studies are summarized by the EDC of interest in Table 1 (steroidal estrogens) and Table 2 (estrogen mimics). Table 3 presents results of studies with androgenic or antiandrogenic EDCs. Finally, Table 4 summarizes the types of reproductive effects that have been observed in fish species exposed to various EDCs in laboratory tests.

#### 2.1.1. Medaka (Oryzias latipes)

Probably the most commonly used species in studies assessing changes in reproductive success is Japanese medaka. This freshwater tropical aquarium species was used as a laboratory model to study effects of estrogenic compounds as long ago as the 1950s (Yamamoto, 1953, 1958). Medaka are suitable to evaluate effects of EDCs because they are daily egg-layers, they have a short time to maturity (6–8 weeks), and their small size (3–4 cm) allows for large sample numbers in treatment groups (Arcand-Hoy and Benson, 1998; Nimrod and Benson, 1998; Patyna et al., 1999). The species is sexually dimorphic. Males typically have a dorsal fin notch and convex anal fin, which has tubercle-like processes on its rays when the males are sexually mature (Papoulias et al., 1999). In addition, a strain of medaka (d-rR) has been developed that possesses a sex-linked color gene which produces orange-red coloration in genetic males, allowing easy identification of genetic sex regardless of functional gender (Yamamoto, 1953, 1958).

Studies with medaka show that dietary exposure to 17β-estradiol can result in sex reversal in developing fish, as well as reduced male sexual behavior and egg production in adults. Patyna et al. (1999) exposed young medaka to 17β-estradiol in their diet starting 2 weeks post-hatch and continuing for 170 days. All fish in the 0.05 and 0.5 mg/kg treatments had female secondary sexual characteristics and immature ovaries. None of the estradiol-exposed fish produced any eggs. Oshima et al. (2003) exposed mating pairs of adult medaka to17β-estradiol in their diet (3 and 30 μg/g body weight) for 2 weeks. In this short-term exposure experiment, both egg production and male sexual behavior decreased significantly in estradiol-treated medaka. Fertility of eggs did not differ between control and estradiol-treated fish, which these researchers interpreted to mean short-term estradiol exposure did not affect quality of egg or sperm.

Table 1
Reproductive effects in fish exposed in the laboratory to steroidal estrogens, organized by chemical

| EDC | Fish species | Life stage | Exposure route | Exposure length | High conc. (ppb) | LOEC (ppb) | Effect | Reference |
|---|---|---|---|---|---|---|---|---|
| Estradiol | Medaka | post-hatch to adult | diet | 170 days | 5 mg/kg (5000) | 0.05 mg/kg (50)<br>5 mg/kg (5000) | no egg production<br>high mortality | Patyna et al., 1999 |
| | | repro. adults | diet | 2 weeks | 30 μg/g (30 000) | 3 μg/g (3000) | reduced egg production; decreased male sexual behavior | Oshima et al., 2003 |
| | | post-hatch | water | 1 month | 1.66 μg/L (1.66) | 0.01 μg/L (0.01)<br>1.66 μg/L (1.66) | all female fish<br>reduced egg production | Nimrod and Benson, 1998 |
| | | repro. adults | water | 21 days | 463 ng/L (0.463) | 29.3 ng/L (0.029)<br>463 ng/L (0.463) | males with testis–ova<br>reduced male GSI, egg production and fertility | Kang et al., 2002 |
| | | post-hatch | water | 100 days | 1 μg/L (1) | 0.01 μg/L (0.01)<br>0.1 μg/L (0.1)<br>1 μg/L (1) | 10% of males with testis–ova<br>all males with testis–ova<br>high mortality with only females surviving | Metcalfe et al., 2001 |
| | | repro. adults | water | 2 weeks | 27.24 μg/L (27.24) | 0.817 μg/L (0.817) | reduced egg prod. and decreased egg hatch | Shioda and Wakabayashi, 2000 |
| | | eggs or young | water | 6 days | 15 μg/L (15) | 15 μg/L (15) | more phenotypic females than males; intersex gonads | Koger et al., 2000 |
| | Fathead minnow | repro. adults | water | 19 days | 2724 ng/L (2.724) | est. 120 ng/L (0.12) | inhibition of egg production | Kramer et al., 1998 |
| | | develop. males | water | 21 days | 1000 ng/L (1) | 320 ng/L (0.320) | reduced testicular growth (GSI) | Panter et al., 1998 |
| | Goldfish | repro. males | diet | 24–28 days | 100 μg/g (100 000) | 10 μg/g (10 000)<br>100 μg/g (100 000) | reduced GSI and courting behavior<br>fewer males with milt and tubercles | Bjerselius et al., 2001 |
| | | repro. males | water | 24–28 days | 10 μg/L (10) | 1 μg/L (1) | reduced GSI and courting; fewer males with tubercles | Bjerselius et al., 2001 |
| | Guppies | repro. adults | water | 26–36 days | 0.85 μg/L (0.85) | NA | no adverse effects on reproduction or young observed | Kinnberg et al., 2003 |
| | | repro. adults | water | 4 weeks | 10 μg/L (10) | 10 μg/L (10) | complete inhibition of male sexual behavior | Bayley et al., 1999 |
| | Common carp | repro. males | water | 3 months | 1 μg/L (1) | 1 μg/L (1) | reduced GSI; no milt production; some testis–ova | Gimeno et al., 1998b |
| | | juvenile males | water | 2 months | 23 μg/L (23) | 9 μg/L (9) | all juvenile males developed into females | Gimeno et al., 1998a |
| Ethynylestradiol | Medaka | post-hatch | water | 100 days | 1 μg/L (1) | 0.1 μg/L (0.1) | all males with testis–ova; more females than males | Metcalfe et al., 2001 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 1 μg/L (1) | high mortality with only females surviving | |
| | | repro. adults | water | 21 days | 488 ng/L (0.488) | 63.9 ng/L (0.064) | males with testis–ova (still fertile) | Seki et al., 2002 |
| | | | | | | 488 ng/L (0.488) | reduced egg production and high male mortality | |
| | | post-hatch | water | 2 months | 100 ng/L (0.1) | 10 ng/L (0.01) | reduced egg production | Scholz and Gutzeit, 2000 |
| | | | | | | 100 ng/L (0.1) | genetic males all developed into functional females | |
| | Fathead minnow | egg to adult | water | 305 days | 64 ng/L (0.064) | 4 ng/L (0.004) | all fish with female gonads (50% fertile) | Lange et al., 2001 |
| | | | | | | 16 ng/L (0.016) | severe physical deformities | |
| | | develop. females | water | 21 days | 100 ng/L (0.1) | 0.1 ng/L (0.0001) | increased egg production | Jobling et al., 2003; Pawlowski et al., 2004 |
| | | | | | | 10 ng/L (0.01) | reduced egg production | |
| | | | | | | 100 ng/L (0.1) | no egg production | |
| | | develop. females | water | 21 days | 100 ng/L (0.1) | 10 ng/L (0.01) | reduced egg fertilization success rate | Pawlowski et al., 2004 |
| | Rainbow trout | develop. males | water | 3 weeks | 2 ng/L (0.002) | 2 ng/L (0.002) | reduced testicular growth (GSI) | Jobling et al., 1996 |
| | Zebrafish | egg to adult | water | 75 days | 10 ng/L (0.01) | 1.67 ng/L (0.00167) | delayed maturation; altered mating behavior | Segner et al., 2003 |
| | | | | | | 3 ng/L (0.003) | reduced egg production and fertilization; altered gonad histology | |
| | | post-hatch | water | 60 days | 15.4 ng/L (0.0154) | 15.4 ng/L (0.0154) | all females; depressed ovarian development | Andersen et al., 2003 |
| | | 20 to 60 days post-hatch | water | 40 days | 25 ng/L (0.025) | 1 ng/L (0.001) | more females than males | Orn et al., 2003 |
| | | | | | | 2 ng/L (0.002) | all females, except at highest dose (25 ng/L) | |
| | Sand goby | juvenile to repro. adult | water | 7 months | 6 ng/L (0.006) | 6 ng/L (0.006) | delayed male maturation; inhibited male sex behavior; reduced egg production and fertilization | Robinson et al., 2003 |
| Estrone | Medaka | post-hatch | water | 100 days | 10 μg/L (10) | 1 μg/L (1) | all males with testis–ova; more females than males | Metcalfe et al., 2001 |
| | Fathead minnow | develop. males | water | 21 days | 992.7 ng/L (0.9927) | 317.7 ng/L (0.3177) | reduced testicular growth (GSI) | Panter et al., 1998 |
| Estriol | Medaka | post-hatch | water | 100 days | 10 μg/L (10) | 0.01 μg/L (0.01) | more males than females (except at high concentration) | Metcalfe et al., 2001 |
| | | | | | | 10 μg/L (10) | all males with testis–ova | |

repro.=actively reproducing fish; develop.=adult fish that are starting to develop into a reproductively active state; High conc.=highest concentration tested in study; LOEC=lowest tested concentration at which noted effect occurred.

Table 2
Reproductive effects in fish exposed in the laboratory to single EDCs with estrogenic activity, organized by chemical

| EDC | Fish species | Life stage | Exposure route | Exposure length | High conc. (ppb) | LOEC (ppb) | Effect | Reference |
|---|---|---|---|---|---|---|---|---|
| Nonylphenol | Medaka | post-hatch | water | 1 month | 1.9 μg/L (1.9) | 0.54 μg/L (0.54)<br>0.77 μg/L (0.77) | increased egg production<br>more males than females | Nimrod and Benson, 1998 |
| | | repro. adults | water | 2 weeks | 64.5 μg/L (64.5) | NA | no significant adverse effects observed | Shioda and Wakabayashi, 2000 |
| | | post-hatch | water | 100 days | 100 μg/L (100) | NA | no significant adverse effects observed | Metcalfe et al., 2001 |
| | Fathead minnow | repro. adults | water | 3 weeks | 100 μg/L (100) | 100 μg/L (100) | reduced egg production<br>reduced male secondary sex characteristics | Harries et al., 2000 |
| | | repro. adults | water | 42 days | 3.4 μg/L (3.4) | NA | no significant adverse effects observed | Geisy et al., 2000 |
| | Rainbow trout | develop. males | water | 3 weeks | 30 μg/L (30) | 30 μg/L (30) | reduced testicular growth (GSI) | Jobling et al., 1996 |
| | | repro. adults | water | 3 weeks | 54.3 μg/L (54.3) | 54.3 μg/L (54.3) | reduced testicular growth (GSI) | |
| | | develop. adults | water | 4 months (10 days/ month) | 10 μg/L (10) | 1 μg/L (1)<br>10 μg/L (10) | reduced egg viability<br>offspring with disrupted hormonal balance | Schwaiger et al., 2002 |
| Octylphenol | Medaka | post-hatch to adult | water | 6 months | 100 μg/L (100) | 10 μg/L (10) | significant developmental abnormalites in 2nd generation | Gray et al., 1999 |
| | | | | | | 25 μg/L (25) | reduced courting activity and fertilization by males | |
| | | | | | | 50 μg/L (50) | low incidence of testis–ova in males | |
| | Rainbow trout | developing males | water | 3 weeks | 30 μg/L (30) | 30 μg/L (30) | reduced testicular growth (GSI) | Jobling et al., 1996 |
| | | repro. males | water | 3 weeks | 43.9 μg/L (43.9) | 4.8 μg/L (4.8) | reduced testicular growth (GSI) at 4.8 μg/L only | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Guppies | repro. adults | water | 26-36 days | 26 μg/L (26) | 26 μg/L (26) | no adverse effects on reproduction or young observed | Kinnberg et al., 2003 |
| | | repro. males | water | 4 weeks | 150 μg/L (150) | 150 μg/L (150) | inhibition of male sexual behavior | Bayley et al., 1999 |
| Pentylphenol | Common carp | repro. males | water | 3 months | 1000 μg/L (1000) | 32 μg/L (32) | reduced GSI | Gimeno et al., 1998b |
| | | | | | | 1000 μg/L (1000) | reduced density of spermatozoa in milt | |
| | | juvenile males | water | 3 months | 256 μg/L (256) | 36 μg/L (36) | feminized testis with oviduct formation in males | Gimeno et al., 1998a |
| Methoxychlor | Medaka | post-hatch | water | 1 month | 2.3 μg/L (2.3) | NA | no adverse effects observed | Nimrod and Benson, 1998 |
| | Fathead minnow | repro. adults | water | 21 days | 3.56 μg/L (3.56) | 3.56 μg/L (3.56) | reduced egg production; altered concentration of plasma steroids | Ankley et al., 2001 |
| Bisphenol A | Medaka | repro. adults | water | 2 weeks | 2282.8 μg/L (2282.8) | 2282.8 μg/L (2282.8) | reduced egg production and reduced hatch | Shioda and Wakabayashi, 2000 |
| | | post-hatch | water | 100 days | 200 μg/L (200) | 10 μg/L (10) | few testis–ova; morphological changes in testes | Metcalfe et al., 2001 |
| | Zebrafish | egg to adult | water | 75 days | 1500 μg/L (1500) | 375 μg/L (375) | altered gonad histology | Segner et al., 2003 |
| | | | | | | 1500 μg/L (1500) | delayed maturation; altered mating behavior; reduced egg production and fertilization | |
| | Fathead minnow | adults | water | 164 days | 1280 μg/L (1280) | 640 μg/L (640) | reduced GSI and egg hatchabilty | Sohoni et al., 2001 |
| | | | | | | 1280 μg/L (1280) | reduced egg production | |
| DEHPhthalate | Medaka | repro. adults | water | 2 weeks | 390.54 μg/L (390.54) | NA | no adverse effects observed | Shioda and Wakabayashi, 2000 |
| | | post-hatch | water | 100 days | 5000 μg/L (5000) | NA | no adverse effects observed | Metcalfe et al., 2001 |
| BBPhthalate | Fathead minnow | repro. adults | water | 3 weeks | 100 μg/L (100) | NA | no adverse effects observed | Harries et al., 2000 |

repro.=actively reproducing fish; develop.=adult fish that are starting to develop into a reproductively active state; High conc.=highest concentration tested in study; LOEC=lowest tested concentration at which noted effect occurred.

Table 3
Reproductive effects in fish exposed in the laboratory to single EDCs with androgenic or antiandrogenic activity

| EDC | Fish species | Life stage | Exposure route | Exposure length | High conc. (ppb) | LOEC (ppb) | Effect | Reference |
|---|---|---|---|---|---|---|---|---|
| Testosterone | Medaka | eggs or young | water | 6 days | 100 μg/L (100) | 100 μg/L (100) | intersex gonads | Koger et al., 2000 |
| Methyltestosterone | Fathead minnow | repro. adults | water | 12 days | 1700 μg/L (1700) | 120 μg/L (120) | no egg production females developed male nuptial tubercles | Ankley et al., 2001 |
| | | juveniles | water | 21 days | 100 μg/L (100) | 10 μg/L (10) | vitellogenin induction in all fish | Zerulla et al., 2002 |
| | Zebrafish | 20 to 60 days post-hatch | water | 40 days | 1000 ng/L (1) | 26 ng/L (0.026) | all developed as males | Orn et al., 2003 |
| | | | | | | 250 ng/L (0.25) | intersex gonads in males (testis–ova) | |
| Vinclozolin | Fathead minnow | egg to juveniles | water | 34 days | 1200 μg/L (1200) | NA | no adverse effects observed | Makynen et al., 2000 |
| | | repro. adults | | 21 days | 700 μg/L (700) | 700 μg/L (700) | reduced female GSI; retarded oocyte development | |
| | Guppies | birth to repro. adult | diet | 24 weeks | 10 μg/mg (1 000 000) | 0.1 μg/mg (100 000) | inhibited male sexual behavior and gonopodium growth; reduced body size and young/female | Bayley et al., 2002 |
| | | | | | | 10 μg/mg (1 000 000) | delayed maturation; reduced sperm count; more females than males; very few young/female | |
| *p*,*p*′-DDE | Guppies | birth to repro. adult | diet | 24 weeks | 0.1 μg/mg (100 000) | 0.01 μg/mg (10 000) | reduced young/female | Bayley et al., 2002 |
| | | | | | | 0.1 μg/mg (100 000) | inhibited gonopodium growth; reduced sperm count; more females than males; reduced body size | |
| Flutamide | Guppies | birth to repro. adult | diet | 24 weeks | 1.0 μg/mg (1 000 000) | 0.01 μg/mg (10 000) | inhibited gonopodium growth | Bayley et al., 2002 |
| | | | | | | 1.0 μg/mg (1 000 000) | inhibited male sexual behavior; more females than males; reduced number of young per female | |

repro.=actively reproducing fish; develop.=adult fish that are starting to develop into a reproductively active state; High conc.=highest concentration tested in study; LOEC=lowest tested concentration at which noted effect occurred.

Table 4
Reproductive effects, ranked from most common to least common,[a] in fish exposed in the laboratory to single EDCs

| Reproductive effect | EDC exposure | Species | References |
|---|---|---|---|
| Reduced egg production (or live young)[1] | estradiol | medaka | Patyna et al., 1999; Oshima et al., 2003; Kang et al., 2002; Nimrod and Benson, 1998; Shioda and Wakabayashi, 2000 |
| | | fathead minnow | Kramer et al., 1998 |
| | ethynylestradiol | medaka | Seki et al., 2002; Scholz and Gutzeit, 2000 |
| | | zebrafish | Segner et al., 2003 |
| | | fathead minnow | Jobling et al., 2003 |
| | | sand goby | Andersen et al., 2003 |
| | nonylphenol | fathead minnow | Harries et al., 2000 |
| | methoxychlor | fathead minnow | Ankley et al., 2001 |
| | bisphenol A | medaka | Shioda and Wakabayashi, 2000 |
| | | fathead minnow | Sohoni et al., 2001 |
| | | zebrafish | Segner et al., 2003 |
| | methyltestosterone | fathead minnow | Ankley et al., 2001 |
| | vinclozolin | guppies | Bayley et al., 2002 |
| | *p*,*p*′-DDE | guppies | Bayley et al., 2002 |
| | flutamide | guppies | Bayley et al., 2002 |
| Skewed sex ratio[1] | estradiol | medaka | Nimrod and Benson, 1998; Koger et al., 2000; Metcalfe et al., 2001 |
| | | carp | Gimeno et al., 1998a |
| | ethynylestradiol | medaka | Metcalfe et al., 2001; Scholz and Gutzeit, 2000 |
| | | fathead minnow | Lange et al., 2001 |
| | | zebrafish | Andersen et al., 2003; Orn et al., 2003 |
| | estrone | medaka | Metcalfe et al., 2001 |
| | estriol | medaka | Metcalfe et al., 2001 |
| | nonylphenol | medaka | Nimrod and Benson, 1998 |
| | methyltestosterone | zebrafish | Orn et al., 2003 |
| | vinclozolin | guppies | Bayley et al., 2002 |
| | *p*,*p*′-DDE | guppies | Bayley et al., 2002 |
| | flutamide | guppies | Bayley et al., 2002 |
| Reduced male Gonadosomatic Index (GSI) | estradiol | medaka | Kang et al., 2002 |
| | | fathead minnow | Panter et al., 1998 |
| | | goldfish | Bjerselius et al., 2001 |
| | | carp | Gimeno et al., 1998b |
| | ethynylestradiol | rainbow trout | Jobling et al., 1996 |
| | estrone | fathead minnow | Panter et al., 1998 |
| | nonylphenol | rainbow trout | Jobling et al., 1996 |
| | octylphenol | rainbow trout | Jobling et al., 1996 |
| | pentylphenol | carp | Gimeno et al., 1998b |
| | bisphenol A | fathead minnow | Sohoni et al., 2001 |
| Decreased sexual behavior in males | estradiol | medaka | Oshima et al., 2003 |
| | | goldfish | Bjerselius et al., 2001 |
| | | guppies | Bayley et al., 1999 |
| | ethynylestradiol | zebrafish | Segner et al., 2003 |
| | | sand goby | Robinson et al., 2003 |
| | octylphenol | medaka | Gray et al., 1999 |
| | | guppies | Bayley et al., 1999 |
| | bisphenol A | zebrafish | Segner et al., 2003 |
| | vinclozolin | guppies | Bayley et al., 2002 |
| | flutamide | guppies | Bayley et al., 2002 |
| Intersex gonads | estradiol | medaka | Kang et al., 2002; Koger et al., 2000; Metcalfe et al., 2001 |

(continued on next page)

Table 4 (*continued*)

| Reproductive effect | EDC exposure | Species | References |
|---|---|---|---|
| Intersex gonads | estradiol | carp | Gimeno et al., 1998b |
| | ethynylestradiol | medaka | Metcalfe et al., 2001; Seki et al., 2002 |
| | estrone | medaka | Metcalfe et al., 2001 |
| | estriol | medaka | Metcalfe et al., 2001 |
| | octylphenol | medaka | Gray et al., 1999 |
| | bisphenol A | medaka | Metcalfe et al., 2001 |
| | testosterone | medaka | Koger et al., 2000 |
| | methyltestosterone | zebrafish | Orn et al., 2003 |
| Reduced egg fertility | estradiol | medaka | Kang et al., 2002 |
| | ethynylestradiol | zebrafish | Segner et al., 2003 |
| | | fathead minnow | Pawlowski et al., 2004 |
| | | sand goby | Robinson et al., 2003 |
| | octylphenol | medaka | Gray et al., 1999 |
| | bisphenol A | zebrafish | Segner et al., 2003 |
| Decrease in male sexual characteristics | estradiol | goldfish | Bjerselius et al., 2001 |
| | nonylphenol | fathead minnow | Harries et al., 2000 |
| | vinclozolin | guppies | Bayley et al., 2002 |
| | *p*,*p*′-DDE | guppies | Bayley et al., 2002 |
| | flutamide | guppies | Bayley et al., 2002 |
| Males with no milt or a reduced sperm count | estradiol | goldfish | Bjerselius et al., 2001 |
| | | carp | Gimeno et al., 1998b |
| | pentylphenol | carp | Gimeno et al., 1998b |
| | vinclozolin | guppies | Bayley et al., 2002 |
| | *p*,*p*′-DDE | guppies | Bayley et al., 2002 |
| Delayed sexual maturation[2] | ethynylestradiol | zebrafish | Segner et al., 2003 |
| | | sand goby | Robinson et al., 2003 |
| | bisphenol A | zebrafish | Segner et al., 2003 |
| | vinclozolin | guppies | Bayley et al., 2002 |
| Alterations in gonad structure[2] | ethynylestradiol | zebrafish | Segner et al., 2003 |
| | pentylphenol | carp | Gimeno et al., 1998a |
| | bisphenol A | medaka | Metcalfe et al., 2001 |
| | | zebrafish | Segner et al., 2003 |
| Decreased egg viability or hatchability[2] | estradiol | medaka | Shioda and Wakabayashi, 2000 |
| | nonylphenol | rainbow trout | Schwaiger et al., 2002 |
| | bisphenol A | medaka | Shioda and Wakabayashi, 2000 |
| | | fathead minnow | Sohoni et al., 2001 |
| Physical deformities[3] | ethynylestradiol | fathead minnow | Lange et al., 2001 |
| | octylphenol | medaka | Gray et al., 1999 |
| Altered plasma steroid hormone concentrations[3] | nonylphenol | rainbow trout | Schwaiger et al., 2002 |
| | methoxychlor | fathead minnow | Ankley et al., 2001 |
| Reduced female Gonadosomatic Index (GSI) | bisphenol A | fathead minnow | Sohoni et al., 2001 |
| | vinclozolin | | Makynen et al., 2000 |
| Females with male sexual characteristics | methyltestosterone | fathead minnow | Ankley et al., 2001 |

Superscript indicates that two effects had identical ranking.

[a] Ranking determined by summing the number of EDCs that caused the effect with the number of species exhibiting the effect.

Exposure to 17β-estradiol in water also affects reproductive capacity in medaka. Kang et al. (2002) exposed reproducing pairs of medaka to waterborne estradiol for 21 days. Both total number of eggs produced and egg fertility were significantly less in the highest estradiol treatment (463 ng/L). Intersex gonads (testis–ova) were observed in males from a range of estradiol treatments (29–463 ng/L). No transgenerational effects on egg hatch or sex ratio were noted in the offspring of the medaka treated with estradiol. In another study with reproductive adult medaka, Shioda and Wakabayashi (2000) found fish

exposed to estradiol at 3 nmol/L or above for 2 weeks produced significantly fewer eggs and had a significantly lower hatching rate than controls. Nimrod and Benson (1998) observed that exposure of young medaka to 0.01, 0.12 or 1.66 μg/L waterborne 17β-estradiol for a month following hatch resulted in development of all females. When these female medaka were paired with untreated males, females from the 1.66 μg/L treatment produced a significantly decreased number of eggs compared with controls. Thus, estradiol can induce sex reversal in developing medaka and intersex gonads in adults, as well as reducing egg production, fertility and hatch.

Seki et al. (2002) exposed mating pairs of medaka to waterborne ethynylestradiol, the xenoestrogen, for 21 days and observed a significant decrease in overall egg production in their 488 ng/L treatment, but no significant differences in mean fertility in any treatment. Scholz and Gutzeit (2000) exposed juvenile medaka to waterborne ethynylestradiol (nominal concentrations of 1, 10 and 100 ng/L) for 2 months, followed by a six-week recovery period in clean water. They observed no differences in survival or growth of the ethynylestradiol-exposed fish compared to controls, although all genetic males (XY) developed into functional females with normal immature ovaries in the 100 ng/L treatment. In genetic females (XX) from the 10 and 100 ng/L treatments, significantly reduced GSI (GSI=gonad weight normalized to body weight) and egg production was observed. These results indicate that ethynylestradiol exposure can decrease egg production in female medaka and cause sex-reversal in genetic male medaka.

Exposure to other estrogenic EDCs has been shown to affect male sexual behavior, egg production, egg fertility and egg hatching in medaka. Adults exposed to 10 μmol/L bisphenol A for 2 weeks exhibited significant decreases in egg production and egg hatchability, but nonylphenol (0.03–0.3 μmol/L) and diethylhexylpthalate (0.1–1 μmol/L) did not significantly affect these parameters (Shioda and Wakabayashi, 2000). Nimrod and Benson (1998) exposed developing medaka to nonylphenol (0.5–1.9 μg/L) and methoxychlor (0.2–2.3 μg/L) for a month following hatch and found no effect on adult sex ratios or egg production, viability and hatchability. Gray et al. (1999) exposed newly hatched medaka to octylphenol (10, 25, 50 and 100 μg/L) for 6 months and found males exposed to octylphenol during development exhibited reductions in courtship activity and egg fertilization rates.

Several studies have investigated the impact of exposure to androgenic EDCs. Seki et al. (2004) exposed medaka to the androgen methyltestosterone from egg through adult and noted that all fish exposed to waterborne methyltestosterone at 27.75 ng/L possessed either testes or testes–ova and exhibited male secondary sex characteristics. In the 9.98 ng/L treatment, 40% of female fish had male secondary sex characteristics, but egg production, fertility and hatchability was not significantly different than controls. Koger et al. (2000) found that some medaka exposed to waterborne testosterone (100 μg/L) for a six-day period during development from eggs to young exhibited intersex gonads, but this exposure did not affect phenotypic sex ratio in adults. These results indicate that exposure to androgenic EDCs can affect both sexual differentiation and secondary sex characteristics in medaka.

Some studies demonstrate that exposure to estrogenic EDCs can cause testis–ova formation in medaka, although presence of such intersex gonads does not necessarily translate into reproductive impairment in affected individuals. Kang et al. (2002) observed testes–ova in reproducing males treated with a range of waterborne estradiol concentrations (29.3–463 ng/L). Male GSI was also reduced in fish exposed to 463 ng/L estradiol. Males from all treatment groups, except 463 ng/L, were able to fertilize eggs, indicating that presence of testes–ova does not necessarily mean reproductive impairment. Metcalfe et al. (2001) also observed testis–ova in medaka exposed to four different estrogens (17β-estradiol, 17α-ethynylestradiol, estrone and estriol) from hatch to approximately 100 days post-hatch, but did not measure reproductive fitness in these fish. Gray et al. (1999) reported that a single male medaka exposed to 100 μg/L octylphenol for 6 months had a testis–ova, but was still able to fertilize eggs from an unexposed female. Koger et al. (2000) induced intersex in medaka by exposing eggs and newly hatched fry to high concentrations of waterborne estradiol (15 μg/L) for a six day period. Estradiol exposure resulted in a phenotypic sex ratio that was greater than 75% female, but estradiol-induced intersex gonads were functionally male and still

produced mature spermatozoa. Seki et al. (2002) examined gonads from mating pairs of medaka exposed to waterborne ethynylestradiol for 21 days. Females from the 488 ng/L ethynylestradiol treatment had lowered egg production and regressed ovaries. Gonads of males exposed to 63.9 ng/L and above showed testis–ova but still appeared to have active spermatogenesis and egg fertility was not affected. Balch et al. (2004) found that the presence of oocytes in testicular tissue in medaka did not affect the ability of males to fertilize eggs. Rather, poor reproductive success was associated with decreased mating behavior in both sexes exposed to ethynylestradiol, suggesting intersex medaka may have poor reproductive success due to altered mating behavior (Balch et al., 2004).

*2.1.2. Fathead minnow (Pimephales promelas)*

The fathead minnow is a freshwater cyprinid fish that has been widely used for aquatic toxicity testing (Kramer et al., 1998) and is endorsed by the U.S. Environmental Protection Agency (1998) for a short-term reproductive assay for screening EDCs. Fathead minnows develop into sexually dimorphic mature adults within 4–5 months and are fractional spawners, producing a clutch of 50–100 eggs every 3 to 5 days (Jensen et al., 2001). In addition, Kahl et al. (2001) found reproductive endpoints in these fish, including fecundity, egg hatching success, egg fertility, gonadal condition and plasma sex steroid concentrations, were not significantly affected by routine laboratory handling.

Reproductive capacity of fathead minnows can be impacted by exposure to waterborne estrogens. Kramer et al. (1998) noted reproductive adults exposed to a nominal concentration of 2724 ng/L estradiol did not produce any eggs. Panter et al. (1998) found that maturing male fathead minnows exposed to estradiol (320 or 1000 ng/L) or estrone (317.7 ng/L) for 21 days exhibited decreased GSI relative to control fish. Lange et al. (2001) conducted a full life cycle test with fathead minnows exposed to waterborne ethynylestradiol. Severe physical deformities were noted in fish treated with 16 or 64 ng/L ethynylestradiol, and only females developed in exposure groups receiving 4 ng/L ethynylestradiol or above. Jobling et al. (2003) exposed adult female fathead minnows to ethynylestradiol for 3 weeks during gonad development and examined subsequent egg production in mating pairs after cessation of exposure. In fish exposed to ethynylestradiol concentrations of 0.1 and 1 ng/L, there was a statistically significant increase in egg production, suggesting exposure to low concentrations of this estrogen may actually stimulate female egg production. However, fish treated with 10 ng/L produced fewer eggs than controls and those treated with 100 ng/L produced no eggs at all. Eggs from the fish that were treated with 10 ng/L exhibited only a 30% fertilization success rate, compared with more than 90% in controls (Pawlowski et al., 2004). Overall, results of these studies with estrogens indicate that GSI, egg production and egg fertility could be impacted by exposure.

Harries et al. (2000) measured fecundity, GSI, plasma vitellogenin and secondary sex characteristics in adult fathead minnows over a three-week pre-exposure and a three-week exposure period to two suspected EDCs, butyl benzyl phthalate and 4-nonylphenol. Waterborne butyl benzyl phthalate (100 μg/L) had no effect on survival, fecundity, egg size, GSI, plasma vitellogenin or secondary sex characteristics in exposed fish. Females exposed to waterborne nonylphenol (1, 10 and 100 μg/L) showed a concentration-dependent decrease in GSI and number of spawnings, as well as significantly reduced egg batch size and overall fecundity in the 100 μg/L treatment. Males demonstrated significant effects on secondary sex characteristics (number of tubercles, thickness of fat pad). Geisy et al. (2000) also exposed adult fathead minnows to waterborne nonylphenol at concentrations ranging from 0.05 to 3.4 μg/L for 42 days. No statistically significant effect of nonylphenol treatment on egg production was found. These studies suggest exposure to high nonylphenol concentrations can alter egg production and male secondary sex characteristics in fathead minnows, but low concentrations have little effect.

Sohoni et al. (2001) investigated effects of long-term exposure to waterborne bisphenol A in sexually mature fathead minnows. Male GSI, female GSI and egg hatching success were lower in fish from the 640 and 1280 μg/L treatments compared to controls. Egg production was decreased in the 1280 μg/L treatment. Ankley et al. (2001) evaluated reproductive endpoints in fathead minnows exposed to the pesticide methoxychlor (measured concentrations of 0.55 and 3.56 μg/L) in water for 21 days. No alteration in sexual behavior,

secondary sex characteristics, fertility rate or egg hatching success was observed, but a significant decrease in fecundity was noted in the 3.56 μg/L treatment. These studies suggest that fathead minnows exposed to estrogenic EDCs such as bisphenol A and methoxychlor may exhibit decreased GSI, female egg production and egg hatching success.

Observations of intersex gonads are scarce in fathead minnows exposed to EDCs. Lange et al. (2001) reported a dose-related increase in occurrence of ova–testes in fathead minnows exposed to waterborne concentrations of ethynylestradiol ranging from 1 to 16 ng/L for 56 days post-hatch. No instances of intersex gonads were found in fish that continued in these exposures until 172 days post-hatch, but all fish were females at concentrations of ethynylestradiol ≥4 ng/L. Other histological changes in gonads from fish exposed to EDCs have been noted. Miles-Richardson et al. (1999a) examined histology of the gonads from fish exposed to waterborne 17β-estradiol for 19 days and found degenerative changes in the testes of males, while ovaries of estradiol-exposed females demonstrated a significant increase in primary follicles versus other stages. Miles-Richardson et al. (1999b) also examined male secondary sex characteristics and gonad histology of adult fathead minnows exposed to waterborne nonylphenol at concentrations ranging from 0.05 to 3.4 μg/L for 42 days. There were no changes in female ovaries or male secondary sex characteristics, but testes of nonylphenol-exposed males exhibited a dose-dependent increase in the number and size of Sertoli cells, necrotic spermatozoa and germ cell synctia. Sohoni et al. (2001) found a reduction in the number of spermatozoa in testis males treated with 16, 640 or 1280 μg/L of waterborne bisphenol A for 164 days. Fish treated with 16 μg/L bisphenol A exhibited over a 28% inhibition in spermatozoa formation, but the ability of males from this treatment to fertilize eggs was not impaired. No intersex gonads were observed in any treatment. In combination, these reports suggest fathead minnows may only exhibit transient formation of intersex gonads during a developmental window following hatch, and EDC exposure beyond that window may be expressed as total sex reversal. Short-term exposure of adults to EDCs may result in histological alterations to gonad structure, but not formation of intersex gonads.

Some androgenic and anti-androgenic EDCs have been tested in fathead minnows. Makynen et al. (2000) exposed fathead minnows to the anti-androgen vinclozolin (75–1200 μg/L) as fertilized eggs through 30 days post-hatch, but found no significant effects on phenotypic sex ratios when these fish were grown out for up to 7 months. When reproductive adults were exposed to waterborne vinclozolin (200 or 700 μg/L) for 21 days, there were no differences in reproductive success, but fish in all treatments spawned very little during this experiment (Makynen et al., 2000). Females from the high vinclozolin treatment had substantially smaller oocytes and reduced GSI. Thus, vinclozolin did not affect sex ratio or reproduction in fathead minnows, but did reduce female GSI. Ankley et al. (2001) found that the androgen methyltestosterone had a profound effect on egg production of fathead minnows, with immediate cessation of spawning observed at a water concentration of 120 μg/L. In contrast, Zerulla et al. (2002) exposed sexually undifferentiated, juvenile fathead minnows to waterborne methyltestosterone (10, 50 and 100 μg/L) for 21 days and noted partial feminization of exposed fish. They hypothesized that methyltestosterone was converted in juvenile fathead minnows to estrogen through endogenous aromatase. Indeed, when they blocked aromatase activity by treating simultaneously with the chemical fadrozole, methyltestosterone exposure resulted in a precocious development of male sex characteristics and behavior in exposed fish (Zerulla et al., 2002). The observation of Ankley et al. (2001) that methyltestosterone abruptly halted egg production in adult female fathead minnows contrasts with the feminizing effect that Zerulla et al. (2002) noted in juvenile fathead minnows, and suggests that the different life stages may metabolize methyltestosterone differently. In reproducing fathead minnows, exposure to methyltestosterone reduces egg production.

### *2.1.3. Carp (Cyprinus carpio)*

Carp, like fathead minnows, are batch-spawning cyprinids with external fertilization. Gimeno et al. (1998a) observed that mature male carp exposed to 1 μg/L estradiol in water failed to produce any milt and had reduced GSI. Gimeno et al. (1998b) also found that immature, genetically male carp exposed to waterborne estradiol (9 or 23 μg/L) from 50 to 110

days post-hatch all developed into females, a result in accordance with similar exposures in medaka (Nimrod and Benson, 1998).

Sexually mature male carp exposed to 1000 μg/L of the alkylphenol, 4-*tert*-pentylphenol, for 3 months had a significantly reduced spermatocrit (i.e., percentage of packed cells in a milt sample) compared to controls (Gimeno et al., 1998a). Histological changes in the testes of these fish included necrosis, inhibition of spermatogenesis, and a few instances of ova–testes. A significant reduction in GSI was also observed in exposures to 32, 100 or 1000 μg/L pentylphenol. Exposure of immature carp from 50 to 110 days post-hatch to pentylphenol at 36, 90 or 256 μg/L induced formation of a female reproductive tract (oviduct) in genetic males, and sporadic oocytes were observed in a few of the testes with oviducts (Gimeno et al., 1998b). The effects of this oviduct formation on male reproductive fitness was not assessed. These results indicate that exposure of genetically male carp to pentylphenol can cause reduced GSI and milt production in adults, or sex reversal in juveniles. Ova–testes formation may occur, but appears to be relatively rare.

#### 2.1.4. Goldfish (*Carassius auratus*)

Goldfish, like fathead minnows and carp, are batch-spawning cyprinids. The sexual behavior of male goldfish was shown by Bjerselius et al. (2001) to be impacted by exogenous exposure to 17β-estradiol. Mature male goldfish were exposed to estradiol either through food (1, 10 or 100 μg/g food) or water (1 or 10 μg/L) for 24–28 days during spawning, then paired with a reproductive female. Regardless of exposure method, male GSI was significantly reduced in most estradiol treatments compared to controls. In all estradiol exposures, significantly fewer males had tubercles, a secondary male sex characteristic, and male sexual activity was dramatically reduced. In addition, significantly fewer males from the estradiol-treated food groups expressed milt. These researchers conclude that the adverse effects of estradiol exposure on male sexual behavior would severely impair reproductive success (Bjerselius et al., 2001). Results from this single study indicate exposure to exogenous estradiol can decrease GSI, milt production, secondary sex characteristics and sexual activity of male goldfish.

#### 2.1.5. Zebrafish (*Danio rerio*)

Tropical zebrafish are in the cyprinid family, have a short life cycle of about 4 months and can be stimulated to breed throughout the year. In zebrafish, both sexes pass through an ovary-like stage before differentiating into a phenotypic sex, which means juvenile males pass through a phase as females before final development of male gonads (Orn et al., 2003). Females spawn once every 2–3 days.

A number of studies in zebrafish have shown ethynylestradiol can impact egg viability, egg production, fertilization success, sexual differentiation and development, and sex ratios. Kime and Nash (1999) report that exposing adult zebrafish to 5 ng/L of waterborne ethynylestradiol for 12 days prior to spawning led to arrested development of all eggs produced. Andersen et al. (2003) exposed zebrafish to 15.4 ng/L waterborne ethynylestradiol during different stages of development and noted a high incidence (59%) of ovo-testis in fish exposed from 20 to 40 days post-hatch, but this is not particularly surprising because this is the normal protogynic stage in developing zebrafish (Segner et al., 2003). In addition, all fish exposed from hatch or 20 days post-hatch through 60 days post-hatch developed into females in which development of ovaries was "severely depressed" (Andersen et al., 2003). Van den Belt et al. (2003) exposed zebrafish from eggs until 3 months post-fertilization to waterborne ethynylestradiol and found testicular development was inhibited in fish from the 0.1 or 1 ng/L treatments, while both testicular and ovarian development was suppressed in fish from the 10 and 25 ng/L exposures. When treated fish were allowed to recover in clean water for five months, gonad development appeared to recover, but females from the 10 or 25 ng/L treatments still exhibited reduced egg production. Orn et al. (2003) exposed zebrafish juveniles from 20 days post-hatch through 60 days post-hatch to waterborne ethynylestradiol (1–25 ng/L) and found a significantly higher proportion of females in groups exposed to ethynylestradiol. All fish in groups exposed to 2, 5 or 10 ng/L developed as females. Segner et al. (2003) exposed zebrafish from eggs through sexual maturity to waterborne ethynylestradiol and found that, at nominal concentrations greater than or equal to 1.67 ng/L, fish exhibited elevated vitellogenin levels, delayed onset of spawning, reduced egg production and reduced fertilization

success. Treatment of zebrafish with either of the estrogenic EDCs bisphenol A or octylphenol also led to altered gonadal differentiation, reduced egg production, reduced fertilization success, and elevated vitellogenin levels. This study indicated the most critical period of exposure to EDCs, with later impacts on the reproductive performance of mature fish, was from 42 until 75 days post-hatch in zebrafish, during the developmental period of bisexual morphological differentiation.

Orn et al. (2003) found zebrafish exposed from 20 to 60 days post-hatch to the androgen methyltestosterone (26–1000 ng/L) developed as males, although intersex gonads occurred at concentrations of 260 ng/L (3% of fish) and 1000 ng/L (27% of fish). Induction of intersex gonads at these high methyltestosterone concentrations may indicate metabolism of the androgen into an estrogen, or alternatively, may indicate a general toxic effect, causing a delay in sexual development and elongating the timeframe for the normal protogynic stage in young zebrafish.

Early life tributyltin (TBT) exposure appears to affect later sperm motility and viability in male zebrafish. McAllister and Kime (2003) exposed zebrafish to TBT during various developmental stages (0–70, 0–30 and 30–60 days post-hatch), then examined sexual development and sperm quality in the mature adults 3 to 5 months later. TBT exposure resulted in an increased number of abnormal sperm, some completely lacking flagella. Sperm motility was significantly decreased in fish from treatments of 1 ng/L or greater. TBT induced a significant male-biased sex ratio at an exposure concentration as low as 0.1 ng/L, indicating TBT can affect sexual differentiation as well.

### *2.1.6. Rainbow trout (Oncorhynchus mykiss)*

Rainbow trout, a salmonid fish, has been used as a test species for some laboratory studies with EDCs. In this species, the male and female simultaneously release gametes into a nest, then the female covers the eggs with a layer of gravel to protect them until hatching. Jobling et al. (1996) exposed male rainbow trout undergoing sexual maturation to 2 ng/L waterborne ethynylestradiol or one of four different waterborne alkylphenolic compounds (nonylphenol, nonylphenoxycarboxylic acid, nonylphenoldiethoxylate and octylphenol), each at a nominal concentration of 30 μg/L, for 3 weeks. GSI was significantly inhibited in all exposed fish, with males exposed to ethynylestradiol or octylphenol exhibiting a 50% reduction. Testicular tissue from treated fish also showed differences in distribution of germ cell stages. Later in the year, when testicular growth was "well underway", males exposed to nonylphenol showed significant reduction in GSI only a concentration of 54.3 μg/L. In an experiment conducted even later, with fully mature trout exposed to octylphenol, a significant decrease in GSI was noted at a concentration of 4.8 μg/L. These researchers did not measure how reduced GSI or observed testicular changes affected reproductive fitness of males. However, this study showed that impacts from exposure to an EDC can depend on the stage of reproductive development during which the exposure occurs.

Schwaiger et al. (2002) exposed pre-spawning adult rainbow trout of both sexes to waterborne nonylphenol (1 and 10 μg/L) for 10 days per month over 4 months in an effort to mimic a realistic environmental exposure scenario. Eggs from treated fish showed a significantly reduced survival to the eyed stage, and eggs from adults exposed to 10 μg/L nonylphenol had a significantly reduced hatch. Offspring of nonylphenol-exposed fish that were grown out in clean water for 3 years still exhibited disrupted hormonal balance when compared to their control counterparts, with male estradiol concentrations increased two-fold and female testosterone concentrations increased almost 13-fold. Results from this study demonstrate that exposure to an EDC can have long-term, cross-generational effects on steroid hormone profiles.

### *2.1.7. Guppies (Poecilia reticulata)*

Guppies are viviparous (produce live young) and have a short generation time. The reproductive adults are sexually dimorphic. Kinnberg et al. (2003) conducted exposure studies with guppies to investigate the effects of EDCs on developing embryos. One female and two males were treated with either waterborne 17β-estradiol (0.85 μg/L) or octylphenol (26 μg/L) for 26 to 36 days until the female gave birth. No significant difference in mortality, brood interval, brood size or male GSI was noted. Young of exposed fish were raised in clean water for 70 days and did not show any adverse effects from parental exposure.

Bayley et al. (1999) investigated the effects of waterborne 17β-estradiol (10 μg/L) and octylphenol (150 μg/L) on the sexual behavior of male guppies exposed for 4 weeks. While no differences were observed in non-sexual swimming behavior, male sexual displays were dramatically decreased in males exposed to octylphenol and completely inhibited in males exposed to estradiol. This inhibition of male sexual behavior in guppies parallel those reported for male goldfish exposed to estradiol by Bjerselius et al. (2001). For fish species that depend on male sexual behavior to successfully reproduce, exposure to EDCs such as estradiol or octylphenol could have dramatic consequences.

One study in guppies indicates dietary exposure to anti-androgenic EDCs can have deleterious effects on both male and female reproduction. Bayley et al. (2002) exposed juvenile guppies through diet to vinclozolin, *p*,*p*′-DDE and flutamide, and then compared male courtship behavior, secondary male sex characteristics and sperm counts when these fish became sexually mature. All three antiandrogens caused a reduced body size and a skewed sex ratio towards females and adults produced fewer young. Males in some exposures exhibited adverse impacts on secondary sex characteristics, sexual behavior and sperm counts. Male sperm counts in guppies have been shown to be affected by exposures to other EDCs. Haubruge et al. (2000) found significant declines of 40–75% in total sperm counts of adult male guppies after 21 days exposure to waterborne tributyltin (11.2 and 22.3 ng/L) or bisphenol A (274 and 549 μg/L). These studies provide direct evidence that exposure to EDCs can adversely affect sperm counts in fish.

#### *2.1.8. Sand gobies (Pomatoschistus minutus)*

The sand goby, an inshore estuarine fish common in Northern Europe, undergoes multiple spawnings during the breeding season. Male sand gobies build a nest, court females to lay eggs, then fertilize the eggs and guard them until hatching. Robinson et al. (2003) evaluated the effects of ethynylestradiol on reproductive success in sand gobies. Fish were exposed to waterborne ethynylestradiol (6 ng/L) and sampled over a total of 7 months, from juveniles through gonadal maturation and then during an eight-week breeding period. During the breeding trials, ethynylestradiol treatment significantly reduced male nest building, percentage of pairs breeding, egg fertility, percentage of females spawning and individual female fecundity. The decrease in male sexual behavior in these ethynylestradiol-exposed sand gobies is consistent with effects observed in goldfish (Bjerselius et al., 2001) and guppies (Bayley et al., 1999) exposed to estradiol or octylphenol.

### *2.2. Laboratory studies with mixtures containing EDCs*

Laboratory studies with effluents containing a mixture of chemicals, including EDCs, represent a hybrid approach between laboratory studies with single EDCs and field studies that sample natural populations of fish at sites likely to be receiving effluents containing EDCs. Ideally, these types of studies are designed to control many of the variables present in field situations (e.g., food availability) while maintaining environmental relevance (van den Heuval et al., 2002).

A number of studies have been conducted to investigate the effects of sewage treatment effluent or sewage sludge on reproductive fitness of fish exposed in the laboratory. Sewage effluent is of interest because chemicals with estrogenic activity are widespread contaminants in such wastewater discharges (Desbrow et al., 1998; Routledge et al., 1998; Snyder et al., 1999; Baronti et al., 2000). Evidence also indicates that androgenic contaminants are present in these effluents (Kirk et al., 2002). Rodgers-Gray et al. (2000) conducted a long-term experiment with the cyprinid fish roach (*Rutilus rutilus*) exposed to sewage treatment effluent over a four-month period. Exposure did not affect survival, growth, GSI, or occurrence of intersexuality in these fish. Robinson et al. (2003) conducted breeding trials with sand gobies (*P. minutus*) exposed to 0.3 or 0.03% v/v sewage effluent over a seven-month period. Treatment did not affect GSI, and no evidence of ovo-testis was seen in males. Fish exposed to either concentration of effluent actually produced larger broods than solvent control fish, but hatched larvae from effluent-treated parents were significantly smaller in size than larvae from solvent control parents. Fish exposed to 0.3% effluent showed a significantly higher cumulative mortality than control

fish. These studies suggest that exposure to non-lethal dilutions of sewage effluent may have little impact on reproductive success in fish, but should be interpreted cautiously because composition of sewage effluent may vary from location to location.

Rodgers-Gray et al. (2001) investigated the effects of exposure to sewage treatment effluent on sexual differentiation and development in juvenile roach (*R. rutilus*) from 50 through 200 days post-hatch. No effects on germ cell differentiation were observed in exposed fish, but a dose-dependent feminization of the gonadal reproductive ducts was noted in exposed males. These researchers admit "that the functional significance of the female-like reproductive ducts in feminized males is not known". Formation of oviducts within male testes was also reported by Gimeno et al. (1998b) in carp (*C. carpio*) exposed from 50 through 110 days post-hatch to pentylphenol. These results suggest that the presence of feminized reproductive ducts in male testes may be indicative of exposure to EDCs, but, because data are lacking, the relationship between such altered gonad structure and reproductive capacity is unclear.

Waring et al. (1996) exposed adult sand gobies (*P. minutus*) to 0.1% sewage sludge for 19 weeks prior to the end of spawning. Individual female fecundity and larval survival were not affected, but there was an inhibition of spawning in about 50% of the sludge-exposed females. These researchers thought that failure of females to spawn could be due to adverse effects of sewage sludge exposure on male courting behavior, and noted both spawning and non-spawning females had similar GSI values. Such effects on male courting behavior are consistent with the single chemical laboratory exposures that demonstrate EDCs affect male mating behavior in goldfish (Bjerselius et al., 2001), guppies (Bayley et al., 1999, 2002) and sand gobies (Robinson et al., 2003). These results further support the idea that EDCs can impact reproductive success of a species by affecting male sexual behavior.

Kraft mill effluent, a type of pulp mill effluent, is known to contain biologically active androgenic compounds (Parks et al., 2001; Jenkins et al., 2001; Durhan et al., 2002). Larsson et al. (2002) found female guppies (*P. reticulata*) exposed to 5%, 10% or 25% pulp mill effluent exhibited evidence of male coloration, but continued to produce fry in normal male:female ratios. van den Heuvel et al. (2002) exposed maturing rainbow trout (*O. mykiss*) to 10% kraft mill effluent for 2 months and observed no reproductive dysfunction in exposed fish. Kovacs et al. (1995) studied the effects of bleached kraft mill effluent (concentrations of 0–20%) on fathead minnows (*P. promelas*) exposed for 275 days, from the egg stage through reproductive maturity. Embryo exposure did not significantly affect hatching success or overall mortality. As reproductive adults, fish exposed to effluent concentrations ≥2.5% had fewer spawns and fewer eggs, with delayed reproduction in minnows treated with 10% effluent and no egg production in fish treated with 20% effluent. The number of phenotypic males significantly increased with effluent concentrations ≥5%, with all fish in 20% effluent having male secondary sexual characteristics. While the specific composition of kraft mill effluent varies because of differences in effluent treatment, phytosterols can be major components (Cody and Bortone, 1997; Denton et al., 1985). Lehtinen et al. (1999) exposed maturing brown trout (*Salmo trutta lacustris*) to a mixture of waterborne phytosterols at 10 and 30 μg/L over a 4.5-month period prior to spawning. Exposed adults showed slight changes in circulating levels of steroid hormones, but fertilization success was not affected. Mortality of eggs from exposed fish was increased and fry were significantly smaller than fry from unexposed fish. Overall, these studies indicate kraft mill effluent is capable of inducing expression of male secondary sexual characteristics in females, decreasing egg production and increasing egg mortality, but exposure does not consistently result in detrimental reproductive effects, probably because composition of kraft mill effluent and receiving water conditions can vary greatly between locations.

Janssen et al. (1997) investigated the effects that exposure to dredged spoil from a harbor polluted with polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) had on flounder (*Platichthys flesus*) in large mesocosms for up to 3 years. Compared to control flounder in a clean mesocosm, dredge-exposed females exhibited precociously elevated vitellogenin concentrations and a doubling in plasma estradiol concentration. These researchers concluded that the increased plasma estradiol was likely caused by a modification of estradiol clearance due to exposure to dredged spoil,

rather than by a direct estrogenic action of contaminants. They did not examine the impact that such altered estradiol concentrations might have on reproduction in these fish.

### 2.3. Field studies and surveys

Field studies have the potential to provide the most direct evidence that fish populations are impacted by EDCs in the aquatic environment. A few field surveys have been conducted to investigate if fish assemblages in the vicinity of sewage outfalls change. Kingsford et al. (1996) conducted a broad-scale survey in Australian waters investigating the abundance of deformed fish larvae in neuston collected from waters in the Sydney area compared to two relatively clean locations of Terrigal and Jervis Bay. The more polluted waters off Sydney did not produce higher numbers of deformed larvae. In the Mediterranean Sea off the coast of Italy, Guidetti et al. (2003) conducted underwater visual censuses four times over 1 year along randomly selected transects at a site impacted by sewage outfall and two adjacent control sites. While species richness and density of herbivorous fish did not show significant differences attributable to the outfall, total fish abundance was always about 5-fold greater at the outfall site than at control sites, mostly because of the greater abundance of particulate feeders and planktivorous fish at the outfall location. Hall et al. (1997) examined total number of fish caught and relative abundance of adult flatfish populations (flounder, plaice and dab) in spring and fall trawls that were taken from 1992 through 1994 at five stations in the lower Tyne Estuary near the Howdon sewage treatment works. Total numbers of fish caught were higher in spring 1994 due to increased populations of small pelagic species. Since the increase in fish numbers was concurrent with a large increase in effluent discharge from Howden, these authors speculate that some species may have been attracted to the increase in sewage effluent as a food resource. These studies indicate that sewage outfall may actually attract and sustain increased numbers of certain fish species, but do not give any information on the reproductive capability of these exposed fish.

Probably the most extensive field studies to assess aquatic EDC contamination have been conducted in inland waters of the United Kingdom. In response to reports from anglers that British sewage treatment lagoons contained hermaphrodite fish, Purdom et al. (1994) placed trout in cages near various sewage treatment discharges and observed elevated levels of the female protein vitellogenin in these male fish. Harries et al. (1996) conducted a study using caged male trout and induction of male plasma vitellogenin to determine the "estrogenic activity" in the British river Lea and a number of sewage treatment reservoirs. They observed a pattern of elevated male vitellogenin concentrations in caged trout that declined with increasing distance from sewage treatment plant outfalls. A larger study in five British rivers found four of these rivers induced significant vitellogenesis in caged male trout (Harries et al., 1997). To investigate effects on an indigenous fish species, Lye et al. (1997) surveyed wild populations of euryhaline flounder (*P. flesus*) from three sites in Northern England that received differing quantities of sewage effluent. They observed that male plasma vitellogenin was induced to some degree at all sites, with both frequency and quantity lowest at the least contaminated site. More extensive field surveys of wild flounder conducted by Allen et al. (1999a,b) in 11 British estuaries and several offshore marine sites confirmed elevated male vitellogenin levels in fish from all but two estuaries. While it is possible that the estrogenic activity that causes elevated vitellogenin concentrations in males may also affect the reproductive potential of some wild fish populations, the effect of vitellogenin production in males on reproductive fitness is unclear. Elevated male plasma vitellogenin levels have been linked with exposure to estrogenic EDCs by numerous researchers, but a reliable linkage with reproductive impairment is still a subject of investigation (Mills et al., 2003).

The effects of pulp and paper mill effluent on indigenous fish populations has been investigated by a number of researchers and is the subject of a government-mandated environmental effects monitoring program in Canada (see Munkittrick et al., 1998, 2002; Courtenay et al., 2002). McMaster et al. (1992) evaluated the reproductive performance of male and female white sucker (*Catostomus commersoni*) from Jackfish Bay in Lake Superior that receives bleached kraft mill effluent. Compared to controls from another bay, these fish showed no differences in mean female fecundity, spawning success or efficiency of egg

release. Males exhibited reduced secondary sexual characteristics and sperm motility, but no differences in milt volume, spermatocrit or fertilization success. Eggs and larvae showed no differences in hatch date or hatchability, deformity rate, developmental rate, or larval behavior. Munkittrick et al. (1991) found that adult white sucker (*C. commersoni*) from Jackfish Bay demonstrated a lower growth rate, delayed maturity, lower estimated fecundity, reduced GSI and inhibited development of male secondary sexual characteristics. In a later study, Munkittrick et al. (1994) examined white sucker in the receiving waters from twelve Canadian pulp mills that used a variety of effluent treatment processes and measured steroid levels, EROD induction, gonad size and liver size. Reduction in GSI of females was the most consistent change and was observed at all sites but one. Oakes et al. (2003) evaluated data from 8 years of studies and observed that reductions in GSI of white sucker from Jackfish Bay and another site receiving mill effluent varied with year and season of sampling. During some seasons and years, no reduction in GSI was noted. Swanson et al. (1994) compared populations of mountain whitefish (*Prosopium williamsoni*) and longnose sucker (*Catostomus catostomus*) exposed to bleached kraft mill effluent to similar populations in a reference river system in Alberta, Canada, and found exposed populations were growing and reproducing successfully, with no evidence of reproductive or recruitment failures in any age class. In fact, species distribution data indicated that the area nearest the pulp mill was actually preferred, presumably because of an abundance of food. Chemical body burdens of persistent chlorinated organics in effluent-exposed fish were not significantly correlated with any population level effects (Kloepper-Sams et al., 1994). In New Brunswick, Canada, Leblanc et al. (1997) monitored reproduction in wild mummichog (*Fundulus heteroclitus*) populations from two estuaries, one of which received kraft mill effluent. These researchers observed that the reproductive season of mummichogs at two sites downstream from a bleached kraft pulp mill was delayed 1 to 2 weeks compared to cleaner sites with comparable water temperatures. However, fish from one of the sites receiving effluent exhibited a notably higher reproductive output (based on a longer spawning period, increased fecundity, and a higher percentage of ripe females) than fish at all other stations. Billiard and Khan (2003) surveyed a population of the estuarine fish cunner (*T. adspersus*) in Humber Arm estuary, Newfoundland, Canada, which receives both pulp mill effluent and raw sewage. They concluded that, based on differences in tissue histology and liver mixed function oxidase activity, Humber Arm cunner were under chronic stress. While these researchers did not measure reproductive success, they reported that GSIs of fish from Humber Arm were greater than from a reference site.

Larsson and Forlin (2002) examined sex ratios of eelpout (or blennies, *Zoarces viviparus*) embryos collected in the vicinity of a large kraft pulp mill on the Swedish Baltic coast. They noted a male bias at sites sampled during 3 out of 4 years. The 1 year that there was no male bias coincided with a shutdown period of the mill, and the strongest male bias (embryos 61% male) occurred during a year that there was an increase in the production capacity of the mill. In the Fenhalloway River, Florida, which receives effluent from a paper mill, the prevalence of female mosquitofish (*Gambusia affinis holbrooki*) exhibiting male secondary sex characteristics (i.e., gonopodium-like anal fins) and elements of male reproductive behavior is well-documented (Howell et al., 1980; Cody and Bortone, 1997; Parks et al., 2001). Bortone and Cody (1999) reported the same type of masculinization in eastern mosquitofish (*Gambusi holbrooki*), least killifish (*Heterandria formosa*), and sailfin molly (*Poecilia latipinna*) from Rice Creek in Florida, which receives effluent from a large wood-pulping facility. To our knowledge, adverse effects of this masculinization on reproductive success have never been reported. In female mosquitofish exposed in the laboratory to plant sterol degradation products such as those found in paper mill effluent, Denton et al. (1985) observed development of a male-like gonopodia, yet found no evidence of testicular tissue in these 'masculinized' females.

Collectively, research on fish populations exposed to kraft pulp or paper mills in situ indicates widely variable impacts on reproductive endpoints. This may in part be due to migratory patterns of the fish species studied, which affects actual level of exposure to effluents (Munkittrick et al., 2002). Other factors that may influence observed effects include effluent composition, species sensitivity and receiving water characteristics. The most dramatic effect reported

appears to be a skewed sex ratio in an exposed population of eelpout (Larsson and Forlin, 2002), but the ecological ramifications of this on maintenance of the eelpout population has not been explored.

Recent reports have uncovered some previously unsuspected sources of EDCs in the aquatic environment. There is evidence that leakage water from refuse dumps can be a source of EDCs that impact fish populations in nearby water bodies. As Noaksson et al. (2001) note: "refuse dumps are not only potential point sources of anthropogenic substances presently in use, but also for substances used previously, including substances whose use is now banned by regulations and laws". In a two-year Swedish study comparing Lake Molnbyggen with a reference lake, Noaksson et al. (2001) found female perch (*Perca fluviatilis*) from Lake Molnbyggen, which receives leakage water from a dump, exhibited an 80% decrease in GSI and males a 36% decrease. Three-quarters of the female perch collected from the contaminated lake were sexually immature, compared with about 8% in the reference lake. However, perch of all different age classes were caught in Lake Molnbyggen, indicating that a population level impact of the large number of immature females was not manifest at the present time. A similar study done on brook trout (*Salvelinus fontinalis*) from a leachate-contaminated stream which feeds into Lake Molnbyggen indicated that only 17% of the female brook trout captured had gonads with mature oocytes, compared with 100% from a reference stream (Noaksson et al., 2003). Another source of EDCs to the aquatic environment is run-off from concentrated animal feedlots or agricultural operations that fertilize with animal manure (Nichols et al., 1997; Schiffer et al., 2001). Orlando et al. (2004) sampled indigenous fathead minnow populations living upstream (reference site) and downstream of both a cattle grazing area and a high density cattle feedlot. These researchers found fish from contaminated waters exhibited decreased GSI and gonadal testosterone synthesis in males, and a decreased estrogen to androgen ratio in females. Reproductive stage of gonads in both sexes was not affected, nor was any pathology evident in gonads of exposed fish, so the reproductive impact, if any, of the noted changes remains unclear.

In a unique study, population and ecosystem effects of the EDC ethynylestradiol are being studied directly. A group of Canadian researchers is investigating the effects that triweekly additions of ethynylestradiol to a small Precambrian lake in northwestern Ontario have on indigenous fish populations over time. Ethynylestradiol additions, started in Spring 2001, have resulted in an average concentration of 6 ng/L in the epilimnetic waters of the experimental lake (Palace et al., 2002). Fathead minnows from the lake exhibited histological abnormalities in liver, kidney and testes after the first 4 months of the ethynylestradiol addition (Palace et al., 2002). Since then, the lake's fathead minnow population, originally numbering about 7000 individuals, has shown a dramatic decline and is expected to disappear by 2004 (Pelley, 2003). Ethynylestradiol exposure is affecting reproductive behavior, nest size and egg development in these fish, leading to overall reproductive failure in the population (Blanchfield et al., 2003). Longer-lived fish species (pearl dace, white sucker and trout) from the experimental lake are not yet showing any effects of ethynylestradiol exposure on their population structures (Pelley, 2003).

Occurrence of intersex individuals in native fish populations have been examined by researchers world-wide. In the United States, male shovelnose sturgeon (*Scaphirhynchus platyorynchus*) collected in a highly contaminated stretch of the Mississippi River below Saint Louis exhibited intersex in two out of seven males (29%) captured, while male sturgeon sampled upstream all had normal testes (Harshbarger et al., 2000). In Japan, 15% of the wild male flounder (*Pleuronectes yokohamae*) from a part of Tokyo Bay which receives a large amount of industrial and domestic sewage effluent had testes that contained scattered oocytes (Hashimoto et al., 2000). In Italy, 50% of barbel (*Barbus plebejus*) collected from a contaminated stretch of the Po River had intersex gonads, while no intersexuality was found in fish from an upstream site (Vigano et al., 2001). De Metrio et al. (2003) noted that 25% of the 162 male swordfish that they captured in the Mediterranean had female germ cells within their testes. Flounder (*P. flesus*) from the Tyne and Mersey estuaries showed occurrence of ova–testis in 7–20% of the males collected at various sites, but no evidence of skewed sex ratios, delayed testicular development, or precocious ovarian maturation was found (Allen et al., 1999b). Abnormal testis morphology was noted in a substantial proportion

(30–53%) of male flounder from two contaminated sites in the Tyne estuary, which receive effluent from a major sewage treatment facility as well as industrial sources (Lye et al., 1997). van Aerle et al. (2001) reported intersex condition in the cyprinid gudgeon (*Gobio gobio*) from several British rivers and lakes, but were unable to determine whether the intersexuality in wild gudgeon was a natural state or had resulted from contaminant exposure. Sole et al. (2003) collected wild carp downstream from a sewage treatment facility on the Anoia River, Spain and noted 43% of the males exhibited testicular atrophy or ova–testes. Nichols et al. (1999) were not able to discern any trends in male vitellogenin concentrations, steroid hormone concentrations, male secondary sex characteristics or gonad abnormalities in male and female fathead minnows caged near seven different central Michigan wastewater treatment plants for 21 days. Stentiford et al. (2003) noted that 25% of male blennies (*Z. viviparus*) captured in the Tyne estuary during the spring spawning season were intersex, but no intersex males were found in the autumn, suggesting that detection of intersex individuals depends on the adult reproductive stage in some fish species. Jobling et al. (1998) collected native roach (*R. rutilus*) that appeared macroscopically to be either male or female from a suite of British rivers, but found that many males actually had intersex gonads. The incidence of intersexuality was as high as 100% in populations at sites downstream from sewage treatment plants in two rivers. These researchers have since linked intersexuality in wild roach with altered sexual maturation, lowered gamete production, and reduced fertility (Jobling et al., 2002a,b), but have not reported any impact of intersexuality on maintenance of wild roach populations. In combination, these results indicate intersex can serve as an indicator of exposure to EDCs, but the significance of intersexuality in various fish species in terms of reproductive success and maintenance of fish populations needs further clarification.

## 3. Discussion

Current literature suggests many environmental contaminants that are considered EDCs have the potential to impact reproductive success of fish. The evidence is strong that EDCs, including steroidal estrogens (Table 1) and estrogen mimics (Table 2), can and do affect reproduction of fish in laboratory exposures. Limited laboratory studies with EDCs that are androgenic (testosterone, methyltestosterone, tributyltin) or antiandrogenic (the fungicide vinclozolin, the DDT metabolite *p*,*p*′-DDE and the therapeutic agent flutamide) also indicate a potential to negatively affect fish reproductive success (Table 3). These laboratory studies have been conducted in a variety of fish species from a number of different families (Adrianichthyidae, Cyprinidae, Salmonidae, Poeciliidae and Gobiidae), but cyprinid fish (fathead minnows, carp, goldfish, zebrafish) are the best represented. Cyprinid fish commonly exhibit secondary sex characteristics and defined mating behavior, which makes them well-suited to studies investigating phenotypic and behavioral changes associated with EDC exposure. Monitoring egg production and egg fertility is also relatively easy in these species, because they typically produce numerous clutches of eggs that adhere to a surface when they are laid. However, several researchers (Lange et al., 2001; Geisy et al., 2000) have noted that large variability in clutch size is an inherent problem in studies that try to compare fecundity in batch-spawning cyprinids. Medaka is not a cyprinid but is a daily egg-layer that has been widely used for reproductive studies with suspected EDCs. Medaka mature in only 6 to 8 weeks, which makes them especially suited for multi-generational studies. Guppies, the one live-bearing fish that has been a choice for laboratory study, are particularly amenable to experiments investigating sperm quality or motility. In this internally fertilizing species, males store mature spermatozoa in the deferent canal of the testis, which allows a researcher to obtain accurate sperm counts with relative ease.

Table 4 lists the reproductive effects observed in laboratory experiments where various fish species were exposed to single EDCs. Of the seven species examined in laboratory reproductive studies, five different species exhibit reduced egg (or live young) production, skewed sex ratio or sex reversal, reduced male GSI and decreased male sexual behavior in response to EDC exposure. In four species, EDC exposure has been shown to adversely impact production of milt or sperm. In laboratory studies, medaka appears to be the most sensitive species for

determining the capability of an EDC to induce intersex, although there are a few reports of intersex gonads in carp and zebrafish. Intersex in adults of the cyprinid fishes roach (Jobling et al., 1998), gudgeon (van Aerle et al., 2001) and carp (Sole et al., 2003) have been reported in field-collected specimens from EDC-contaminated environments with a high frequency.

Despite strong evidence that single EDCs can disrupt fish reproduction in laboratory studies, the evidence that EDCs in the aquatic environment are impacting reproductive success of indigenous fish populations is less clear. One approach to try to address whether an EDC has the potential to impact wild fish populations is to compare the concentration of an EDC that has observable reproductive effects in laboratory studies with concentrations of that EDC in aquatic environments. Such comparisons probably underestimate effects of environmental exposures, however, because direct comparisons do not account for possible bioconcentration of chemicals within exposed organisms, or for possible interactive effects (additive, antagonistic or synergistic) resulting from exposure of an organism to multiple chemicals. These caveats need to be remembered when comparing laboratory exposure data with measured environmental concentrations of single EDCs. Still, a review of such information provides insight into the types of reproductive effects that might be expected in fish exposed to EDCs in situ. Concentrations of EDCs present in aquatic environments that originate from sewage effluent have been especially well-characterized, and are reviewed below.

In the United States, Snyder et al. (1999) measured 17β-estradiol concentrations as high as 2.67 ng/L in Lake Mead Nevada, 1.29 ng/L in tributaries leading into the Detroit River Michigan and 3.66 ng/L in wastewater treatment effluent from a plant in south central Michigan. Kolpin et al. (2002) found a median concentration of 160 ng/L estradiol in samples from 139 streams throughout the United States that are impacted by wastewater from human, industrial and agricultural sources. In the United Kingdom, Desbrow et al. (1998) detected as much as 48 ng/L of estradiol in sewage treatment effluent discharging into British waters. In Italy, Baronti et al. (2000) noted a maximum concentration of 3.5 ng/L and a median of 1.0 ng/L for estradiol in sewage treatment effluent from six Roman plants over a six-month period. In Sweden, Larsson et al. (1999) measured 1.1 ng/L in effluent water from a Swedish sewage treatment works. Comparing these reported estradiol concentrations (0.001 to 0.160 ppb) with the laboratory data in Table 1, effects from laboratory exposures of this magnitude include female-skewed sex ratios (Nimrod and Benson, 1998), males with testis–ova (Kang et al., 2002; Metcalfe et al., 2001) and inhibition of egg production (Kramer et al., 1998).

Estrone is an oxidation product of 17β-estradiol (Ternes et al., 1999). In the United States, a median concentration of 27 ng/L was measured in samples from 139 streams that are impacted by wastewater from human, industrial and agricultural sources (Kolpin et al., 2002). In British sewage effluent, as much as 76 ng/L of estrone has been detected (Desbrow et al., 1998). In Italy, up to 82.1 ng/L of estrone, with a median of 9.3 ng/L, has been measured in sewage treatment effluent (Baronti et al., 2000). Larsson et al. (1999) found 5.8 ng/L of estrone in effluent water from a Swedish sewage treatment works, while Belfroid et al. (1999) detected up to 47 ng/L in effluent from a wastewater treatment plant in The Netherlands. These concentrations (0.006 to 0.082 ppb) are lower than the concentrations showing effects in laboratory exposure experiments (Table 1). The limited laboratory data on estriol, which Baronti et al. (2000) measured at a maximum concentration of 18 ng/L (0.018 ppb) in effluent leaving sewage treatment plants in Rome, Italy, suggests a possible effect of environmental exposure of this magnitude could be sex ratios skewed in the male direction (Metcalfe et al., 2001).

Snyder et al. (1999) found a maximum concentration of the xenoestrogen ethynylestradiol in Lake Mead Nevada to be 520 pg/L, in a tributary leading into the Detroit River Michigan to be 359 pg/L, and in effluent from a Michigan treatment plant to be 759 pg/L. In effluent from British sewage treatment works, the highest concentration of ethynylestradiol detected was 7000 pg/L (Desbrow et al., 1998). In Italian effluent the maximum was 1700 pg/L, with a median of 450 pg/L (Baronti et al., 2000). Data from laboratory experiments (Table 1) indicate effects of ethynylestradiol associated with this level of exposure (0.0004 to 0.007 ppb) include fish with all female gonads (Lange et al., 2001), reduced testicular growth

(Jobling et al., 1996) or delayed maturation, altered mating behavior, reduced egg production and fertilization, and altered gonad structure (Segner et al., 2003).

Biodegradation products of alkyl polyethoxylate detergents, such as nonylphenol and octylphenol, are found in sewage effluent at high ng/L to low μg/L concentrations (Lye et al., 1999). Rudel et al. (1998) measured concentrations of nonylphenol above 1000 μg/L, octylphenol up to 42 μg/L and alkylphenol polyethoxylates as high as 11000 μg/L in domestic wastewater, especially from septic systems. Alkylphenol polyethoxylates were reduced to less than 30 μg/L by sewage treatment (Rudel et al., 1998). Snyder et al. (1999) reported concentrations of nonylphenol polyethoxylate up to 332 μg/L, nonylphenol up to 37 μg/L and octylphenol up to 0.7 μg/L in effluent from a Michigan wastewater treatment plant. Larsson et al. (1999) found 0.84 μg/L of nonylphenol in effluent water from a Swedish sewage treatment works. In the United Kingdom, a maximal concentration of nonylphenol measured in water discharged from a sewage treatment facility was 3 μg/L (ppb), while concentrations in tissues of flounder (*P. flesus*) from these waters ranged from 5 to 180 ng/g (ppb) wet weight (Lye et al., 1999), suggesting some degree of bioconcentration in flounder. Data from laboratory exposure experiments with nonylphenol (Table 2) indicate concentrations lower than those reported may actually increase egg production and skew sex ratios toward males (Nimrod and Benson, 1998). However, concentrations of nonylphenol within the range of the maximums reported (3 to 1000 ppb) have resulted in reduced egg production and male secondary sex characteristics (Harries et al., 2000), reduced testicular growth (Jobling et al., 1996), or reduced egg viability and production of offspring with a permanently disrupted hormonal balance (Schwaiger et al., 2002). Effects noted in laboratory experiments with fish exposed to octylphenol in the range reported for environmental samples (0.7 to 42 ppb) include reduced male sexual behavior and fertilization success (Gray et al., 1999), testis–ova (Gray et al., 1999), reduced male GSI (Jobling et al., 1996) and significant abnormalities in offspring (Gray et al., 1999).

Larsson et al. (1999) measured 490 ng/L of bisphenol A in effluent water from a Swedish sewage treatment works. Bisphenol A has been found at concentrations as high as 258 ng/L in effluent from a sewage treatment plant on the Danube River in Germany, while 8–33 ng/L has been detected in effluent from another German sewage plant (Hansen et al., 1998). These reported concentrations (0.008 to 0.49 ppb) are much lower than the concentrations that have shown effects in laboratory studies with fish (Shioda and Wakabayashi, 2000; Metcalfe et al., 2001; Segner et al., 2003). Di-(ethyl-hexyl)-phthalate (DEHP) was measured at concentrations between 0.622 and 40 μg/L (ppb) in effluents from the Berlin–Ruhleben sewage plant in Germany (Hansen et al., 1998), which is also lower than the concentrations tested in laboratory exposure experiments. Even at the higher concentrations used in laboratory studies, DEHP had no adverse reproductive effects in fish (Shioda and Wakabayashi, 2000; Metcalfe et al., 2001).

Sewage effluent contains a mixture of many of the EDCs discussed above. Can effects observed in the laboratory with single EDCs predict effects that are seen in field situations where exposures are to mixtures? Skewed sex ratio and decreased egg production are the two most frequently reported reproductive effects associated with single EDC exposure experiments in the laboratory (Table 4). Skewed sex ratio and decreased egg production in a population clearly have potential for disrupting population structure. However, we could find no studies that examined egg production in situ, and it is easy to imagine that accurately measuring egg production of a fish population in situ is a daunting, if not impossible, task. Researchers investigating effects of exposure to pulp and paper mill effluents have estimated female egg production by counting the number of developing eggs in subsamples of ovarian tissue taken from field-collected prespawning fish (Munkittrick et al., 1991; Swanson et al., 1994), but this approach is only accurate for determinate spawning species. Field surveys have revealed one population of fish (eelpout), from the vicinity of a large kraft pulp mill on the Swedish Baltic coast, that exhibited a skewed sex ratio (Larsson and Forlin, 2002). The reports of ‘masculinized’ females in the vicinity of pulp and paper mills in Florida appear to be purely a phenotypic disruption with no actual reproductive consequences. It is perplexing that skewed sex ratios, which have been reported so many times as an effect

in laboratory experiments exposing fish to single EDCs (Table 4), should so rarely be noted in field surveys of exposed populations. Because sex ratio in a population is relatively easy and straightforward to measure, lack of such reports likely reflects lack of occurrence. Perhaps sex ratio is less affected in field situations because exposures are generally to mixtures containing multiple EDCs. For example, sewage treatment effluent contains both 17β-estradiol, which has been shown to cause female skewed sex ratios, and estriol, which has resulted in male skewed sex ratios in laboratory exposures. Simultaneous exposure to both of these EDCs could theoretically result in an interaction where each chemical offsets the action of the other.

Researchers conducting field surveys of fish populations exposed to EDCs commonly appraise indicators of reproductive status such as GSI, secondary sex characteristics, intersexuality and other gonad abnormalities. As noted in Table 4, reduced GSI, decreases in male mating behavior, and altered gonad structure are effects that have been observed in a number of laboratory exposures to single EDCs (Table 4). In field surveys, GSI of exposed fish (including intersex individuals) sometimes is no different than in reference fish of the same sex (Kloepper-Sams et al., 1994; van Aerle et al., 2001; Jobling et al., 2002a; De Metrio et al., 2003), while in other instances, GSIs have been reported to be lower in exposed fish (Munkittrick et al., 1994; Jobling et al., 2002a). Billard and Khan (2003) caution that "GSI may not be a reliable index of maturation, particularly when comparing fish of different sizes". They also note that, in their study, lower GSI in reference fish was consistent with fish that had already spawned, which emphasizes the importance of sampling fish from different sites at identical stages of reproductive development. In laboratory studies exposing fish to sewage effluent, researchers have found that exposure does not adversely impact GSI (Rodgers-Gray et al., 2000; Robinson et al., 2003), although exposure to single EDCs normally found in sewage effluent (e.g., estrogens, alkylphenols) impacts GSI in a number of fish species (Jobling et al., 1996; Panter et al., 1998; Gimeno et al., 1998a; Harries et al., 2000; Bjerselius et al., 2001). Collectively, the evidence suggests that GSI in fish may or may not be affected by exposure to EDCs. In addition, numerous other factors such as age and size of fish, water temperature, food supply, disease and direct toxicity can impact reproductive status and individual GSI. GSI should be used cautiously as an indicator of endocrine disruption, and always in conjunction with data from reference fish at the same stage of gonadal development as fish in the population being evaluated. Further, the nature of any relationship between reduced GSI and reproductive impairment needs to be established (e.g., how much does GSI need to be reduced before adverse reproductive effects occur?).

Alteration of normal secondary sex characteristics in a fish population may be an indicator of exposure to EDCs in field situations. In Canadian studies examining fish exposed to kraft mill effluent in situ, no changes in male secondary sex characteristics were observed in longnose sucker from contaminated and reference rivers (Swanson et al., 1994; Kloepper-Sams et al., 1994), but white sucker from Jackfish Bay, which also receives kraft mill effluent, displayed a significant decrease in male secondary sex characteristics. Such contrasting findings may be due to differences in effluent composition, receiving environments, species sensitivity or other complicating factors. In any case, alterations in secondary sex characteristics have not been associated with reproductive effects. Acquisition of male secondary sex characteristics (i.e., masculinized anal fin) in female mosquitofish exposed to kraft mill effluent in Florida rivers is a well-known phenomena (Howell et al., 1980; Cody and Bortone, 1997; Parks et al., 2001), yet no impact on reproductive success or maintenance of affected populations has been reported. As for male sexual behavior, Toft et al. (2003) investigated changes in sexual behavior in male mosquitofish collected from a lake contaminated with EDCs, but found no statistically significant differences, although these fish had a reduced number of sperm cells per milligram testis. These results with mosquitofish exposed in situ are in sharp contrast to laboratory studies with fish exposed to single EDCs, where goldfish (Bjerselius et al., 2001), guppies (Bayley et al., 1999) and sand gobies all exhibited reduced male sexual behavior. While neither secondary sex characteristics nor male sexual behavior can be consistently linked with EDC exposure in field situations, a change in either parameter can be taken as a warning sign that EDC exposure is occurring. For monitoring exposure to EDCs, selecting

a sexually dimorphic fish species when feasible would be advantageous, but because not all fish species exhibit secondary sex characteristics, this parameter may be of limited use in assessing reproductive status in many fish populations.

Intersexuality and other gonad abnormalities have been reported by numerous researchers conducting field studies on fish from sites receiving EDC contamination. Lye et al. (1997) noted male flounder (*P. flesus*) exposed to sewage and industrial effluents in the Tyne estuary in northern England displayed testicular abnormalities in 30% to 53% of the fish sampled. Intersexuality has been observed in wild populations of roach (Jobling et al., 1998), shovelnose sturgeon (Harshbarger et al., 2000), flounder (Allen et al., 1999b; Hashimoto et al., 2000), barbel (Vigano et al., 2001), gudgeon (van Aerle et al., 2001) and carp (Sole et al., 2003). Field surveys conducted in British waters by Jobling et al. (1998), Allen et al. (1999a,b) and van Aerle et al. (2001) found increased incidence of intersexuality in fish from waters receiving sewage treatment effluents, and intersexuality has been detected in some species exposed to single EDCs that are found in sewage effluent (Table 4). Unfortunately, the reproductive or ecological significance of these intersex fish is unclear. In laboratory studies with medaka exposed to single EDCs, intersexuality did not result in reproductive impairment (Kang et al., 2002; Gray et al., 1999). As stated succinctly by Sumpter (1998), "until it is established whether intersex fish can, or cannot, breed successfully, the population-level consequences of intersexuality cannot be quantified". Jobling et al. (2002a,b) report that sexual maturity, gamete production, ability of sperm to fertilize eggs and number of viable offspring are all reduced in native intersex roach when compared to normal male roach. These researchers conclude that widespread intersexuality in roach populations is likely to influence their reproductive success, but acknowledge that many other factors can influence reproductive success in situ. The significance of other gonadal abnormalities in fish is also uncertain. In a laboratory study, Rodgers-Gray et al. (2000) noted a dose-dependent feminization of reproductive ducts in the testes of young male roach exposed to increasing percentages of sewage effluent from 50 to 200 days post-hatch, although no differences in germ cell differentiation were observed. Similarly, while a number of researchers have noted gonadal changes associated with exposing various fish species to single EDCs (Christiansen et al., 1998; Metcalfe et al., 2000; Zaroogian et al., 2001; Orn et al., 2003; Kinnberg et al., 2003; Kinnberg and Toft, 2003; Islinger et al., 2003), a link between specific histological alterations in gonad structure and reproductive success remains to be elucidated.

There are limited studies in which a population assessment of fish living in waters that receive inputs containing EDCs has been conducted. Most have been done by Canadian researchers evaluating the effects of pulp and paper mill effluents on fish populations (Munkittrick et al., 1991; Swanson et al., 1994; Gagnon et al., 1995; Leblanc et al., 1997; Munkittrick et al., 1998). For example, Swanson et al. (1994) evaluated fish habitat, species distribution and abundance, migration and other population parameters downstream of a bleached-kraft pulp mill in Alberta, Canada. Some habitat alterations, including greater algal cover and abundance of benthic invertebrates, were noted as a result of nutrient input from the pulp mill and municipal sewage treatment effluent, but habitat data indicated mill effluent had not eliminated spawning, overwintering or rearing habitat. In fact, species distribution data suggested that the area near the pulp mill was actually preferred, especially in fall and winter, presumably because of greater food abundance. Age structure analysis of fish populations demonstrated that no loss of age classes had occurred due to reproductive or recruitment failures, and presence of young-of-year confirmed that successful reproduction was taking place. Another study by Noaksson et al. (2001) in Sweden found that all appropriate age classes of perch were present in a lake contaminated with dump leachate suspected of containing EDCs. Beyond these reports, there exists a notable lack of data on population parameters, such as age class structure in exposed and unexposed fish populations, make linking endocrine disruption and reproductive impairment with a meaningful assessment of ecological risk difficult.

## 4. Conclusion

This review has collated a wealth of data from laboratory experiments that support the hypothesis

that EDCs in the aquatic environment have the potential to impact the reproductive health and persistence of various fish species. However, evidence from field surveys that EDCs in the aquatic environment are actually impacting the reproductive health and sustainability of indigenous fish populations is much less convincing. The scarcity of evidence to link exposure to environmental EDCs with impacts on reproduction of indigenous fish populations reflects a critical need for a dependable method or indicator to assess reproductive success of fish in situ. Two indicators from laboratory studies that appear to be consistently associated with decreased reproductive success, female egg production and male sexual behavior, are difficult to measure in situ. A method used by Munkittrick et al. (1991) to estimate individual fecundity based on the number of eggs in ovarian tissue from prespawning females may help fill this critical need. One indicator commonly assessed at present in field surveys, induction of male plasma vitellogenin, is not adequately linked to reproductive or population level effects. Severe intersexuality in males has recently been linked with decreased reproductive success in the fish roach (Jobling et al., 2002a,b), but similar research on other species is needed, as well as an examination of the actual effects of intersex in individuals on the maintenance of a fish population in situ. Less dramatic gonad alterations such as changes in GSI or histology have been presented as evidence of EDC exposure, but there is little information to link such alterations in gonad structure with reproductive impairment. Like many other indicators, abnormal gonad histology needs to be tied to reproductive output if it is to be a valid indicator of reproductive impairment. Studies examining age structure of fish populations in areas impacted by EDCs, compared with unaffected areas, are very limited, yet such information would seem to be of practical importance when trying to establish whether EDCs are impacting an exposed population. In order to address conclusively the question of whether EDCs in the aquatic environment are impacting health and sustainability of wild fish populations, more emphasis needs to be placed on investigating if fish populations routinely exposed to EDCs are experiencing changes in population structure. This approach, however, might be fruitless in areas that have experienced drastic or long-term EDC exposure, because sensitive fish species may already be gone. Comparative community assessment studies may be most useful in these instances.

In summary, there appears to be sufficient evidence from laboratory experiments with single chemicals to link exposure to exogenous EDCs with reproductive impairment in many fish species. The next necessary step, being able to accurately assess the reproductive fitness of fish exposed to EDCs in their natural environment, is dependent on the establishment of a reliable method or indicator to determine reproductive success of fish in situ. There are some promising indicators, but linkages between these indicators and reproductive success need to be established or strengthened. Linking endocrine disruption and reproductive impairment with an ecologically relevant impact on a real fish population remains, with few exceptions, an open challenge.

## Acknowledgments and disclaimer

We thank R.E. Gutjahr-Gobell, G.E. Zaroogian, D. Nacci and three anonymous reviewers who provided valuable comments to an earlier version of this manuscript. This manuscript has been reviewed and approved for publication by the U.S. Environmental Protection Agency's Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, contribution number AED-04-076. Approval does not signify that the contents of this report necessarily reflect the views and policies of the agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by EPA.

## References

Allen Y, Scott AP, Matthiessen P, Haworth S, Thain JE, Feist S. Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effect on gonadal development of the flounder *Platichthys flesus*. Environ Toxicol Chem 1999a;18: 1791–800.

Allen Y, Matthiessen P, Scott AP, Haworth S, Feist S, Thain JE. The extent of oestrogenic contamination in the UK estuarine and marine environments—further surveys of flounder. Sci Total Environ 1999b;233:5–20.

Andersen L, Holbech H, Gessbo A, Norrgren L, Petersen GI. Effects of exposure to 17α-ethinylestradiol during early development on sexual differentiation and induction of vitellogenin in zebrafish (*Danio rerio*). Comp Biochem Physiol, Part C Pharmacol Toxicol 2003;134:365–74.

Ankley GT, Jensen KM, Kahl MD, Korte JJ, Makynen EA. Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 2001;20:1276–90.

Arcand-Hoy LD, Benson WH. Fish reproduction: an ecologically relevant indicator of endocrine disruption. Environ Sci Technol 1998;17:49–57.

Balaguer P, Francois F, Comunale F, Fenet H, Boussioux A-M, Pons M, et al. Reporter cell lines to study the estrogenic effects of xenoestrogens. Sci Total Environ 1999;233:47–56.

Balch GC, Mackenzie CA, Metcalfe CD. Alterations to gonadal development and reproductive success in Japanese medaka (*Oryzias latipes*) exposed to 17α-ethinylestradiol. Environ Toxicol Chem 2004;23:782–91.

Baronti C, Curini R, D'Ascenzo G, Di Corcia A, Gentili A, Samperi R. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environ Sci Technol 2000;34:5059–66.

Bayley M, Nielsen JR, Baatrup E. Guppy sexual behavior as an effect biomarker of estrogen mimics. Ecotoxicol Environ Saf 1999;43:68–73.

Bayley M, Junge M, Baatrup E. Exposure of juvenile guppies to three antiandrogens causes demasculization and a reduced sperm count in adult males. Aquat Toxicol 2002;56:227–39.

Belfroid AC, Van der Horst A, Vethaak AD, Schafer AJ, Rijs GBJ, Wegener J, et al. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. Sci Total Environ 1999;225:101–8.

Billiard SM, Khan RA. Chronic stress in cunner, *Tautogolabrus adspersus*, exposed to municipal and industrial effluents. Ecotoxicol Environ Saf 2003;55:9–18.

Bjerselius R, Lundstedt-Enkel K, Olsen H, Mayer I, Dimberg K. Male goldfish reproductive behaviour and physiology are severely affected by exogenous exposure to 17β-estradiol. Aquat Toxicol 2001;53:139–52.

Blanchfield P, Majewski A, Palace V, Kidd K, Mills K. Reproductive and population-level impacts of a synthetic estrogen on the fathead minnow. Twenty-fourth Annual Meeting of the Society of Environmental Toxicology and Chemistry, 9–13 November 2003, Austin, TX, USA, vol. 24; 2003. p. 289.

Bolger R, Wiese TE, Ervin K, Nestich S, Checovich W. Rapid screening of environmental chemicals for estrogen receptor binding capacity. Environ Health Perspect 1998;106:551–7.

Bortone SA, Cody RP. Morphological masculinization in Poeciliid females from a paper mill effluent receiving tributary of the St Johns River, Florida, USA. Bull Environ Contam Toxicol 1999;63:150–6.

Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N. Xenoestrogens released from laquer coatings in food cans. Environ Health Perspect 1995;103:608–12.

Cheek AO, Vonier PM, Oberdorster E, Burow BC, McLachlan JA. Environmental signaling: a biological context for endocrine disruption. Environ Health Perspect 1998;106(Suppl 1): 5–10.

Christiansen T, Korsgaard B, Jespersen A. Induction of vitellogenin synthesis by nonylphenol and 17β-estradiol and effects on the testicular structure in the eelpout *Zoarces viviparus*. Mar Environ Res 1998;46:141–4.

Cody RP, Bortone SA. Masculinization of mosquitofish as an indicator of exposure to kraft mill effluent. Bull Environ Contam Toxicol 1997;58:429–36.

Colburn T, Dumanoski D, Myers JP. Our stolen future: are we threatening our fertility, intelligence, and survival?—A scientific detective story. NY, NY: Penguin Books; 1996. 306 pp.

Cooper RL, Kavlok RJ. Endocrine disruptors and reproductive development: a weight-of-evidence overview. J Endocrinol 1997;152:159–66.

Courtenay SC, Munkittrick KR, Dupuis HMC, Parker R, Boyd J. Quantifying impacts of pulp mill effluent on fish in Canadian marine and estuarine environments: problems and progress. Water Qual Res J Can 2002;37:79–99.

Dawson A. Comparative reproductive physiology of non-mammalian species. Pure Appl Chem 1998;70:1657–69.

Denton TE, Howell WM, Allison JJ, McCollum J, Marks B. Masculinization of female mosquito fish by exposure to plant sterols and *Mycobacterium smegmatis*. Bull Environ Contam Toxicol 1985;35:627–32.

Desbrow C, Routledge EJ, Brighty GC, Sumpter JP, Waldock M. Identification of estrogenic chemicals in STW effluent: 1. Chemical fractionation and in vitro biological screening. Environ Sci Technol 1998;32:1549–58.

De Metrio G, Corriero A, Desantis S, Zubani D, Cirillo F, Deflorio M, et al. Evidence of a high percentage of intersex in the Mediterranean swordfish (*Xiphias gladius* L). Mar Pollut Bull 2003;46:358–61.

Durhan EJ, Lambright C, Wilson V, Butterworth BC, Kuehl DW, Orlando EF, et al. Evaluation of androstenedione as an adrogenic component of river water downstream of a pulp and paper mill effluent. Environ Toxicol Chem 2002;21: 1973–6.

Gagnon MM, Bussieres D, Dodson JJ, Hodson PV. White sucker (*Catostomus commersoni*) growth and sexual maturation in pulp mill-contaminated and reference rivers. Environ Toxicol Chem 1995;14:317–27.

Geisy JP, Pierens SL, Snyder ER, Miles-Richardson S, Kramer VJ, Snyder SA, et al. Effects of 4-nonylphenol on fecundity and biomarkers of estrogenicity in fathead minnows (*Pimephales promelas*). Environ Toxicol Chem 2000;19:1368–77.

Gimeno S, Komen H, Jobling S, Sumpter J, Bowmer T. Demasculinisation of sexually mature male common carp, *Cyprinus carpio*, exposed to 4-*tert*-pentylphenol during spermatogenesis. Aquat Toxicol 1998a;43:93–109.

Gimeno S, Komen H, Gerritsen AGM, Bowmer T. Feminisation of young males of the common carp, *Cyprinus carpio*, exposed to 4-*tert*-pentylphenol during sexual differentiation. Aquat Toxicol 1998b;43:77–92.

Gray MA, Teather KL, Metcalfe CD. Reproductive success and behavior of Japanese medaka (*Oryzias latipes*) exposed to 4-*tert*-octylphenol. Environ Toxicol Chem 1999;18:2587–94.

Guidetti P, Terlizzi A, Fraschetti S, Boero F. Changes in Mediterranean rocky-reef fish assemblages exposed to sewage pollution. Mar Ecol Prog Ser 2003;253:269–78.

Hall JA, Frid CL, Gill ME. The response of estuarine fish and benthos to an increasing discharge of sewage effluent. Mar Pollut Bull 1997;34:527–35.

Hansen P-D, Dizer H, Hock B, Marx A, Sherry J, McMaster M, et al. Vitellogenin—a biomarker for endocrine disruptors. Trends Anal Chem 1998;17:448–51.

Harries JE, Sheahan DA, Jobling S, Matthiessen P, Neall P, Routledge EJ, et al. A survey of estrogenic activity in United Kingdom inland waters. Environ Toxicol Chem 1996;15:1993–2002.

Harries JE, Sheahan DA, Jobling S, Matthiessen P, Neall P, Sumpter JP, et al. Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. Environ Toxicol Chem 1997;16:534–42.

Harries JE, Runnalls T, Hill E, Harris CA, Maddix S, Sumpter JP, et al. Development of a reproductive performance test for endocrine disrupting chemicals using pair-breeding fathead minnows (*Pimephales promelas*). Environ Sci Technol 2000;34:3003–11.

Harshbarger JC, Coffey MJ, Young MY. Intersexes in Mississippi River shovelnose sturgeon sampled below Saint Louis, Missouri, USA. Mar Environ Res 2000;50:247–50.

Hashimoto S, Bessho H, Hara A, Nakamura M, Iguchi T, Fujita K. Elevated serum vitellogenin levels and gonadal abnormalities in wild male flounder (*Pleuronectes yokohamae*) from Tokyo Bay, Japan. Mar Environ Res 2000;49:37–53.

Haubruge E, Petit F, Gage MJG. Reduced sperm counts in guppies (*Poecilia reticulata*) following exposure to low levels of tributyltin and bisphenol A. Proc R Soc Lond, B 2000;267: 2333–7.

Howell WM, Black DA, Bortone SA. Abnormal expression of secondary sex characteristics in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence for environmentally induced masculinization. Copeia 1980;4:676–81.

Hutchinson TH, Pickford DB. Ecological risk assessment and testing for endocrine disruption in the aquatic environment. Toxicology 2002;181–182:383–7.

Islinger M, Willimski D, Volkl A, Braunbeck T. Effects of 17α-ethinylestradiol on the expression of three estrogen-responsive genes and cellular ultrastructure of liver and testes in male zebrafish. Aquat Toxicol 2003;62:85–103.

Janssen PAH, Lambert JGD, Vethaak AD, Goos HJT. Environmental pollution caused elevated concentrations of oestradiol and vitellogenin in the female flounder, *Platichthys flesus* (L). Aquat Toxicol 1997;39:195–214.

Jenkins R, Angus RA, McNatt H, Howell WM, Kemppainen JA, Kirk M, et al. Identification of androstenedione in a river containing paper mill effluent. Environ Toxicol Chem 2001;20: 1325–31.

Jensen KM, Korte JJ, Kahl MD, Pasha MS, Ankley GT. Aspects of basic reproductive biology and endocrinology in the fathead minnow (*Pimephales promelas*). Comp Biochem Physiol, Part C Pharmacol Toxicol 2001;128:127–41.

Jobling S, Sumpter JP. Detergent components in sewage effluent are weakly oestrogenic to fish: an in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquat Toxicol 1993;27: 361–72.

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environ Toxicol Chem 1996;15:194–202.

Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. Widespread sexual disruption in wild fish. Environ Sci Technol 1998;32: 2498–506.

Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty GC, Sumpter JP, et al. Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. Biol Reprod 2002a;66: 272–81.

Jobling S, Coey S, Whitmore JG, Kime DE, Van Look KJW, McAllister BG, et al. Wild intersex roach (*Rutilus rutilus*) have reduced fertility. Biol Reprod 2002b;67:515–24.

Jobling S, Casey D, Rodgers-Gray T, Oehlmann J, Schulte-Oehlmann U, Pawlowski S, et al. Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquat Toxicol 2003;65:205–20.

Kahl MD, Jensen KM, Korte JJ, Ankley GT. Effects of handling on endocrinology and reproductive performance of the fathead minnow. J Fish Biol 2001;59:515–23.

Kang IJ, Yokota H, Oshima Y, Tsuruda Y, Yamaguchi T, Maeda M, et al. Effect of 17β-estradiol on the reproduction of Japanese medaka (*Oryzias latipes*). Chemosphere 2002;47:71–80.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA-sponsored workshop. Environ Health Perspect 1996;104(Suppl 4):715–40.

Kime DE, Nash JP. Gamete viability as an indicator of reproductive endocrine disruption in fish. Sci Total Environ 1999;233:123–9.

Kingsford MJ, Suthers IM, Gray CA. Exposure to sewage plumes and the incidence of deformities in larval fishes. Mar Pollut Bull 1996;33:201–12.

Kinnberg K, Toft G. Effects of estrogenic and antiandrogenic compounds on the testis structure of the adult guppy (*Poecilia reticulata*). Ecotoxicol Environ Saf 2003;54:16–24.

Kinnberg K, Korsgaard B, Bjerregaard P. Effects of octylphenol and 17β-estradiol on the gonads of guppies (*Poecilia reticulata*) exposed as adults via the water or as embryos via the mother. Comp Biochem Physiol, Part C Pharmacol Toxicol 2003; 134:45–55.

Kirby MF, Allen YT, Dyer RA, Feist SW, Katsiadaki I, Matthiessen P, et al. Surveys of plasma vitellogenin and intersex in male flounder (*Platichthys flesus*) as measures of endocrine disruption by estrogenic contamination in United Kingdom estuaries: temporal trends, 1996 to 2001. Environ Toxicol Chem 2004;23:748–58.

Kirk LA, Tyler CR, Lye CM, Sumpter JP. Changes in estrogenic and androgenic activities at different stages of treatment in wastewater treatment works. Environ Toxicol Chem 2002;21:972–9.

Kloepper-Sams PJ, Swanson SM, Marchant T, Schryer R, Owens JW. Exposure of fish to biologically treated bleached-kraft effluent: 1. Biochemical, physiological, and pathological assessment of Rocky Mountain whitefish (*Prosopium williamsoni*)

and longnose sucker (*Catostomus catostomus*). Environ Toxicol Chem 1994;13:1469–82.

Koger CS, Teh SJ, Hinton DE. Determining the sensitive developmental stages of intersex induction in medaka (*Oryzias latipes*) exposed to 17β-estradiol or testosterone. Mar Environ Res 2000;50:201–6.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ Sci Technol 2002;36:1202–11.

Korner W, Bolz U, Sußmuth W, Hiller G, Schuller W, Hanf V, et al. Input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany. Chemosphere 2000;40: 1131–42.

Kovacs TG, Gibbons JS, Tremblay LA, O'Connor BI, Martel PH, Voss RH. The effects of a secondary-treated bleached kraft mill effluent on aquatic organisms as assessed by short-term and long-term laboratory tests. Ecotoxicol Environ Saf 1995;31:7–22.

Kramer VJ, Miles-Richardson S, Pierens SL, Giesy JP. Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of estrogen exposure, in fathead minnows (*Pimephales promelus*) exposed to waterborne 17β-estradiol. Aquat Toxicol 1998;40:335–60.

Lange R, Hutchinson TH, Croudace CP, Siegmund F, Schweinfurth H, Hampe P, et al. Effects of the synthetic estrogen 17α-ethinylestradiol on the life-cycle of the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 2001;20: 1216–27.

Larsson DGJ, Forlin L. Male-biased sex ratios of fish embryos near a pulp mill: temporary recovery after a short-term shutdown. Environ Health Perspect 2002;110:739–42.

Larsson DGJ, Adolfsson-Erici M, Parkkonen J, Pettersson M, Berg AH, Olsson P-E, et al. Ethynyl estradiol—an undesired fish contraceptive? Aquat Toxicol 1999;45:91–7.

Larsson DGJ, Kinnberg K, Sturve J, Stephensen E, Skon M, Forlin L. Studies of masculinization, detoxification, and oxidative stress responses in guppies (*Poecilia reticulata*) exposed to effluent from a pulp mill. Ecotoxicol Environ Saf 2002;52:13–20.

Leblanc J, Couillard CM, Brethes J-CF. Modifications of the reproductive period in mummichog (*Fundulus heteroclitus*) living downstream from a bleached kraft pulp mill in the Miramichi Estuary, New Brunswick, Canada. Can J Fish Aquat Sci 1997;54:2564–73.

Lehtinen KJ, Mattsson K, Tana J, Engstrom C, Lerche O, Hemming J. Effects of wood-related sterols on reproduction, egg survival, and offspring of brown trout (*Salmo trutta lacustris* L). Ecotoxicol Environ Saf 1999;42:40–9.

Loomis AK, Thomas P. Binding characteristics of estrogen receptor (ER) in Atlantic croaker (*Micropogonias undulatus*) testis: different affinity for estrogens and xenobiotics from that of hepatic ER. Biol Reprod 1999;61:51–60.

Lye CM, Frid CLJ, Gill ME, McCormick D. Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works. Mar Pollut Bull 1997;34:34–41.

Lye CM, Frid CLJ, Gill ME, Cooper DW, Jones DM. Estrogenic alkylphenols in fish tissues, sediments, and waters from the UK Tyne and Tees estuaries. Environ Sci Technol 1999;33: 1009–14.

Makynen EA, Kahl MD, Jensen KM, Tietge JE, Wells KL, Van Der Kraak G, et al. Effects of the mammalian antiandrogen vinclozolin on development and reproduction in the fathead minnow (*Pimephales promelus*). Aquat Toxicol 2000;48:461–75.

Matthiessen P, Gibbs PE. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. Environ Toxicol Chem 1998;17:37–43.

McAllister BG, Kime DE. Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinisation and irreversible sperm damage in zebrafish. Aquat Toxicol 2003;65:309–16.

McMaster ME, Portt CB, Munkittrick KR, Dixon DG. Milt characteristics, reproductive performance, and larval survival and development of white sucker exposed to bleached kraft mill effluent. Ecotoxicol Environ Saf 1992;23:103–17.

Metcalfe TL, Metcalfe CD, Kiparissis Y, Niimi AJ, Foran CM, Benson WH. Gonadal development and endocrine responses in Japanese medaka (*Oryzias latipes*) exposed to *o,p′*-DDT in water or through maternal transfer. Environ Toxicol Chem 2000;19:1893–900.

Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, et al. Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 2001;20:297–308.

Miles-Richardson SR, Kramer VJ, Fitzgerald SD, Render JA, Yamini B, Barbee SJ, et al. Effects of waterborne exposure of 17β-estradiol on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelus*). Aquat Toxicol 1999a;47:129–45.

Miles-Richardson SR, Pierens SL, Nichols KM, Kramer VJ, Snyder EM, Synder SA, et al. Effects of waterborne exposure to 4-nonylphenol and nonylphenol ethoxylate on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelus*). Environ Res, Section A 1999b;80:S122–37.

Mills LJ, Gutjahr-Gobell RE, Horowitz DB, Denslow ND, Chow MC, Zaroogian GE. Relationship between reproductive success and male plasma vitellogenin concentrations in cunner, *Tautogolabrus adspersus*. Environ Health Perspect 2003;111:93–9.

Munkittrick KR, Portt CB, Van Der Kraak GJ, Smith IR, Rokosh DA. Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. Can J Fish Aquat Sci 1991;48:1371–80.

Munkittrick KR, Van Der Kraak GJ, McMaster ME, Portt CB, van den Heuvel MR, Servos MR. Survey of receiving-water environmental impacts associated with discharges from pulp mills: 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. Environ Toxicol Chem 1994;13:1089–101.

Munkittrick KR, McMaster ME, McCarthy LH, Servos MR, Van Der Kraack GJ. An overview of recent studies on the potential of pulp-mill effluents to alter reproductive parameters in fish. J Toxicol Environ Health, Part B Crit Rev 1998;1:347–71.

Munkittrick KR, McGeachy SA, McMaster ME, Courtenay SC. Overview of freshwater fish studies from the pulp and paper environmental effects monitoring program. Water Qual Res J Can 2002;37:49–77.

Nichols DJ, Daniel TC, Moore PA, Edwards DR, Pote DH. Runoff of estrogen hormone 17β-estradiol from poultry litter applied to pasture. J Environ Qual 1997;26:1002–6.

Nichols KM, Miles-Richardson SR, Synder EM, Giesy JP. Effects of exposure to municipal wastewater in situ on the reproductive physiology of the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 1999;18:2001–12.

Nimrod AC, Benson WH. Xenobiotic interaction with and alteration of channel catfish estrogen receptor. Toxicol Appl Pharmacol 1997;147:381–90.

Nimrod AC, Benson WH. Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens. Aquat Toxicol 1998;44:141–56.

Noaksson E, Tjarnlund U, Bosveld AT, Balk L. Evidence for endocrine disruption in perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) in a remote Swedish lake in the vicinity of a public refuse dump. Toxicol Appl Pharmacol 2001;174:160–76.

Noaksson E, Linderoth M, Bosveld ATC, Norrgren L, Zebuhr Y, Balk L. Endocrine disruption in brook trout (*Salvelinus fontinalis*) exposed to leachate from a public refuse dump. Sci Total Environ 2003;305:87–103.

Oakes KD, McMaster ME, Pryce AC, Munkittrick KR, Portt CB, Hewitt LM, et al. Oxidative stress and bioindicators of reproductive function in pulp and paper mill effluent exposed white sucker. Toxicol Sci 2003;74:51–65.

Oberdorster E, Cheek AO. Gender benders at the beach: endocrine disruption in maine and estuarine organisms. Environ Toxicol Chem 2000;20:23–36.

Orlando EF, Kolok AS, Binzcik GA, Gates JL, Horton MK, Lambright CS, et al. Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. Environ Health Perspect 2004;112:353–8.

Orn S, Holbech H, Madsen TH, Norrgren L, Petersen GI. Gonad development and vitellogenin production in zebrafish (*Danio rerio*) exposed to ethinylestradiol and methyltestosterone. Aquat Toxicol 2003;65:397–411.

Oshima Y, Kang IJ, Kobayashi M, Nakayama K, Imada N, Honjo T. Suppression of sexual behavior in male Japanese medaka (*Oryzias latipes*) exposed to 17β-estradiol. Chemosphere 2003;50:429–36.

Pait AS, Nelson JO. Vitellogenesis in male *Fundulus heteroclitus* (killifish) induced by selected estrogenic compounds. Aquat Toxicol 2003;64:331–42.

Palace V, Evans RE, Wautier K, Baron C, Vandenbyllardt L, Vandersteen W, et al. Induction of vitellogenin and histological effects in wild fathead minnows from a lake experimentally treated with the synthetic estrogen, ethynylestradiol. Water Qual Res J Can 2002;37:637–50.

Palace V, Kidd K, Blanchfield P, Mills K, Evans RE, Baron CL, et al. Vitellogenin induction and histopathological effects in pearl dace (*Semotilus margarita*) captured from a lake experimentally treated with the synthetic estrogen ethynylestradiol. Twenty-fourth Annual Meeting of the Society of Environmental Toxicology and Chemistry, 9–13 November 2003, Austin, TX, USA, vol. 24; 2003. p. 289.

Panter GH, Thompson RS, Sumpter JP. Adverse reproductive effects in male fathead minnows exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. Aquat Toxicol 1998;42:243–53.

Panter GH, Hutchinson TH, Lange R, Lye CM, Sumpter JP, Zerulla M, et al. Utility of a juvenile fathead minnow screening assay for detecting (anti-)estrogenic substances. Environ Toxicol Chem 2002;21:319–26.

Papoulias DM, Noltie DB, Tillitt DE. An in vivo model fish system to test chemical effects on sexual differentiation and development: exposure to ethinyl estradiol. Aquat Toxicol 1999;48:37–50.

Parks LG, Lambright CS, Orlando EF, Guillette Jr LJ, Ankley GT, Gray Jr LE. Masculinization of female mosquito fish in kraft mill effluent-contaminated Fenholloway River water is associated with androgen receptor agonist activity. Toxicol Sci 2001;62:257–67.

Patyna PJ, Davi RA, Parkerton TF, Brown RP, Cooper KR. A proposed multigeneration protocol for Japanese medaka (*Oryzias latipes*) to evaluate effects of endocrine disruptors. Sci Total Environ 1999;233:211–20.

Pawlowski S, van Aerle R, Tyler CR, Braunbeck T. Effects of 17α-ethinylestradiol in a fathead minnow (*Pimephales promelas*) gonadal recrudescence assay. Ecotoxicol Environ Saf 2004;57:330–45.

Pelley J. Estrogen knocks out fish in whole-lake experiment. Environ Sci Technol 2003;37:313–4.

Purdom CE, Hardiman PA, Bye VJ, Eno NC, Tyler CR, Sumpter JP. Estrogenic effects of effluents from sewage treatment works. Chem Ecol 1994;8:275–85.

Robinson CD, Brown E, Craft JA, Davies IM, Moffat CF, Pirie D, et al. Effects of sewage effluent and ethynyl oestradiol upon molecular markers of oestrogenic exposure, maturation and reproductive success in the sand goby (*Pomatoschistus minutus*, Pallas). Aquat Toxicol 2003;62:119–34.

Rodgers-Gray TP, Jobling S, Morris S, Kelly C, Kirby S, Janbakhsh A, et al. Long-term temporal changes in the estrogenic composition of treated sewage effluent and its biological effects on fish. Environ Sci Technol 2000;34:1521–8.

Rodgers-Gray TP, Jobling S, Kelly C, Morris S, Brighty G, Waldock MJ, et al. Exposure of juvenile roach (*Rutilus rutilus*) to treated sewage effluent induces dose-dependent and persistent disruption in gonadal duct development. Environ Sci Technol 2001;35:462–70.

Routledge EJ, Sheahan D, Desbrow C, Brighty GC, Waldock M, Sumpter JP. Identification of estrogenic chemicals in STW effluent: 2. In vivo responses in trout and roach. Environ Sci Technol 1998;32:1559–65.

Rudel RA, Melly SJ, Geno PW, Sun G, Brody JG. Identification of alkylphenols and other estrogenic phenolic compounds in wastewater, septage, and groundwater on Cape Cod, Massachusetts. Environ Sci Technol 1998;32:861–9.

Schiffer B, Daxenberger A, Meyer K, Meyer HHD. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. Environ Health Perspect 2001;109:1145–51.

Scholz S, Gutzeit HO. 17-α-Ethinylestradiol affects reproduction, sexual differentiation and aromatase gene expression of the medaka (*Oryzias latipes*). Aquat Toxicol 2000;50:363–73.

Schwaiger J, Mallow U, Ferling H, Knoerr S, Braunbeck Th, Kalbfus W, et al. How estrogenic is nonylphenol? A trans-generational study using rainbow trout (*Oncorhynchus mykiss*) as a test organism. Aquat Toxicol 2002;59:177–89.

Segner H, Caroll K, Fenske M, Janssen CR, Maack G, Pascoe D, et al. Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project. Ecotoxicol Environ Saf 2003;54:302–14.

Seki M, Yokota H, Matsubara H, Tsuruda Y, Maeda M, Tadokoro H, et al. Effect of ethinylestradiol on the reproduction and induction of vitellogenesis and testis–ova in medaka (*Oryzias latipes*). Environ Toxicol Chem 2002;21:1692–8.

Seki M, Yokota H, Matsubara H, Maeda M, Tadokoro H, Kobayashi K. Fish full life-cycle testing for androgen methyltestosterone on medaka (*Oryzias latipes*). Environ Toxicol Chem 2004;23:774–81.

Shioda T, Wakabayashi M. Effect of certain chemicals on the reproduction of medaka (*Oryzias latipes*). Chemosphere 2000;40:239–43.

Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. J Endocrinol 1998;158:327–39.

Sohoni P, Tyler CR, Hurd K, Caunter J, Hetheridge M, Williams T, et al. Reproductive effects of long-term exposure to bisphenol A in the fathead minnow (*Pimephales promelas*). Environ Sci Technol 2001;35:2917–25.

Sole M, Raldua D, Piferrer F, Barcelo D, Porte C. Feminization of wild carp, *Cyprinus carpio*, in a polluted environment: plasma steroid hormones, gonadal morphology and xenobiotic metabolizing system. Comp Biochem Physiol, Part C Pharmacol Toxicol 2003;136:145–56.

Sonnenschein C, Soto AM. An updated review of environmental estrogen and androgen mimics and antagonists. J Steroid Biochem Mol Biol 1998;65:143–50.

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 1995;103(Suppl 7):113–22.

Stentiford GD, Longshaw M, Lyons BP, Jones G, Green M, Feist SW. Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. Mar Environ Res 2003;55:137–59.

Sumpter JP. Xenoendocrine disrupters—environmental impacts. Toxicol Lett 1998;102–103:337–42.

Snyder SA, Keith TL, Verbrugge DA, Snyder EM, Gross TS, Kannan K, et al. Analytical methods for detection of selected estrogenic compounds in aqueous mixtures. Environ Sci Technol 1999;33:2814–20.

Swanson SM, Schryer R, Shelast R, Kloepper-Sams PJ, Owens JW. Exposure of fish to biologically treated bleached-kraft mill effluent: 3. Fish habitat and population assessment. Environ Toxicol Chem 1994;13:1497–507.

Taylor MR, Harrison PTC. Ecological effects of endocrine disruption: current evidence and research priorities. Chemosphere 1999;39:1237–48.

Ternes TA, Kreckel P, Mueller J. Behaviour and occurance of estrogens in municipal sewage treatment plants: II. Aerobic batch experiments with activated sludge. Sci Total Environ 1999;225:91–9.

Thompson S, Tilton F, Schlenk D, Benson WH. Comparative vitellogenic responses in three teleost species: extrapolation to in situ field studies. Mar Environ Res 2000;51:185–9.

Toft G, Edwards TM, Baatrup E, Guillette Jr LJ. Disturbed sexual characteristics in male mosquito fish (*Gambusia holbrooki*) from a lake contaminated with endocrine disruptors. Environ Health Perspect 2003;111:695–701.

Tran DQ, Ide CF, McLachlan JA, Arnold SF. The anti-estrogenic activity of selected polynuclear aromatic hydrocarbons in yeast expressing human estrogen receptor. Biochem Biophys Res Commun 1996;229:102–8.

Tyler CR, Jobling S, Sumpter JP. Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol 1998;28:319–61.

U.S. Environmental Protection Agency. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Report. 1998. OPPTS, Washington, DC.

van Aerle R, Nolan M, Jobling S, Christiansen LB, Sumpter JP, Tyler CR. Sexual disruption in a second species of wild cyprinid fish (the gudgeon, *Gobio gobio*) in United Kingdom freshwaters. Environ Toxicol Chem 2001;20:2841–7.

Van den Belt K, Verheyen R, Witters H. Effects of 17α-ethynylestradiol in a partial life-cycle test with zebrafish (*Danio rerio*): effects on growth, gonads and female reproductive success. Sci Total Environ 2003;309:127–37.

van den Heuval MR, Ellis RJ, Tremblay LA, Stuthridge TR. Exposure of reproductively maturing rainbow trout to a New Zealand pulp and paper mill effluent. Ecotoxicol Environ Saf 2002;51:65–75.

Van Der Kraack G, Hewitt M, Lister A, McMaster ME, Munkittrick KR. Endocrine toxicants and reproductive success in fish. Hum Ecol Risk Assess 2001;7:1017–25.

Vigano L, Arillo A, Bottero S, Massari A, Mandich A. First observation of intersex cyprinids in the Po River (Italy). Sci Total Environ 2001;269:189–94.

Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, et al. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicol 2000;30:71–133.

Waring CP, Stagg RM, Fretwell K, McLay HA, Costello MJ. The impact of sewage sludge exposure on the reproduction of the sand goby, *Pomatoschistus minutus*. Environ Pollut 1996;93:17–25.

White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 1994;135:175–82.

Yamamoto T. Artificially induced sex-reversal in genotypic males of the medaka (*Oryzias latipes*). J Exp Zool 1953;123:571–94.

Yamamoto T. Artificial induction of the functional sex reversal in genotypic females of the medaka (*Oryzias latipes*). J Exp Zool 1958;137:227–63.

Zacharewski T. In vitro bioassays for assessing estrogenic substances. Environ Sci Technol 1997;31:613–23.

Zaroogian G, Gardner G, Borsay Horowitz D, Gutjahr-Gobell R, Haebler R, Mills L. Effect of 17β-estradiol, *o*,*p*′-DDT, octylphenol and *p*,*p*′-DDE on gonadal development and liver and kidney pathology in juvenile male summer flounder (*Paralichthys dentatus*). Aquat Toxicol 2001;54:101–12.

Zerulla M, Lange R, Steger-Hartmann T, Panter G, Hutchinson T, Dietrich DR. Morphological sex reversal upon short-term exposure to endocrine modulators in juvenile fathead minnows (*Pimephales promelas*). Toxicol Lett 2002;131:51–63.