

Effects of Endocrine Disrupters on Sexual, Gonadal Development in Fish

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Key Words

Endocrine disruption · Environmental chemicals · Estrogens · Gonad · Gonadosomatic index · Intersex · Sex ratio

Abstract

Steroid sex hormones play an important role in the sexual differentiation of fish. Thus, it is not surprising that chemical contaminants with steroid-like activities were considered as responsible for the unusual occurrence of gonadal intersex conditions and other gonadal aberrations in feral fish. In this review, we give an overview about field data and summarise and categorise experimental evidence that links disruption of gonadal development in gonochoristic fish to contaminations by endocrine disrupting chemicals. A comprehensive overview on laboratory studies using water-borne exposures and histopathological analysis is given. Parameters ranging from simple quantitative characteristics such as sex ratio, number of sex reversed fish, and gonadosomatic index (GSI) to detailed morphometric analyses have been considered. Categorisation of the data indicates 2 major groups of chemicals with apparently conserved effects across species, i.e. estrogenic/anti-androgenic or androgenic/anti-estrogenic compounds. Since gross morphological parameters and histological analysis are often the first parameters

measured in field campaigns for sampling of feral fish, the review supports the critical evaluation of present and future field studies and the confirmation or rejection of causative links to exposure with endocrine disrupting chemicals. Furthermore, in combination with the analysis of molecular endpoints the processed data will be useful to deduce mechanistic information on potential endocrine disrupting compounds.

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In fish, sexual determination and differentiation exhibit an enormous variety and plasticity [Francis, 1992]. Multiple ways of determining the sex have been described including sex chromosomal and autosomal coding or dependency on environmental and social factors (see also other reviews in this issue). Hermaphroditism (simultaneous and sequential) is also known for a number of particularly marine teleosts. Furthermore, in some species different modes of inheritance can be present within the same strain. The molecular mechanism underlying sex determination has been identified thus far for only one teleost species with XY-chromosomal inheritance, the medaka (*Oryzias latipes*) [see review of M. Kondo in this issue]. Interestingly, the *dmrt1bY* (or *dmy*) gene is not the master regulating gene in other even closely related spe-

cies [Matsuda et al., 2002; Kondo et al., 2003; Tanaka et al., 2007]. Apart from this variability in triggering sex determination it is anticipated that the downstream sex determination/differentiation cascade shows a much higher conservation of molecular marker genes. Some of the potential candidate genes such as *dmrt1*, *amh* or *sox9* are known to be involved in the sex determination and differentiation cascade of many vertebrates including teleosts [Zarkower, 2001; Morrish and Sinclair, 2002; Scharlt, 2004].

Steroidogenic sex hormones (estrogens and androgens) play an important role in sexual differentiation. They are secreted by the developing gonads and are required to manifest primary and secondary sex characteristics. In contrast to higher vertebrates, treatment of fish with natural hormones can functionally override the genetic sex. This finding stems back from very early experiments by Yamamoto [e.g. 1953] using estrogen or androgen supplemented diets for the induction of functional sex reversal in medaka. The detection of complete sex reversal by hormonal treatments in fish has been of great impact in aquaculture, since one sex is often preferred by breeders for economic reasons [Hunter and Donaldson, 1983].

Bearing in mind the variety and plasticity of sexual determination and differentiation it was not surprising that chemical contaminants were considered as responsible for the unusual occurrence of gonadal intersex conditions or reversed secondary sex characteristics in gonochoristic feral fish, firstly reported in the late 70s in roach (*Rutilus rutilus*, testis with embedded oocytes) [Jafri and Ensor, 1979] and mosquito fish (*Gambusia affinis*, females with male gonopodia) [Howell et al., 1980]. The potential responsible contaminants were later termed endocrine disrupting chemicals (EDCs), i.e. 'exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes' [Kavlock et al., 1996]. Based on the early findings of potential endocrine disruption in the 1970s, systematic field surveys have revealed sexual disruption in fish in many locations that are contaminated by industrial or municipal discharges (table 1).

Intersexuality and reduced gonadal weights have been the most widely reported gonadal aberration presumably caused by exposure to EDCs. However, the natural background of intersex or other sexual aberration is not known for some fish species. In species such as the sea bass, intersex (intratesticular oocytes) is frequently observed and

known for cultured as well as wild fish without any indication that exposure to EDCs could have caused the effect [Saillant et al., 2003]. Furthermore the observation of intersex conditions in several fish species (e.g. eelpout, stickleback, perch) in areas with probably low levels of contamination indicate that a certain degree of intersex in gonochoristic fish could be natural [Gercken and Sordyl, 2002 and citations therein].

Infection with parasites has also been discussed to affect the endocrine status and cause EDC-like effects. For instance, in breams along the river Elbe, distinct rates of infection with the tapeworm *Ligula intestinalis* were observed [Hecker and Karbe, 2005]. The rates of infection were highest at sampling sites that were heavily contaminated with complex mixtures of organic chemicals and metals. Gonadal impairment potentially associated with infection by parasites was also observed in old roach of Finnish brackish waters along the Baltic Sea [Wiklund et al., 1996]. In fish infected by the microsporid *Pleistophora mirandella* a degeneration of ovaries up to complete absence and destruction was observed. Some of the infected fish were hermaphrodites. Thus, other factors than EDCs (which may, however, be related to pollution and/or effect the endocrine status) cannot be excluded to be responsible for the occurrence of sexual disorders in wildlife fish [Hecker and Karbe, 2005]. Hypoxia might be another confounding factor that has recently been reported in a field and laboratory study in the Pensacola bay in Florida [Thomas et al., 2007]. Atlantic croaker (*Micropogonias undulatus*) sampled from hypoxic regions had significantly reduced gonadal weight indices (GSI). Laboratory studies confirmed the hypoxia effect on gonadal growth and indicate that reproduction may have been impaired via hypothalamic serotonin levels. Another laboratory study using the zebrafish as model organism demonstrated that the increased male proportion induced by hypoxia was associated with the downregulation of various genes controlling the synthesis of sex hormones (i.e., 3- β -hydroxysteroid dehydrogenase, *cyp11a*, *cyp19a*, and *cyp19b*) [Shang et al., 2006].

The migratory and nutritional background, the low number of specimens (<20 individuals per site in many studies) and the composition of several age groups in sampled feral fish represent other factors that could impede establishing a significant link to exposure with EDCs. Appropriate reference sites are also difficult to identify. Usually, sites upstream of an effluent discharge or other lakes and rivers with known or anticipated lower contamination levels are selected for control groups.

Table 1. Examples of studies indicating aberrations in gonadal development in feral fish potentially associated with endocrine disruption. The cited studies provide evidence for a link of elevated pollution levels or point sources of discharge to the observed effects in fish which are normally gonochoristic. Keywords describing the gonadal aberration are highlighted in bold.

Field sites	Species	Description	Reference
USA, California, Estuaries of the Gulf of St. Lawrence	Atlantic tomcod (<i>Microgadus tomcod</i>)	Fish were sampled from 5 sites of which 3 were located in the vicinity of paper mills. GSI (males and females) strongly differs between the different sampling sites and is highest at 2 sites receiving paper mill effluents. Increase in GSI is associated with advanced maturity of testis.	Couillard et al., 1999
USA, California, BKME site	White sucker (<i>Catostomus commersoni</i>)	Fish collected from a site receiving primary-treated bleached kraft mill effluent (BKME) exhibited an increased age to maturity, smaller gonads and females failed to show an increase in egg size with age (compared to a reference site).	Munkittrick et al., 1991
USA, California, Lake Ontario	White perch (<i>Morone americana</i>)	Gonadal intersex was observed in male white perch collected from several locations in western Lake Ontario. Intersex was not observed in hatchery-reared white perch or in white perch collected from an uncontaminated reference site.	Kavanagh et al., 2004
Germany, Baltic Sea, North Sea	Eelpout (<i>Zoarces viviparus</i>)	In 1999 a high incidence of fish with a low degree of intersex (testis-ova) was reported for inner coastal sampling sites of the Baltic Sea. No intersexes were reported for an outer coastal sampling site (Darss). Sampling performed 7 years later revealed 15–43% of fish with intersex at all of the previously tested sites and an additional sampling site in the North Sea. Up to 100% of females of sampling sites showed ovarian atresia . It is not clear whether the intersexes could be associated with endocrine disruption. However, reference sites in Sweden show lower incidence of intersex.	Gercken and Sordyl, 2002; Gercken, 2007
Denmark, Rivers	Roach (<i>Rutilus rutilus</i>)	A tendency to an average higher severity of the intersex indices was seen in males from streams impacted by sewage effluent compared to males from reference sites. Infection with the parasite <i>Pleistophora mirandellae</i> , causing degenerative changes in testes and ovaries did not appear to correlate with intersex.	Bjerregaard et al., 2006
Spain, Ebro river	Carp (<i>Cyprinus carpio</i>)	GSI was reduced in male fish of the Ebro river if compared to fish of a reference site in the tributary Segre river. Weak incidence for morphologic alterations has been identified at sampling sites downstream of a sewage treatment plant or in an industrialised area (testis-ova in 1 fish, severe structural changes and necrotic areas were found in 2 fish).	Lavado et al., 2004
Italy, Po river	Barbel (<i>Barbus plebejus</i>)	Intersex fish were found only downstream (8 out of 16 samples) the Lambro tributary, a highly contaminated river draining a heavily industrialised and populated area and receiving mostly untreated waste water.	Viganò et al., 2001
India, Bangalore	Indian major carp (<i>Labeo rohita</i>)	Carps sampled in 2 lakes (Hebbal and Chowkalli) that receive domestic effluents showed structurally abnormal lobes , not observed in cultured fish obtained from a farm.	Prasad and Zutshi, 2007
Japan, Tokio bay	Japanese flounder (<i>Pleuronectes yokohamae</i>)	Three out of 20 male flounders collected in the inner part of Tokyo Bay, showed testes-ova . This intersex condition was not found among males collected from the reference site at Hokkaido.	Hashimoto et al., 2000
The Netherlands, estuaries	Bream (<i>Abramis abramis</i>)	A location near an STP in the river Dommel was reported with the highest incidence of intersex . Some other sites showed significantly altered GSI.	Vethaak et al., 2002, 2005; Gerritsen et al., 2003
South Africa, DDT-sprayed area	Sharptooth catfish (<i>Clarias gariepinus</i>) Tilapia (<i>Oreochromis mossambicus</i>)	Tilapia from a previously DDT-sprayed area showed intersex (males). GSI was reduced (tilapia) or increased (catfish) if compared to the reference site.	Marchand et al., 2008
South Africa, Marais and Rietvlei Dam	Sharptooth catfish (<i>Clarias gariepinus</i>)	Catfish were sampled from a stream that receives effluents from industrial sites, agricultural activities, informal settlements, and municipal treatment plants. 7–8% (n = 100) of the catfish had testis-ova . A putative reference site has not been sampled nor is it known if catfish have a natural background of intersex incidence.	Barnhoorn et al., 2004
Sweden, Lakes and streams in Dalarna	Perch (<i>Perca fluviatilis</i>) Roach (<i>Rutilus rutilus</i>) Brook trout (<i>Salvelinus fontinalis</i>)	Decreased GSI (females) associated with a low percentage of mature female fish was observed in a lake, which receives a stream contaminated by leachate of a public refuse dump. In a later study – after a treatment plant for the leachate has been installed – decreased GSIs could no longer be detected.	Noaksson et al., 2003, 2005, 2001

Table 1 (continued)

Field sites	Species	Description	Reference
Sweden, Baltic Sea	Perch (<i>Perca fluviatilis</i>)	A long-term study over 8–13 years indicated a trend for a decreasing female GSI that is discussed to be associated with environmental factors, e.g. pollutants. The trend was also observed in the reference site.	Hansson et al., 2006
Sweden, Stockholm	Perch (<i>Perca fluviatilis</i>)	Perch in the Stockholm archipelago have been shown to be in bad health condition including an increased frequency of sexually immature females and a low GSI . An upstream and downstream gradient of effects with Stockholm as a point source of pollution was observed.	Linderöth et al., 2006
Sweden, Baltic coast	Eelpout (<i>Zoarces viviparus</i>)	Sex ratios in the viviparous, gonochoristic eelpout of large pulp mill effluents were significantly male biased compared to reference sites. After a temporary shut-down of the paper mill, sex ratios returned to normal levels.	Larsson et al., 2000; Larsson and Forlin, 2002
UK, Tyne and Solway estuary	Flounder (<i>Platichthys flesus</i>)	A high percentage (up to 53%) of flounders from the Tyne estuary showed malformed (non-elongated) testis . These aberrations were not observed at the Solway firth, the control site.	Lye et al., 1997
UK, estuaries	Flounder (<i>Platichthys flesus</i>)	In 2 subsequent studies, vitellogenin levels and the incidence of intersexes were studied in contaminated estuaries. At most locations – including the control site Alde estuary – testis-ova were not present, except in rivers Mersey and Tyne (9–20% and 7%, respectively).	Allen et al., 1999a, b
UK, Nene and Aire rivers	Roach (<i>Rutilus rutilus</i>)	Intersex fish at downstream sites had reduced GSI (intersexes were also observed at reference sites). A large increase in the ovarian area occupied by atretic follicles was observed at sites downstream of STPs. Percentage of cysts with spermatogonia B and spermatocyte A were reduced in males if compared to reference sites.	Jobling et al., 2002a
UK, rivers and lakes	Gudgeon (<i>Gobio gobio</i>)	Gudgeons sampled at various rivers and lakes (including putative control sites) showed a low incidence of intersex . The difference in severity of the intersex is discussed to be associated with discharge of (industrial) effluents.	van Aerle et al., 2001
UK, rivers, lakes and canals	Roach (<i>Rutilus rutilus</i>)	Intersex incidence in roach was increased downstream of sewage treatment plants of various rivers with respect to upstream and control sites. In juvenile fish from 7 UK rivers, the majority of fish had female-like reproductive tracts.	Jobling et al., 1998; Beresford et al., 2004
USA, Mississippi river	Shovelnose sturgeon (<i>Scaphirhynchus platyrhynchus</i>)	Two of seven (29%) mature male shovelnose sturgeons sampled downstream of Saint Louis were found to have testis-ova . In upstream parts of the river which are characterised by significant lower levels of pollution (e.g. organochlorines) no intersex fish were found.	Harshbarger et al., 2000
USA, Oregon, Columbia river	White sturgeon (<i>Acipenser transmontanus</i>)	Fish were sampled behind hydroelectric dams in the Columbia river. The site with highest burden (Bonneville) of various contaminants exhibited a reduced GSI if compared to other sites and the estuary. In contrast to other sites at Bonneville no mature fish could be captured. Two of three fish with intersex were also found at this site.	Feist et al., 2005
USA, Colorado, Boulder creek	White sucker (<i>Catostomus commersoni</i>)	Intersex fish were present downstream of waste water treatment effluent but not at upstream reference sites. The male-to-female ratio was skewed toward females downstream of the WWTP effluent. Abnormalities in gross gonadal morphology, smaller ovaries, delayed follicular maturation and increased asynchrony of oocyte development were noted in females.	Woodling et al., 2006; Vajda et al., 2008
USA, Florida	Mosquitofish (<i>Gambusia holbrooki</i>)	Differences were observed with respect to the gonadal mass between a river (Fenholloway) receiving paper mill effluents and a reference site (Econfina). However, gonadal mass difference showed opposing effects . It was either increased or decreased depending on the sampling time and gender.	Orlando et al., 2007
USA, Nevada	Carp (<i>Cyprinus carpio</i>)	Contamination associated site differences of GSI between fish from Las Vegas Bay and a reference site were observed only in males. The apparent lack of association between contaminant level and gonadal condition in female carp from mildly mesotrophic Lake Mead may indicate a lack of contaminant effects in females or a confounding effect of the higher nutrient loads in the Las Vegas Bay environment.	Patino et al., 2003
USA, Potomac river	Smallmouth bass	Intersexes (testicular oocytes) were predominantly found at sites with the highest human population density and the most farming intensities.	Blazer et al., 2007

However, these sites may show different habitat conditions and food availability which could affect the endocrine status of the fish. Furthermore, as was discussed for whitefish (*Coregonus lavaretus* spp.) from Lake Thun in Switzerland, distinct genetic differences in fish populations from different sites could also be responsible for the occurrence of different levels of gonadal malformations [Bernet et al., 2008].

Changes in the GSI may also represent systemic toxic responses in highly contaminated areas not related to a primary interference of chemical contamination with the endocrine system. However, molecular and biochemical biomarkers such as the induction of the female-specific and estrogen-regulated vitellogenin were often found to be associated with changes in the GSI and other gonadal aberrations in male fish [Tyler et al., 1998; and for a review with focus on model organisms and experimental studies see Scholz and Mayer, 2008]. This indicates that gonadal disorders are likely to be related to an interference with the endocrine system.

In this review, we summarise the experimental evidence that links disruption of the gonadal development in gonochoristic fish to contaminations by EDCs. A comprehensive overview on laboratory studies using water-borne exposures and histopathological analysis is given. Parameters ranging from simple quantitative characteristics such as sex ratio, number of sex reversed fish, and gonadosomatic index (GSI) to detailed morphometric analyses have been considered. Effects of exposure during early phases of differentiation and to mature adults are compared. Furthermore, we discuss the reversibility of effects. Since gross morphological parameters and histological analysis are often the first parameters measured in field campaigns for sampling of feral fish, the review supports the critical evaluation of present and future field studies and the confirmation or rejection of causative links to exposure with endocrine disrupting chemicals.

Data Sources

The review covers journal articles, book chapters and reports published since the first report on EDC effects in feral fish in 1979. The Web of Science (<http://portal.isiknowledge.com/>) and references have been searched for publications (up to September 2008) on endocrine disrupting effects in teleost fish. Combinations of the terms (and trunks of these terms) 'endocrine', 'disruption' and/or 'GSI', 'intersex', 'hermaphroditism' as well as partial or

entire names of hormones following a manual selection of relevant articles have been used for the literature survey. Only in vivo studies using water-borne exposure have been considered. Studies of short-term exposures of a few hours were not considered. This type of exposure can be regarded as environmentally not relevant and has been predominantly used in studies of sex reversal for aquaculture. The lowest concentrations that had an effect on the gonadal structure were extracted to compare the sensitivity between different chemicals. In case only one concentration was tested or the lowest tested concentrations already caused an effect, this was mentioned in the supplementary table S1 (e.g. marked by ' \leq ') (see www.karger.com/doi/10.1159/000223078). Molar concentrations have been converted to $\mu\text{g/l}$.

Field studies indicating a potential link between contamination and gonadal aberrations (table 1) were selected. Generally, only studies were cited, which have sampled control sites as well. If histological data were only qualitatively described, we considered these studies only if strong incidence (non-singular observations) was provided.

In order to allow a better overview of the effects, data were categorised. For the interested reader we provide a table with detailed descriptions as supplementary information (table S1, including tested range of concentrations, exposure time and a brief summary of the study).

Gonadal Development

The gonad consists of 2 major cell lineages, germ cells and the somatic gonadal mesoderm that surrounds the germ cells. Gonadal development can be divided into 2 phases. The first phase is the development of the indifferent gonad, which usually arises as paired structure within the intermediate mesoderm. The formation of the structural and supporting elements of the indifferent gonad is identical in males and females. The second phase is the development of either testis or ovary, which is triggered by the process of sex determination and followed by sex differentiation.

During early gonadal development different strategies exist among gonochoristic teleost species [see also reviews in this issue]. For example, the formation of the indifferent gonad proceeds directly to testis or ovary, e.g. in medaka [Matsuda, 2005]. An alternative mode of gonadal development is that all gonads initially develop undifferentiated ovary-like structures and in half of the population these ovary-like structures degenerate, which

has been described in zebrafish [Uchida et al., 2002; Maack and Segner, 2004; Wang et al., 2007]. This type of gonadal development is termed juvenile hermaphroditism.

Recently, it has been shown that cross-talk between germ cells and gonadal somatic cells is important for sex differentiation of the gonads. Germ cell depleted fish develop into phenotypic males [Slanchev et al., 2005; Kurokawa et al., 2007; Tanaka et al., 2008]. The female-specific aromatase expression in theca cells is lost during the gonadal development without germ cells and male-specific genes are expressed in the gonadal mesoderm cells, e.g. *dmrt1* [Tanaka et al., 2008].

In later stages of gonadal development the endocrine control plays an important role during sex differentiation and this is a complex interaction of the brain-pituitary-gonadal (BPG) axis and reproductive hormones [Devlin and Nagahama, 2002]. Sex steroids have direct effects on germ-cell development and influence other cell types and organs involved in sex differentiation. They are secreted by the developing gonads (within the follicular layer of ovaries or interstitial/Leydig cells in testes) and are required to manifest primary and secondary sex characteristics. Exposure to endogenous steroidal hormones but EDCs as well (for references see tables 3 and 4) are capable of redirecting development partially or completely and functionally to the opposite of the genotypic sex. The type and severity of effect depend on the exposure period, exposure concentration and the compound to which the fish have been exposed.

Mode of Action of EDC Effects on the Reproductive Axis

The definition of EDCs given by Kavlock and colleagues [1996] principally refers to any hormonal system. However, with respect to sexual development, interaction with the BPG axis and reproductive hormones is of primary concern.

In common with all vertebrates, reproduction in teleost fish is under the control of the BPG axis. In seasonally spawning fish external factors such as light and water temperature control the timing of gonad recrudescence and maturation via the brain. Signals from the brain control the hypothalamic secretion of gonadotropin-releasing hormones which stimulate the release of gonadotropins from the pituitary. The gonadotropins in turn control the production and secretion of steroid hormones from the gonads.

Table 2. Examples of studies demonstrating aberrations in gonadal development in fish exposed to effluents in laboratory experiment or in situ exposure (caged fish in rivers or effluents)

Sample	Species	P	SR	IS	TSI	OSI	Sperm.	Oog.	AF	Notes	Reference
Pulp mill effluent	<i>Danio rerio</i>	J	m	x			S			Dilution series of effluents	Örn et al., 2006a
STE-exposure	<i>Danio rerio</i>	J	m								Vethaak et al., 2005
	<i>Rutilus rutilus</i>	J		x						Intersex = feminisation of reproductive duct (no testis-ova)	Rodgers-Gray et al., 2001
	<i>Rutilus rutilus</i>	J		x						Effects observed for undiluted samples only	Liney et al., 2005
	<i>Carassius carassius</i>	A	x	x	R	R	D			Effects observed at 50% dilution or less	Diniz et al., 2005a
	<i>Cyprinus carpio</i>	A	x	x	R	R	D	x			Diniz et al., 2005b
Caging downstream of an STE	<i>Oncorhynchus mykiss</i>	A			R					Compared to upstream site	Harries et al., 1997
Caging experiment in and downstream of paper mill effluent	<i>Oncorhynchus mykiss</i>	A		I				S		Compared to upstream sites	Orrego et al., 2006

A = Exposure restricted to adult fish, AF = atretic follicles, D = delayed, I = increased, IS = intersex (testis-ova, feminised gonads), J = exposure at least during larval and juvenile period, m = unusual male-biased sex ratio, Oog = oogenesis, OSI = ovarian somatic index, P = period of exposure, R = reduced, S = stimulated, Sperm. = spermatogenesis, SR = sex ratio/reversal, STE = sewage treatment effluent, TSI = testicular somatic index, x = induction of the appropriate phenotype in exposed fish has been observed.

Table 3. Effects of (potential) estrogenic and anti-androgenic (flutamide, vinclozoline) chemicals on the development of the teleost gonad. Based on their predominant effect and comparison to the effects of 17- β -estradiol and its most potent agonists, the compounds have been classified as estrogenic. Compounds are ordered according to the lowest observed concentrations that show the effect.

Compound	Species	P	Effect level ($\mu\text{g/l}$)	SR	IS	TSI	OSI	Sperm.	Oog.	AF	PGC	Other effects (number of studies)	Reference
Ethinyl-estradiol	<i>Danio rerio</i>	J	0.001–0.1	ffff	xx			DDS	D	x		No gonads or undifferentiated (2)	Hill and Janz, 2003; Örn et al., 2003, 2006b; Van den Belt et al., 2003; Weber et al., 2003; Maack and Segner, 2004; Nash et al., 2004; Schulz et al., 2007
	<i>Pimephales promelas</i>	J	0.001–0.01	ffff	xxx		R	D	D			Fibrosis in testis (1); no testicular tissue (1)	Lattier et al., 2002; Parrott and Wood, 2002; van Aerle et al., 2002; Parrott and Blunt, 2005
	<i>Gasterosteus aculeatus</i>	J	0.002–0.05	ff	x		R		D				Hahlbeck et al., 2004; Maunder et al., 2007
	<i>Oryzias latipes</i>	J	0.002–0.1	fff	xxx								Scholz and Gutzeit, 2000; Metcalfe et al., 2001; Balch et al., 2004; Örn et al., 2006b
	<i>Rutilus rutilus</i>	J	0.004		x								Katsu et al., 2007
	<i>Cyprinodon variegatus</i>	J	0.002	f	x					x		Testicular fibrosis	Zillioux et al., 2001
	<i>Danio rerio</i>	A	0.008–0.01			RRRR	RRRR	DD	D	x			Van den Belt et al., 2001, 2002, 2004; Versommen et al., 2003
	<i>Pimephales promelas</i>	A	0.01			R	RR	D				Necrotic spermatogonia (1)	Pawlowski et al., 2004b; Filby et al., 2007
	<i>Oryzias latipes</i>	A			x				D				Seki et al., 2002
	<i>Oncorhynchus mykiss</i>	A				R		D					Jobling et al., 1996
17- β -estradiol	<i>Fundulus heteroclitus</i>	A	0.1			I							Peters et al., 2007
	<i>Cyprinus carpio</i>	J	1		x						R		Gimeno et al., 1998
	<i>Danio rerio</i>	J	0.1–0.27	ff	x							Reduced testis cyst size	Wester et al., 2003; Brion et al., 2004
	<i>Oryzias latipes</i>	J	0.01–0.10	ffff	xxx								Nimrod and Benson, 1998; Metcalfe et al., 2001; Knörr and Braunbeck, 2002; Hirai et al., 2006
	<i>Gasterosteus aculeatus</i>	J	1	f	x				D				Hahlbeck et al., 2004
Estrone	<i>Pimephales promelas</i>	A	0.14			R	R	D	D	x	R	Sertoli cell hyperplasia and hypertrophy	Miles-Richardson et al., 1999; Halm et al., 2002
	<i>Danio rerio</i>	A	0.1			R							Brion et al., 2004
	<i>Oryzias latipes</i>	A	0.029	x	x	X						Increased connective tissue in testis	Kang et al., 2002
Eqol	<i>Melanotaenia fluviatilis</i>	A	1							x			Pollino et al., 2007
	<i>Oryzias latipes</i>	J	0.01	f	x								Metcalfe et al., 2001
	<i>Danio rerio</i>	A	1				R						Van den Belt et al., 2004
Diethyl-stilbestrol	<i>Oryzias latipes</i>	J	0.4	f	x			D	D	x		Fibrosis in testis, enlarged ovarian lumen and somatic tissue (1)	Kiparissis et al., 2003
	<i>Gobiocypris rarus</i>	J	0.05	f	x								Zhong et al., 2005
	<i>Oryzias latipes</i>	J	1		x								Metcalfe et al., 2001
Genistein	<i>Oryzias latipes</i>	J	1	f	x			D	D	x		Connective tissue in males and stromal tissue in females enlarged (1)	Scholz and Gutzeit, 2001; Kiparissis et al., 2003
	<i>Pimephales promelas</i>	A	3					D	D	x		Enlarged seminiferous tubules	Kunz et al., 2006
	<i>Oryzias latipes</i>	J	1		x			D	D			Increased testicular fibrosis	Kiparissis et al., 2003
3-benzylidene camphor	<i>Oryzias latipes</i>	J	3	f	x								Metcalfe et al., 2000
	<i>Oryzias latipes</i>	J	20		x	R							Zhang and Hu, 2008
	<i>Melanotaenia fluviatilis</i>	J	5			R							Holdway et al., 2008

4-tert-octylphenol	<i>Danio rerio</i> <i>Oryzias latipes</i>	A J	25 2–100	f xxxx	R R	D x	Increased interstitial tissue in males (1)	Van den Belt et al., 2001 Gray et al., 1999a, b; Scholz and Gutzeit, 2001; Seki et al., 2003 Jobling et al., 1996
4-Nonylphenol	<i>Oncorhynchus mykiss</i> <i>Danio rerio</i> <i>Oryzias latipes</i>	A J J	39 100 11.6–50	R f ff xx	R D D	D x		Hill and Janz, 2003 Gray and Metcalfe, 1997; Seki et al., 2003
	<i>Gambusia holbrooki</i>	J	50		D		Undifferentiated atrophied gonads (0.5 µg/l)	Dréze et al., 2000
	<i>Rivulus marmoratus</i>	J	150	f	D		Abnormal gonadal lumen in testis	Tanaka and Grizzle, 2002
	<i>Danio rerio</i>	A	100–500		RR			Van den Belt et al., 2004; Yang et al., 2006
Nonylphenol-diethoxylate	<i>Oncorhynchus mykiss</i>	A	37		R	D		Jobling et al., 1996
Nonylphenol-carboxylic acid	<i>Oncorhynchus mykiss</i>	A	38		R	D		Jobling et al., 1996
4-tert-pentylphenol	<i>Oncorhynchus mykiss</i>	A	32		R	D		Jobling et al., 1996
Nonylphenol-ethoxylate/octylphenol-ethoxylate	<i>Pimephales promelas</i>	J	76		R			Bistodeau et al., 2006
4-tert-pentylphenol	<i>Pimephales promelas</i> <i>Cyprinus carpio</i>	J J	180 36–320	x x	R R	D RR	Undifferentiated fish Reduced diameter of seminiferous tubules	Panter et al., 2006 Gimeno et al., 1996
	<i>Danio rerio</i>	A	100	x		D		OECD, 2006
	<i>Pimephales promelas</i>	A	100			D		OECD, 2006
	<i>Oryzias latipes</i>	A	1000			D		OECD, 2006
Bisphenol A	<i>Pimephales promelas</i> <i>Oryzias latipes</i>	J J	16 50–1820	x	R R	D DD	Abnormal connective tissue in males (1); loss of testicular structure and increase in interstitial spaces, increase in fibrotic tissue (1)	Sohni et al., 2001 Yokota et al., 2000; Metcalfe et al., 2001
	<i>Cyprinus carpio</i>	A	1	x		x	Loss of typical lobular structure	Mandich et al., 2007
β-HCH	<i>Oryzias latipes</i>	J	100–180	f	x		X adipose tissue in testis	Wester and Canton, 1986; Scholz and Gutzeit, 2001
Flutamide	<i>Danio rerio</i> <i>Danio rerio</i>	A J	500 10	m		D D	Sertoli cell hypertrophy Increased number of interstitial cells, Sertoli cell hypertrophy, increased size of early spermatogonia	OECD, 2006 Wester et al., 2003
	<i>Oryzias latipes</i>	A	500				Decreased number of postovulatory follicles	OECD, 2006
Vinclozoline	<i>Pimephales promelas</i>	A	100		R	D		OECD, 2006; Filby et al., 2007
	<i>Pimephales promelas</i>	A	250–700		I	R	Increased ovarian stages score	Makynen et al., 2000; Martinovic et al., 2008
Benzo-phenone-2	<i>Oryzias latipes</i>	A	2500	x		D	Increased testicular fibrosis	Kiparissis et al., 2003
4-tert-butylphenol	<i>Pimephales promelas</i>	A	1200			D		Weisbrod et al., 2007
p-dichloro-benzene	<i>Cyprinus carpio</i> <i>Danio rerio</i>	A A	2300 10000		R		Histo-architectural changes	Barse et al., 2006 Versnennen et al., 2003

A = Exposure restricted to adult fish, AF = atretic follicles, D = delayed, f = unusual female-biased sex ratio, I = increased, IS = intersex (testis-ova, feminised gonads), J = exposure at least during larval and juvenile period, m = unusual male-biased sex ratio, Oog. = oogenesis, OSI = ovarian somatic index, P = period of exposure, PGC = primordial germ cells, R = reduced, S = stimulated, Sperm. = spermatogenesis, SR = (gonadal) sex ratio and/or sex reversal, TSI = testicular somatic index, x = induction of the appropriate phenotype in exposed fish has been observed. The number of letters indicates the numbers of studies that report the appropriate effect.

Table 4. Effects of (potential) androgenic, anti-estrogenic (fadrozole, tamoxifen, tributyltin, ZM 189,156) and steroid-synthesis inhibiting compounds (ketoconazole) on the development of the teleost gonad. Based on their predominant effect and comparison to the effects of 17- α -methyltestosterone and its most potent agonists, the compounds listed have been classified as androgenic. Compounds are ordered according to the lowest observed concentrations that show the effect.

Compound	Species	P	Effect level ($\mu\text{g/l}$)	Sex ratio	IS	TSI	OSI	Sp	Oo	AF	Other effects (number of studies) and notes	Reference
Tributyltin	<i>Danio rerio</i>	J	0.0001	m							Flagella lacking sperm (1)	McAllister and Kime, 2003
	<i>Fundulus heteroclitus</i>	J	2.1			R		S			Epithelial cells of seminal ducts were shrunken	Mochida et al., 2007
Trenbolone	<i>Danio rerio</i>	J	0.05	m				S				Örn et al., 2006b
	<i>Oryzias latipes</i>	J	0.05					S				Örn et al., 2006b
	<i>Gambusia affinis</i>	J	1		x			S				
	<i>Danio rerio</i>	A	0.05				R					Seki et al., 2006
	<i>Pimephales promelas</i>	A	0.5					S	D	x	Sertoli cell hypertrophy and hyperplasia; in females accumulation of vitellogenic oocytes; at higher concentrations estrogenic effects	Ankley et al., 2003
	<i>Danio rerio</i>	J	0.1	m (f)				S				Wester et al., 2003
	<i>Pimephales promelas</i>	J	0.1	m	x			S	D		Dysplasia in testis	Bogers et al., 2006
17- α -methyl-testosterone	<i>Danio rerio</i>	J	0.1		x			S				Örn et al., 2003
	<i>Gasterosteus aculeatus</i>	J	1	m	x				D		Severe testis abnormalities: large branched cavities	Hahlbeck et al., 2004
	<i>Kryptolebias marmoratus</i>	J	5	m								Kanamori et al., 2006
	<i>Pimephales promelas</i>	A	0.1				R			x		Pawlowski et al., 2004a
Ketoconazole	<i>Pimephales promelas</i>	A	6			I	I				Increased proliferation of Leydig cells	Ankley et al., 2007
Fadrozole	<i>Danio rerio</i>	J	10	m								Andersen et al., 2004
	<i>Pimephales promelas</i>	J	24.8			I	R					OECD, 2006
	<i>Danio rerio</i>	A	100					S	D		Interstitial fibrosis; oocyte membrane folding affected	OECD, 2006
	<i>Pimephales promelas</i>	A	50–100			I	R	S	D		Sertoli cell hypertrophy and increased number of interstitial cells (2)	OECD, 2006; Panter et al., 2004
	<i>Oryzias latipes</i>	A	20						D	x	Increased number of interstitial cells	OECD, 2006
Tamoxifen	<i>Danio rerio</i>	J	32						D	x	Increased number of Leydig cells and granulosa cell height; in males asynchronous meiotic maturation	Wester et al., 2003
Diazonin	<i>Lepomis macrochirus</i>	A	60							x	Paralleled by reduced estradiol serum levels	Maxwell and Dutta, 2005
ZM 189,156	<i>Danio rerio</i>	J	100	m							Undifferentiated fish	Andersen et al., 2004
Letrozole	<i>Oryzias latipes</i>	A	125			I	I	S	D		Enlarged lumen of seminiferous tubules	Sun et al., 2007
Prochloraz	<i>Oryzias latipes</i>	A	20						D		Increased number of interstitial cells	OECD, 2006
	<i>Pimephales promelas</i>	A	300							x	Increased number of late and post-vitellogenic oocytes	OECD, 2006
	<i>Danio rerio</i>	A	20–202		x			SS	D		Interstitial fibrosis, altered oocyte membrane folding; occurrence of undifferentiated gonads	OECD, 2006

A = exposure restricted to adult fish, AF = atretic follicles, D = delayed, f = unusual female-biased sex ratio, I = increased, IS = intersex (testis-ova, feminised gonads), J = exposure at least during larval and juvenile period, m = unusual male-biased sex ratio, Oo = oogenesis, OSI = ovarian somatic index, P = Period of exposure, R = reduced, S = stimulated, Sp = spermatogenesis, TSI = testicular somatic index, x = induction of the appropriate phenotype in exposed fish has been observed. The number of letters indicates the numbers of studies that report the appropriate effect.

These hormones (17- β estradiol and testosterone in females, and testosterone and predominantly 11-ketotestosterone in males) initiate changes in secondary sex characteristics, behaviour as well as development and maturation of gametes. Via negative or positive feedback mechanisms, depending on the physiological stage, reproductive steroids regulate the secretion of pituitary gonadotropins [Peter and Yu, 1997; Schulz and Goos, 1999].

The BPG axis provides several potential modes of interaction for EDCs by either (1) mimicking steroid hormones, (2) modulating steroid synthesis, transport, and catabolism, and (3) by influencing the neuroendocrine system and the involved regulatory feedback mechanisms. Eventually, endocrine disrupting effects are often mediated via the available level of steroid hormones or their analogues and the effects resemble those provoked by natural estrogens and androgens.

Experimental Evidence of EDC Effects on Gonadal Development

Experimental evidence for chemicals as the primary cause for the occurrence of gonadal malformations in the environment is provided by exposure experiments with caged fish (in situ exposure), fish exposed to effluents under laboratory conditions (table 2) or by exposure of fish to single putative EDCs (tables 3 and 4). Results obtained from exposure to reference compounds (tables 3 and 4), i.e. natural reproductive steroidogenic hormones and their agonists/antagonists, are particularly helpful to elucidate the effect of complex environmental samples or unknown compounds. According to these patterns, effects observed for exposure to STE (sewage treatment effluent) from municipal sources, appear to be predominantly estrogenic. Exposures to paper mill effluents exhibit overlapping effects or are androgenic. Hence, these data confirm earlier findings of masculinisation of secondary sex characters (formation of a gonopodium) in female mosquitofish living downstream of paper mill discharges [Howell et al., 1980; Orlando et al., 2007] or male biased sex-ratios near paper mills [Larsson and Forlin, 2002].

The major morphological aberrations reveal that estrogenic or anti-androgenic and androgenic or anti-estrogenic compounds, respectively, exhibit very similar effects. Gonadal development in fish exposed to estrogenic chemicals is characterised by sex-reversal to males (or male biased population), intersex (primarily testis-

ova or feminised seminiferous ducts), reduction in GSI and delayed gametogenesis (tables 3 and 4). An increase in the number of spermatogonia or previtellogenic early oocytes and a decrease in spermatocytes, mature sperm or vitellogenic oocytes indicate this delay. Furthermore, an increased number of atretic oocytes and a reduced number of primordial germ cells are frequently observed (table 3). Only 2 studies revealed deviations (one for ethinylestradiol showing an increased testicular somatic index, the other for bisphenol A with increased ovarian somatic index). The observed patterns appear also to be very consistent among different species. Interestingly, androgenic compounds show very similar effects, including sex reversal (male biased sex proportions, respectively), intersex, delayed oocyte development and occurrence of atretic follicles as well. The major difference to estrogenic compounds is, however, the stimulation of spermatogenesis which clearly marks an androgenic effect. Furthermore, an increased number of interstitial/Leydig cells or Sertoli cell hypertrophy is frequently observed. Few of the listed compounds show deviations from the general patterns, namely, fadrozole, ketoconazole and letrozole. These deviations might be explained by their mechanism of action. Fadrozole and letrozole are both aromatase inhibitors. Thus, they reduce the level of estrogens and increase the levels of androgens. The increased testicular weight was not described for androgenic compounds and may result from an interaction with the hypothalamic/pituitary feedback regulation by androgens and estrogens [OECD, 2006]. Ketoconazole is neither an estrogen nor androgen (or an antagonist, respectively) but a systemic inhibitor of steroidogenesis. Hence, it reduces the level of androgens and estrogens. This may explain – in contrast to other compounds – the stimulation of ovarian weight. It has been shown that FSH levels were increased in ketoconazole-exposed fathead minnows [Villeneuve et al., 2007] indicating that interference with the feedback regulation of gonadotropins was involved in mediating the observed effects on gonadal development. Thus, the different patterns of gonadal aberrations may allow postulating first hypotheses on the mode of action of unknown compounds.

Relevance of Exposure Periods

Interaction with gonadal development can be identified not only for exposure during critical time windows of early development. Intersex, altered GSI and other effects have also been reported for experiments with exposure restricted to adult fish. This confirms early findings that even the adult gonad retains some degree of bipo-

tentiality [Shibata and Hamaguchi, 1988]. However, sex reversal is dependent on an interaction of EDCs during larval or juvenile development referring to the important and environmentally relevant exposure of early life stages.

The reversibility of effects following a recovery period is reported in some studies [Hill and Janz, 2003; Seki et al., 2003; Van den Belt et al., 2003] although the interference with reproductive capacity observed in parallel may not be restored to control levels [Maack and Segner, 2004; Nash et al., 2004; Fenske et al., 2005]. However, examples (for fish exposed during early development) can be found in which intersex gonads or other gonadal disorders are retained also after relatively long recovery periods [Maunder et al., 2007]. Particularly severe effects such as sex reversal are permanent [Scholz and Gutzeit, 2000]. This might be different for sequential hermaphroditic fish [Francis, 1992], which are not considered in this review.

Relevance with Respect to Environmental Concentrations

Many of the tested compounds, e.g. ethinylestradiol, β -HCH, several alkylphenols, bisphenol A and DDT-derivatives, are known to be frequent contaminants in effluents, surface and marine waters. The concentration levels of non-steroidogenic compounds for typical estrogen or androgen-related effects in gonadal development are several orders of magnitude above those of natural steroid hormones and potent agonists. Effect levels of individual compounds can be close to or well above environmental concentrations. For instance, the very potent estrogen agonist ethinylestradiol is generally reported to occur below 1 ng/l or analytical detection limits. Only in effluents of sewage treatment works it may reach concentrations that are known to provoke the development of gonadal disorders [Heberer, 2002]. 4-Nonylphenol concentrations were found to be generally below 1 μ g/l, but could reach more than 100 μ g/l in a few surface waters and STEs [Blackburn and Waldock, 1995]. This concentration is close to the lowest observed effect concentrations reported for changes in sex reversal or intersex incidence. Bisphenol A is detected in the ng/l range and μ g/l concentrations are reached only in exceptional cases [Belfroid et al., 2002]. However, environmental samples consist of a mixture of many compounds bearing the potential to lead to additive or synergistic interaction with the endocrine system. Furthermore, natural estrogens may provide the major contribution to estrogenic effects [Desbrow et al., 1998].

Particularly in densely populated regions, they reach aquatic surface waters due to excretion via human urine and are detected in significant concentrations [Hohenblum et al., 2004].

Conclusions

Reproductive performance of fish with gonadal aberrations such as ovotestis can be reduced [Jobling et al., 2002a, b]. Thus, monitoring of structural changes in feral fish can indicate potential effects on the reproduction and survival of populations. Field studies provide many examples with a probable link between alterations in gonadal disorders and exposure to chemicals introduced by anthropogenic activity. Comparison of the effect levels of histological alterations with molecular and reproductive endpoints in model organisms reveals the different endpoints to exhibit a similar sensitivity [Scholz and Mayer, 2008]. Although molecular endpoints are of similar predictivity and easier to analyse they may not be available for less characterised feral fish. Furthermore, the expression of molecular markers can be anticipated to be related to the short-term exposure condition. In contrast, histological data can be regarded as a more integrative parameter in an environment with fluctuating exposure concentrations. The great strength of histological analysis is, however, the combined analysis with molecular markers. Together, they are able to deduce mechanistic information and to deliver important information for the risk assessment of chemical contamination.

References

- 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* 233:5–20 (1999a).
- 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 effects on gonadal development of the flounder *Platichthys flesus*. *Environ Toxicol Chem* 18:1791–1800 (1999b).
- Andersen L, Kinnberg K, Holbech H, Korsgaard B, Bjerregaard P: Evaluation of a 40 day assay for testing endocrine disruptors: Effects of an anti-estrogen and an aromatase inhibitor on sex ratio and vitellogenin concentrations in juvenile zebrafish (*Danio rerio*). *Fish Physiol Biochem* 30:257–266 (2004).

- Ankley GT, Jensen KM, Makynen EA, Kahl MD, Korte JJ, et al: Effects of the androgenic growth promoter 17-beta-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. *Environ Toxicol Chem* 22:1350–1360 (2003).
- Ankley GT, Jensen KM, Kahl MD, Makynen EA, Blake LS, et al: Ketoconazole in the fathead minnow (*Pimephales promelas*): Reproductive toxicity and biological compensation. *Environ Toxicol Chem* 26:1214–1223 (2007).
- Balch GC, Mackenzie CA, Metcalfe CD: Alterations to gonadal development and reproductive success in Japanese medaka (*Oryzias latipes*) exposed to 17a-ethinylestradiol. *Environ Toxicol Chem* 23:782–791 (2004).
- Barnhoorn I, Bornman M, Pieterse G, van Vuren J: Histological evidence of intersex in feral sharp-tooth catfish (*Clarias gariepinus*) from an estrogen-polluted water source in Gauteng, South Africa. *Environ Toxicol* 19:603–608 (2004).
- Barse A, Chakrabarti T, Ghosh TK, Pal AK, Jadhao SB: One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in *Cyprinus carpio*. *Pestic Biochem Physiol* 86:172–179 (2006).
- Belfroid A, van Velzen M, van der Horst B, Vethaak D: Occurrence of bisphenol A in surface water and uptake in fish: evaluation of field measurements. *Chemosphere* 49:97–103 (2002).
- Beresford N, Jobling S, Williams R, Sumpter J: Endocrine disruption in juvenile roach from English rivers: a preliminary study. *J Fish Biol* 64:580–586 (2004).
- Bernet D, Liedtke A, Bittner D, Eggen RIL, Kipfer S, et al: Gonadal malformations in whitefish from Lake Thun: Defining the case and evaluating the role of EDCs. *Chimia* 62:383–388 (2008).
- Bistodeau TJ, Barber LB, Bartell SE, Cediell RA, Grove KJ, et al: Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. *Aquat Toxicol* 79:268–277 (2006).
- Bjerregaard LB, Korsgaard B, Bjerregaard P: Intersex in wild roach (*Rutilus rutilus*) from Danish sewage effluent-receiving streams. *Ecotoxicol Environ Safe* 64:321–328 (2006).
- Blackburn MA, Waldock MJ: Concentrations of alkylphenols in rivers and estuaries in England and Wales. *Water Res* 29:1623–1629 (1995).
- Blazer VS, Iwanowicz LR, Iwanowicz DD, Smith DR, Young JA, et al: Intersex (testicular oocytes) in smallmouth bass from the Potomac River and selected nearby drainages. *J Aquat Animal Health* 19:242–253 (2007).
- Bogers R, De Vries-Buitenweg S, Van Gils M, Baltussen E, Hargreaves A, et al: Development of chronic tests for endocrine active chemicals – Part 2: An extended fish early-life stage test with an androgenic chemical in the fathead minnow (*Pimephales promelas*). *Aquat Toxicol* 80:119–130 (2006).
- Brion F, Tyler CR, Palazzi X, Laillet B, Porcher JM, et al: Impacts of 17 beta-estradiol, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in zebrafish (*Danio rerio*). *Aquat Toxicol* 68:193–217 (2004).
- Couillard CM, Williams PJ, Courtenay SC, Rawn GP: Histopathological evaluation of Atlantic tomcod (*Microgadus tomcod*) collected at estuarine sites receiving pulp and paper mill effluent. *Aquat Toxicol* 44:263–278 (1999).
- Desbrow C, Routledge EJ, Brighty G, Sumpter JP, Waldock M: Identification of estrogenic chemicals in STW effluent. I. Chemical fractionation and in vitro biological screening. *Environ Sci Technol* 32:1549–1558 (1998).
- Devlin RH, Nagahama Y: Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* 208:191 (2002).
- Diniz MS, Peres I, Magalhaes-Antoine I, Falla J, Pihan JC: Estrogenic effects in crucian carp (*Carassius carassius*) exposed to treated sewage effluent. *Ecotoxicol Environ Safe* 62:427–435 (2005a).
- Diniz MS, Peres I, Pihan JC: Comparative study of the estrogenic responses of mirror carp (*Cyprinus carpio*) exposed to treated municipal sewage effluent (Lisbon) during two periods in different seasons. *Sci Total Environ* 349:129–139 (2005b).
- Dréze V, Monod G, Cravedi J-P, Biagianti-Risbourg S, Le Gac F: Effects of 4-nonylphenol on sex differentiation and puberty in mosquitofish (*Gambusia holbrooki*). *Ecotoxicology* 9:93–103 (2000).
- Feist GW, Webb MAH, Gundersen DT, Foster EP, Schreck CB, et al: Evidence of detrimental effects of environmental contaminants on growth and reproductive physiology of white sturgeon in impounded areas of the Columbia River. *Environ Health Perspect* 113:1675–1682 (2005).
- Fenske M, Maack G, Schafers C, Segner H: An environmentally relevant concentration of estrogen induces arrest of male gonad development in zebrafish, *Danio rerio*. *Environ Toxicol Chem* 24:1088–1098 (2005).
- Filby AL, Thorpe KL, Maack G, Tyler CR: Gene expression profiles revealing the mechanisms of anti-androgen- and estrogen-induced feminization in fish. *Aquat Toxicol* 81:219–231 (2007).
- Francis RC: Sexual lability in teleosts: developmental factors. *Quart Rev Biol* 67:1–18 (1992).
- Gercken J: Biologisches Effektmonitoring mit Aalmuttern aus Nord- und Ostsee – Histologische Gonadenuntersuchung. Report for the German Federal Environmental Agency (UBA). FKZ 301 02 023 (2007).
- Gercken J, Sordyl H: Intersex in feral marine and freshwater fish from northeastern Germany. *Mar Environ Res* 54:651–655 (2002).
- Gerritsen AAM, Rijs GBJ, Klein Breteler JGP, Lahr J: Estrogenic effects in fishes in regional waters (Oestrogene effecten in vissen in regionale wateren). Report no. 2003.019. RIZA – Dutch National Institute of Inland Water Management and Waste Water Treatment, Lelystad, the Netherlands (in Dutch) (2003).
- Gimeno S, Gerritsen A, Bowmer T, Komen H: Feminization of male carp. *Nature* 384:212–222 (1996).
- Gimeno S, Komen H, Gerritsen A, Bowmer T: Feminisation of young males of the common carp, *Cyprinus carpio*, exposed to 4-tert-pentylphenol during sexual differentiation. *Aquat Toxicol* 43:77–92 (1998).
- Gray MA, Metcalfe CD: Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to p-nonylphenol. *Environ Toxicol Chem* 16:1082–1086 (1997).
- Gray MA, Niimi AJ, Metcalfe CD: Factors affecting the development of testis-ova in medaka, *Oryzias latipes*, exposed to octylphenol. *Environ Toxicol Chem* 18:1835–1842 (1999a).
- Gray MA, Teather KL, Metcalfe CD: Reproductive success and behavior of Japanese medaka (*Oryzias latipes*) exposed to 4-tert-octylphenol. *Environ Toxicol Chem* 18:2587–2594 (1999b).
- Hahlbeck E, Griffiths R, Bengtsson B-E: The juvenile three-spined stickleback (*Gasterosteus aculeatus* L.) as a model organism for endocrine disruption: I. Sexual differentiation. *Aquat Toxicol* 70:287 (2004).
- Halm S, Pounds N, Maddix S, Rand-Weaver M, Sumpter JP, et al: Exposure to exogenous 17 beta-estradiol disrupts P450aromB mRNA expression in the brain and gonad of adult fathead minnows (*Pimephales promelas*). *Aquat Toxicol* 60:285–299 (2002).
- Hansson T, Lindesjö E, Förlin L, Balk L, Bignert A, Larsson Å: Long-term monitoring of the health status of female perch (*Perca fluviatilis*) in the Baltic Sea shows decreased gonad weight and increased hepatic EROD activity. *Aquat Toxicol* 79:341–355 (2006).
- Harries JE, Sheahan DA, Jobling S, Matthiessen P, Neali P, et al: Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environ Toxicol Chem* 16:534–542 (1997).
- Harshbarger JC, Coffey MJ, Young MY: Intersexes in Mississippi River shovelnose sturgeon sampled below Saint Louis, Missouri, USA. *Mar Environ Res* 50:247–250 (2000).

- 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* 49:37–53 (2000).
- Heberer T: Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol Lett* 131:5–17 (2002).
- Hecker M, Karbe L: Parasitism in fish – an endocrine modulator of ecological relevance? *Aquat Toxicol* 72:195–207 (2005).
- Hill JRL, Janz DM: Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. Effects on sex ratio and breeding success. *Aquat Toxicol* 63:417–429 (2003).
- Hirai N, Nanba A, Koshio M, Kondo T, Morita M, Tatarazako N: Feminization of Japanese medaka (*Oryzias latipes*) exposed to 17[beta]-estradiol: formation of testis-ova and sex-transformation during early-ontogeny. *Aquat Toxicol* 77:78–86 (2006).
- Hohenblum P, Gans O, Moche W, Scharf S, Lorbeer G: Monitoring of selected estrogenic hormones and industrial chemicals in groundwaters and surface waters in Austria. *Sci Total Environ* 333:185–193 (2004).
- Holdway DA, Heffernan J, Smith A: Multigeneration assessment of nonylphenol and endosulfan using a model Australian freshwater fish, *Melanotaenia fluviatilis*. *Environ Toxicol* 23:253–262 (2008).
- Howell WM, Black D, Bortone SA: Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence for environmentally-induced masculinization. *Copeia* 1980:676–681 (1980).
- Hunter GA, Donaldson EM: Hormonal sex control and its application to fish culture, in Hoar WS, Randall DJ, Donaldson EM (eds): *Fish Physiology*, Vol 9, Reproduction, Part B, Behavior and Fertility Control, pp 223–303 (Academic Press, New York 1983).
- Jafri SIH, Ensor DM: Occurrence of an intersex condition in the roach *Rutilus rutilus* (L.). *J Fish Biol* 14:547–549 (1979).
- Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP: Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to environmental estrogens. *Environ Toxicol Chem* 15:194–202 (1996).
- Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP: Widespread sexual disruption in wild fish. *Environ Sci Technol* 32:2498–2506 (1998).
- Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty GC, et al: Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. *Biol Reprod* 66:272–281 (2002a).
- Jobling S, Coey S, Whitmore JG, Kime DE, Van Look KJW, et al: Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Biol Reprod* 67:515–524 (2002b).
- Kanamori A, Yarnamura A, Koshiba S, Lee JS, Orlando EF, Hori H: Methyltestosterone efficiently induces male development in the self-fertilizing hermaphrodite fish, *Kryptolebias marmoratus*. *Genesis* 44:495–503 (2006).
- Kang I, Yokota H, Oshima Y, Tsuruda Y, Yamaguchi T, et al: Effect of 17beta-estradiol on the reproduction of Japanese medaka (*Oryzias latipes*). *Chemosphere* 47:71–80 (2002).
- Katsu Y, Lange A, Urushitani H, Ichikawa R, Paull GC, et al: Functional associations between two estrogen receptors, environmental estrogens, and sexual disruption in the roach (*Rutilus rutilus*). *Environ Sci Technol* 41:3368–3374 (2007).
- Kavanagh RJ, Balch GC, Kiparissis Y, Niimi AJ, Sherry J, et al: Endocrine disruption and altered gonadal development in white perch (*Morone americana*) from the lower Great Lakes region. *Environ Health Perspect* 112:898–902 (2004).
- Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, et al: Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ Health Perspect* 104 Suppl 4:715–740 (1996).
- Kiparissis Y, Balch GC, Metcalfe TL, Metcalfe CD: Effects of the isoflavones genistein and equol on the gonadal development of Japanese medaka *Oryzias latipes*. *Environ Health Perspect* 111:1158–63 (2003).
- Knörr S, Braunbeck T: Decline in reproductive success, sex reversal, and developmental alterations in Japanese medaka (*Oryzias latipes*) after continuous exposure to octylphenol. *Ecotoxicol Environ Safe* 51:187–196 (2002).
- Kondo M, Nanda I, Hornung U, Asakawa S, Shimizu N, et al: Absence of the candidate male sex-determining gene *dmrt1b(Y)* of medaka from other fish species. *Curr Biol* 13:416–420 (2003).
- Kunz PY, Gries T, Fent K: The ultraviolet filter 3-benzylidene camphor adversely affects reproduction in fathead minnow (*Pimephales promelas*). *Toxicol Sci* 93:311–321 (2006).
- Kurokawa H, Saito D, Nakamura S, Katoh-Fukui Y, Ohta K, et al: Germ cells are essential for sexual dimorphism in the medaka gonad. *Proc Natl Acad Sci USA* 104:16958–16963 (2007).
- Larsson DG, Forlin L: Male-biased sex ratios of fish embryos near a pulp mill: temporary recovery after a short-term shutdown. *Environ Health Perspect* 110:739–742 (2002).
- Larsson DGJ, Hällman H, Förlin L: More male fish embryos near a pulp mill. *Environ Toxicol Chem* 19:2911–2917 (2000).
- Lattier D, Reddy T, Gordon D, Lazorchak JM, Smith M, et al: 17alpha-ethynylestradiol induced vitellogenin gene transcription quantified in livers of adult males, larvae, and gills of fathead minnows (*Pimephales promelas*). *Environ Toxicol Chem* 21:2385–2393 (2002).
- Lavado R, Thibaut R, Raldua D, Martin R, Porte C: First evidence of endocrine disruption in feral carp from the Ebro River. *Toxicol Appl Pharmacol* 196:247–257 (2004).
- Linderoth M, Hansson T, Liewenborg B, Sundberg H, Noaksson E, et al: Basic physiological biomarkers in adult female perch (*Perca fluviatilis*) in a chronically polluted gradient in the Stockholm recipient (Sweden). *Mar Pollut Bull* 53:437–450 (2006).
- Liney KE, Jobling S, Shears JA, Simpson P, Tyler CR: Assessing the sensitivity of different life stages for sexual disruption in roach (*Rutilus rutilus*) exposed to effluents from wastewater treatment works. *Environ Health Perspect* 113:1299–1307 (2005).
- 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* 34:34–41 (1997).
- Maack G, Segner H: Life-stage-dependent sensitivity of zebrafish (*Danio rerio*) to estrogen exposure. *Comp Biochem Physiol C* 139:47–55 (2004).
- Makynen EA, Kahl MD, Jensen KM, Tietje JE, Wells KL, et al: Effects of the mammalian anti-androgen vinclozolin on development and reproduction of the fathead minnow (*Pimephales promelas*). *Aquat Toxicol* 48:461–475 (2000).
- Mandich A, Bottero S, Benfenati E, Cevasco A, Erratico C, et al: In vivo exposure of carp to graded concentrations of bisphenol A. *Gen Comp Endocrinol* 153:15–24 (2007).
- Marchand MJ, Pieterse GM, Barnhoorn IEJ: Preliminary results on sperm motility and testicular histology of two feral fish species, *Oreochromis mossambicus* and *Clarias gariepinus*, from a currently DDT-sprayed area, South Africa. *J Appl Ichthyol* 24:423–429 (2008).
- Martinovic D, Blake LS, Durhan EJ, Greene KJ, Kahl MD: Reproductive toxicity of vinclozolin in the fathead minnow: confirming an anti-androgenic mode of action. *Environ Toxicol Chem* 27:478–488 (2008).
- Matsuda M: Sex determination in the teleost medaka, *Oryzias latipes*. *Annu Rev Genet* 39:293–307 (2005).
- Matsuda M, Nagahama Y, Shinomiya A, Sato T, Matsuda C, et al: *DMY* is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* 417:559–563 (2002).

- Maunder RJ, Matthiessen P, Sumpter JP, Pottinger TG: Impaired reproduction in three-spined sticklebacks exposed to ethinyl estradiol as juveniles. *Biol Reprod* 77:999–1006 (2007).
- Maxwell LB, Dutta HM: Diazinon-induced endocrine disruption in bluegill sunfish, *Lepomis macrochirus*. *Ecotoxicol Environ Safe* 60:21–27 (2005).
- McAllister BG, Kime DE: Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinisation and irreversible sperm damage in zebrafish (*Danio rerio*). *Aquat Toxicol* 65:309–316 (2003).
- Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, 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* 20:297–308 (2001).
- 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* 19:1893–1900 (2000).
- Miles-Richardson SR, Kramer VJ, Fitzgerald SD, Render JA, Yamini B, et al: Effects of waterborne exposure of 17 beta-estradiol on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelas*). *Aquat Toxicol* 47:129–145 (1999).
- Mochida K, Ito K, Kono K, Onduka T, Kakuno A, Fujii K: Molecular and histological evaluation of tributyltin toxicity on spermatogenesis in a marine fish, the mummichog (*Fundulus heteroclitus*). *Aquat Toxicol* 83:73–83 (2007).
- Morrish BC, Sinclair AH: Vertebrate sex determination: many means to an end. *Reproduction* 124:447–457 (2002).
- 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* 48:1371–1380 (1991).
- Nash JP, Kime DE, Van der Ven LTM, Wester PW, Brion F, et al: Long-term exposure to environmental concentrations of the pharmaceutical ethinylestradiol causes reproductive failure in fish. *Environ Health Perspect* 112:1725–1733 (2004).
- Nimrod AC, Benson WH: Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens. *Aquat Toxicol* 44:141–156 (1998).
- Noaksson E, Tjärnlund U, Bosveld ATC, 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* 174:160–176 (2001).
- Noaksson E, Linderöth M, Bosveld ATC, Norrgren L, Zebühr Y, Balk L: Endocrine disruption in brook trout (*Salvelinus fontinalis*) exposed to leachate from a public refuse dump. *Sci Total Environ* 305:87–103 (2003).
- Noaksson E, Linderöth M, Tjärnlund U, Balk L: Toxicological effects and reproductive impairments in female perch (*Perca fluviatilis*) exposed to leachate from Swedish refuse dumps. *Aquat Toxicol* 75:162–177 (2005).
- OECD: Report of the validation of the 21-day fish screening assay for the detection of endocrine substances (phase 1b), series on testing and assessment, Number 61, ENV/JM/MONO(2006)29 (2006).
- Orlando EF, Bass DE, Caltabiano LM, Davis WP, Gray LE, Guillette LJ: Altered development and reproduction in mosquitofish exposed to pulp and paper mill effluent in the Fenholway River, Florida USA. *Aquat Toxicol* 84:399–405 (2007).
- Örn 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* 65:397–411 (2003).
- Örn S, Svensson A, Viktor T, Holbech H, Norrgren L: Male-biased sex ratios and vitellogenin induction in zebrafish exposed to effluent water from a Swedish pulp mill. *Arch Environ Contam Toxicol* 51:445–451 (2006a).
- Örn S, Yamani S, Norrgren L: Comparison of vitellogenin induction, sex ratio, and gonad morphology between zebrafish and Japanese medaka after exposure to 17-alpha-ethinylestradiol and 17beta-trenbolone. *Arch Environ Contam Toxicol* 51:237–243 (2006b).
- Orrego R, Burgos A, Moraga-Cid G, Inzunza B, Gonzalez M, et al: Effects of pulp and paper mill discharges on caged rainbow trout (*Oncorhynchus mykiss*): Biomarker responses along a pollution gradient in the Biobio River, Chile. *Environ Toxicol Chem* 25:2280–2287 (2006).
- Panther GH, Hutchinson TH, Hurd KS, Sherren A, Stanley RD, Tyler CR: Successful detection of (anti-) androgenic and aromatase inhibitors in pre-spawning adult fathead minnows (*Pimephales promelas*) using easily measured endpoints of sexual development. *Aquat Toxicol* 70:11–21 (2004).
- Panther GH, Hutchinson TH, Hurd KS, Bamforth J, Stanley RD, et al: Development of chronic tests for endocrine active chemicals Part 1. An extended fish early-life stage test for oestrogenic active chemicals in the fathead minnow (*Pimephales promelas*). *Aquat Toxicol* 77:279–90 (2006).
- Parrott JL, Blunt BR: Life-cycle exposure of fathead minnows (*Pimephales promelas*) to an ethinylestradiol concentration below 1 ng/l reduces egg fertilization success and demasculinizes males. *Environ Toxicol* 20:131–141 (2005).
- Parrott JL, Wood CS: Fathead minnow lifecycle tests for detection of endocrine-disrupting substances in effluents. *Water Qual Res J Can* 37:651–667 (2002).
- Patino R, Goodbred SL, Draugelis-Dale R, Barry CE, Foott JS, et al: Morphometric and histopathological parameters of gonadal development in adult common carp from contaminated and reference sites in Lake Mead, Nevada. *J Aquat Animal Health* 15:55–68 (2003).
- Pawlowski S, Sauer A, Shears JA, Tyler CR, Braunbeck T: Androgenic and estrogenic effects of the synthetic androgen 17 alpha-methyltestosterone on sexual development and reproductive performance in the fathead minnow (*Pimephales promelas*) determined using the gonadal recrudescence assay. *Aquat Toxicol* 68:277–291 (2004a).
- Pawlowski S, van Aerle R, Tyler CR, Braunbeck T: Effects of 17 alpha-ethinylestradiol in a fathead minnow (*Pimephales promelas*) gonadal recrudescence assay. *Ecotoxicol Environ Safe* 57:330–345 (2004b).
- Peter RE, Yu KL: Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Rev Fish Biol Fisheries* 7:173–197 (1997).
- Peters REM, Courtenay SC, Cagampan S, Hewitt ML, MacLatchy DL: Effects on reproductive potential and endocrine status in the mummichog (*Fundulus heteroclitus*) after exposure to 17 alpha-ethinylestradiol in a short-term reproductive bioassay. *Aquat Toxicol* 85:154–166 (2007).
- Pollino CA, Georgiades E, Holdway DA: Use of the Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) as a model test species for investigating the effects of endocrine disruptors. *Environ Toxicol Chem* 26:2171–2178 (2007).
- Prasad S, Zutshi B: Alterations in structural morphology of brain and ovary in *Labeo rohita* (ham.) from lakes of Bangalore. *J Eco-physiol Occup Health* 7:1 (2007).
- Rodgers-Gray TP, Jobling S, Kelly C, Morris S, Brighty G, 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* 35:462–470 (2001).
- Saillant E, Chatain B, Menu B, Fauvel C, Vidal MO, Fostier A: Sexual differentiation and juvenile intersexuality in the European sea bass (*Dicentrarchus labrax*). *J Zool* 260:53–63 (2003).
- Schartl M: Sex chromosome evolution in non-mammalian vertebrates. *Curr Opin Genet Dev* 14:634–641 (2004).
- Scholz S, Gutzeit HO: 17-alpha-ethinylestradiol affects reproduction, sexual differentiation and aromatase gene expression of the medaka (*Oryzias latipes*). *Aquat Toxicol* 50:363–373 (2000).

- Scholz S, Gutzeit HO: Lasting effects of xeno- and phytoestrogens on sex differentiation and reproduction of fish. *Environ Sci* 8:57–73 (2001).
- Scholz S, Mayer I: Molecular biomarkers of endocrine disruption in small model fish. *Mol Cell Endocrinol* 293:57–70 (2008).
- Schulz RW, Goos HJT: Puberty in male fish: concepts and recent developments with special reference to the African catfish (*Clarias gariepinus*). *Aquaculture* 177:5–12 (1999).
- Schulz RW, Bogerd J, Male R, Ball J, Fenske M, et al: Estrogen-induced alterations in *amh* and *dmrt1* expression signal for disruption in male sexual development in the zebrafish. *Environ Sci Technol* 41:6305–6310 (2007).
- Seki M, Yokota H, Matsubara H, Tsuruda Y, Maeda N, et al: Effect of ethinylestradiol on the reproduction and induction of vitellogenin and testis-ova in medaka (*Oryzias latipes*). *Environ Toxicol Chem* 21:1692–1698 (2002).
- Seki M, Yokota H, Maeda M, Tadokoro H, Kobayashi K: Effects of 4-nonylphenol and 4-tert-octylphenol on sex differentiation and vitellogenin induction in medaka (*Oryzias latipes*). *Environ Toxicol Chem* 22:1507–1516 (2003).
- Seki M, Fujishima S, Nozaka T, Maeda M, Kobayashi K: Comparison of response to 17 beta-estradiol and 17 beta-trenbolone among three small fish species. *Environ Toxicol Chem* 25:2742–2752 (2006).
- Shang EHH, Yu RMK, Wu RSS: Hypoxia affects sex differentiation and development, leading to a male-dominated population in zebrafish (*Danio rerio*). *Environ Sci Technol* 40:3118–3122 (2006).
- Shibata N, Hamaguchi S: Evidence for the sexual bipotentiality of spermatogonia in the fish, *Oryzias latipes*. *J Exp Zool* 245:71–77 (1988).
- Slanchev K, Stebler J, de la Cueva-Mendez G, Raz E: Development without germ cells: the role of the germ line in zebrafish sex differentiation. *Proc Natl Acad Sci USA* 102:4074–4079 (2005).
- Sohoni P, Tyler CR, Hurd K, Caunter J, Hetheridge M, et al: Reproductive effects of long-term exposure to bisphenol A in the fathead minnow (*Pimephales promelas*). *Environ Sci Technol* 35:2917–2925 (2001).
- Sun LW, Zha JM, Spear PA, Wang ZJ: Toxicity of the aromatase inhibitor letrozole to Japanese medaka (*Oryzias latipes*) eggs, larvae and breeding adults. *Comp Biochem Physiol C* 145:533–541 (2007).
- Tanaka JN, Grizzle JM: Effects of nonylphenol on the gonadal differentiation of the hermaphroditic fish, *Rivulus marmoratus*. *Aquat Toxicol* 57:117–125 (2002).
- Tanaka K, Takehana Y, Naruse K, Hamaguchi S, Sakaizumi M: Evidence for different origins of sex chromosomes in closely related *Oryzias* fishes: substitution of the master sex-determining gene. *Genetics* 177:2075–2081 (2007).
- Tanaka M, Saito D, Morinaga C, Kurokawa H: Cross talk between germ cells and gonadal somatic cells is critical for sex differentiation of the gonads in the teleost fish, medaka (*Oryzias latipes*). *Dev Growth Differ* 50:273–278 (2008).
- Thomas P, Rahman MS, Khan IA, Kummer JA: Widespread endocrine disruption and reproductive impairment in an estuarine fish population exposed to seasonal hypoxia. *Proc R Soc B Biol Sci* 274:2693–2702 (2007).
- Tyler CR, Jobling S, Sumpter JP: Endocrine disruption in wildlife: a critical review of the evidence. *Crit Rev Toxicol* 28:319–361 (1998).
- Uchida D, Yamashita M, Kitano T, Iguchi T: Oocyte apoptosis during the transition from ovary-like tissue to testes during sex differentiation of juvenile zebrafish. *J Exp Biol* 205:711–718 (2002).
- Vajda AM, Barber LB, Gray JL, Lopez EM, Woodling JD, Norris DO: Reproductive disruption in fish downstream from an estrogenic wastewater effluent. *Environ Sci Technol* 42:3407–3414 (2008).
- 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* 20:2841–2847 (2001).
- van Aerle R, Pounds N, Hutchinson TH, Maddix S, Tyler CR: Window of sensitivity for the estrogenic effects of ethinylestradiol in early life-stages of fathead minnow, *Pimephales promelas*. *Ecotoxicology* 11:423–434 (2002).
- Van den Belt K, Verheyen R, Witters H: Reproductive effects of ethinylestradiol and 4-tert-octylphenol on the zebrafish (*Danio rerio*). *Arch Environ Contam Toxicol* 41:458–467 (2001).
- Van den Belt K, Wester PW, van der Ven LT, Verheyen R, Witters H: Effects of ethinylestradiol on the reproductive physiology in zebrafish (*Danio rerio*): time dependency and reversibility. *Environ Toxicol Chem* 21:767–775 (2002).
- Van den Belt K, Verheyen R, Witters H: Effects of 17 alpha-ethinylestradiol in a partial life-cycle test with zebrafish (*Danio rerio*): effects on growth, gonads and female reproductive success. *Sci Total Environ* 309:127–137 (2003).
- Van den Belt K, Berckmans P, Vangenechten C, Verheyen R, Witters H: Comparative study on the in vitro/in vivo estrogenic potencies of 17beta-estradiol, estrone, 17alpha-ethinylestradiol and nonylphenol. *Aquat Toxicol* 66:183–195 (2004).
- Versonnen BJ, Arijs K, Verslycke T, Lema W, Janssen CR: In vitro and in vivo estrogenicity and toxicity of o-, m-, and p-dichlorobenzene. *Environ Toxicol Chem* 22:329–335 (2003).
- Vethaak AD, Rijs GBJ, Schrap SM, Ruiter H, Gerritsen A, Lahr J: Estrogens and xeno-estrogens in the aquatic environment of The Netherlands, occurrence, potency and biological effects. Dutch National Institute of Inland Water Management and Waste Water Treatment (RIZA) and the Dutch National Institute for Coastal and Marine Management (RIKZ), RIZA/RIKZ Report No. 2002.001, February 2002, ISBN 9036954010 (2002).
- Vethaak AD, Lahr J, Schrap SM, Belfroid AC, Rijs GBJ, et al: An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands. *Chemosphere* 59:511–524 (2005).
- Viganò L, Arillo A, Bottero S, Massari A, Mandich A: First observation of intersex cypriids in the Po River (Italy). *Sci Total Environ* 269:189–194 (2001).
- Villeneuve DL, Miracle AL, Jensen KM, Degitz SJ, Kahl MD, et al: Development of quantitative real-time PCR assays for fathead minnow (*Pimephales promelas*) gonadotropin beta subunit mRNAs to support endocrine disruptor research. *Comp Biochem Physiol C* 145:171–183 (2007).
- Wang XG, Bartfai R, Sleptsova-Freidrich I, Orban L: The timing and extent of ‘juvenile ovary’ phase are highly variable during zebrafish testis differentiation. *J Fish Biol* 70:33–44 (2007).
- Weber LP, Hill J, Robert L, Janz DM: Developmental estrogenic exposure in zebrafish (*Danio rerio*): II. Histological evaluation of gametogenesis and organ toxicity. *Aquat Toxicol* 63:431–446 (2003).
- Weisbrod CJ, Kunz PY, Zenker AK, Fent K: Effects of the UV filter benzophenone-2 on reproduction in fish. *Toxicol Appl Pharmacol* 225:255–266 (2007).
- Wester PW, Canton JH: Histopathological study of *Oryzias latipes* (medaka) after long term beta-hexachlorocyclohexane exposure. *Aquat Toxicol* 9:21–45 (1986).
- Wester P, van den Brandhof E, Vos J, van der Ven L: Identification of endocrine disruptive effects in the aquatic environment: a partial life cycle assay in zebrafish. RIVM report 640920001/2003 (2003).
- Wiklund T, Lounasheimo L, Lom J, Bylund G: Gonadal impairment in roach *Rutilus rutilus* from Finnish coastal areas of the northern Baltic Sea. *Dis Aquat Org* 26:163–171 (1996).

- Woodling JD, Lopez EM, Maldonado TA, Norris DO, Vajda AM: Intersex and other reproductive disruption of fish in wastewater effluent dominated Colorado streams. *Comp Biochem Physiol C Toxicol Pharmacol* 144:10–15 (2006).
- Yamamoto T-O: Artificially induced sex-reversal in genotypic males of the medaka (*Oryzias latipes*). *J Exp Zool* 123:571–594 (1953).
- Yang FX, Xu Y, Hui Y: Reproductive effects of prenatal exposure to nonylphenol on zebrafish (*Danio rerio*). *Comp Biochem Physiol C* 142:77–84 (2006).
- Yokota H, Tsuruda Y, Maeda M, Oshima Y, Tadokoro H, et al: Effect of bisphenol A on the early life stage in Japanese medaka (*Oryzias latipes*). *Environ Toxicol Chem* 19:1925–1930 (2000).
- Zarkower D: Establishing sexual dimorphism: conservation amidst diversity? *Nat Rev Genet* 2:175–185 (2001).
- Zhang ZB, Hu JY: Effects of p,p'-DDE exposure on gonadal development and gene expression in Japanese medaka (*Oryzias latipes*). *J Environ Sci-China* 20:347–352 (2008).
- Zhong XP, Xu Y, Liang Y, Liao T, Wang JW: The Chinese rare minnow (*Gobiocypris rarus*) as an in vivo model for endocrine disruption in freshwater teleosts: a full life-cycle test with diethylstilbestrol. *Aquat Toxicol* 71:85–95 (2005).
- Zillioux EJ, Johnson IC, Kiparissis Y, Metcalfe CD, Wheat JV, et al: The sheepshead minnow as an in vivo model for endocrine disruption in marine teleosts: A partial life-cycle test with 17 α -ethynylestradiol. *Environ Toxicol Chem* 20:1968–1978 (2001).