Review Article

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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.

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-

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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; Schartl, 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 *(Microgadus tomcod)*	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 *(Catostomus commersoni)*	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 *(Morone americana)*	**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 *(Zoarces viviparous)*	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 *(Rutilus rutilus)*	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** *Pleistophora mirandellae*, causing degenerative changes in testes and ovaries did not appear to correlate with intersex.	Bjerregaard et al., 2006
Spain, Ebro river	Carp *(Cyprinus carpio)*	**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 *(Barbus plebejus)*	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 *(Labeo rohita)*	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 *(Pleuronectes yokohamae)*	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 *(Abramis abramis)*	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 *(Clarias gariepinus)* Tilapia *(Oreochromis mossambicus)*	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 *(Clarias gariepinus)*	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 *(Perca fluviatilis)* Roach *(Rutilus rutilus)* Brook trout *(Salvelinus fontinalis)*	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 damp. 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 *(Perca fluviatilis)*	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 *(Perca fluviatilis)*	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.	Linderoth et al., 2006
Sweden, Baltic coast	Eelpout *(Zoarces viviparous)*	**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 *(Platichthys flesus)*	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 *(Platichthys flesus)*	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 *(Rutilus rutilus)*	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 *(Gobio gobio)*	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 *(Rutilus rutilus)*	**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 *(Scaphirhynchus platyorynchus)*	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 *(Acipenser transmontanus)*	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 *(Catostomus commersoni)*	**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 *(Gambusia holbrooki)*	Differences were observed with respect to the **gonadal mass** between a river (Fenhoholloway) 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 *(Cyprinus carpio)*	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 '≤') (see www.karger.com/doi/10.1159/000223078). Molar concentrations have bee converted to μg/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 in 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	*Danio rerio*	J	m	x			S			Dilution series of effluents	Örn et al., 2006a
STE-exposure	*Danio rerio*	J	m								Vethaak et al., 2005
	Rutilus rutilus	J		x							Rodgers-Gray et al., 2001
	Rutilus rutilus	J		x						Intersex = feminisation of reproductive duct (no testis-ova)	Liney et al., 2005
	Carassius carassius	A		x	R	R	D			Effects observed for undiluted samples only	Diniz et al., 2005a
	Cyprinus carpio	A		x	R	R	D		x	Effects observed at 50% dilution or less	Diniz et al., 2005b
Caging downstream of an STE	*Oncorhynchus mykiss*	A			R					Compared to upstream site	Harries et al., 1997
Caging experiment in and downstream of paper mill effluent	*Oncorhynchus mykiss*	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 (μg/l)	SR	IS	TSI	OSI	Sperm.	Oog.	AF	PGC	Other effects (number of studies)	Reference
Ethinyl-estradiol	*Danio rerio*	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
	Pimephales promelas	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
	Gasterosteus aculeatus	J	0.002–0.05	ff	x		R		D				Hahlbeck et al., 2004; Maunder et al., 2007
	Oryzias latipes	J	0.002–0.1	fff	xxx								Scholz and Gutzeit, 2000; Metcalfe et al., 2001; Balch et al., 2004; Örn et al., 2006b
	Rutilus rutilus	J	0.004		x								Katsu et al., 2007
	Cyprinodon variegatus	J	0.002	f	x					x		Testicular fibrosis	Zillioux et al., 2001
	Danio rerio	A	0.008–0.01			RRRR	RRRR	DD	D	x			Van den Belt et al., 2001, 2002, 2004; Versonnen et al., 2003
	Pimephales promelas	A	0.01			R	RR	D				Necrotic spermatogonia (1)	Pawlowski et al., 2004b; Filby et al., 2007
	Oryzias latipes	A			x				D				Seki et al., 2002
	Oncorhynchus mykiss	A				R		D					Jobling et al., 1996
	Fundulus heteroclitus	A	0.1			I							Peters et al., 2007
17-β-estradiol	*Cyprinus carpio*	J	1		x						R		Gimeno et al., 1998
	Danio rerio	J	0.1–0.27	ff	x							Reduced testis cyst size	Wester et al., 2003; Brion et al., 2004
	Oryzias latipes	J	0.01–0.10	ffff	xxx								Nimrod and Benson, 1998; Metcalfe et al., 2001; Knörr and Braunbeck, 2002; Hirai et al., 2006
	Gasterosteus aculeatus	J	1	f	x				D				Hahlbeck et al., 2004
	Pimephales promelas	A	0.14			R	R	D	D	x	R	Sertoli cell hyperplasia and hypertrophy	Miles-Richardson et al., 1999; Halm et al., 2002
	Danio rerio	A	0.1			R							Brion et al., 2004
	Oryzias latipes	A	0.029		x	X						Increased connective tissue in testis	Kang et al., 2002
	Melanotaenia fluviatilis	A	1							x			Pollino et al., 2007
Estrone	*Oryzias latipes*	J	0.01	f	x								Metcalfe et al., 2001
	Danio rerio	A	1				R						Van den Belt et al., 2004
Equol	*Oryzias latipes*	J	0.4	f	x			D	D	x		Fibrosis in testis, enlarged ovarian lumen and somatic tissue (1)	Kiparissis et al., 2003
Diethyl-stilbestrol	*Gobiocypris rarus*	J	0.05	f	x								Zhong et al., 2005
Estriol	*Oryzias latipes*	J	1		x								Metcalfe et al., 2001
Genistein	*Oryzias latipes*	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
3-benzylidene camphor	*Pimephales promelas*	A	3					D		x		Enlarged seminiferous tubules	Kunz et al., 2006
Cyproterone	*Oryzias latipes*	J	1		x			D	D			Increased testicular fibrosis	Kiparissis et al., 2003
o,p-DDT	*Oryzias latipes*	J	3	f	x								Metcalfe et al., 2000
p,p-DDE	*Oryzias latipes*	J	20		x	R							Zhang and Hu, 2008
Endosulfan	*Melanotaenia fluviatilis*	J	5			R							Holdway et al., 2008

4-tert-octylphenol	*Danio rerio*	A	25				R						Van den Belt et al., 2001
	Oryzias latipes	J	2–100	f	xxxx		R		D	x		Increased interstitial tissue in males (1)	Gray et al., 1999a, b; Scholz and Gutzeit, 2001; Seki et al., 2003
	Oncorhynchus mykiss	A	39			R		D					Jobling et al., 1996
4-Nonylphenol	*Danio rerio*	J	100	f				D	D	x			Hill and Janz, 2003
	Oryzias latipes	J	11.6–50	ff	xx								Gray and Metcalfe, 1997; Seki et al., 2003
	Gambusia holbrooki	J	50						D			Undifferentiated atrophied gonads (0.5 µg/l)	Dréze et al., 2000
	Rivulus marmoratus	J	150	f					D			Abnormal gonadal lumen in testis	Tanaka and Grizzle, 2002
	Danio rerio	A	100–500				RR						Van den Belt et al., 2004; Yang et al., 2006
	Oncorhynchus mykiss	A	37			R		D					Jobling et al., 1996
Nonylphenol-diethoxylate	*Oncorhynchus mykiss*	A	38			R		D					Jobling et al., 1996
Nonylphenoxy-carboxylic acid	*Oncorhynchus mykiss*	A	32			R		D					Jobling et al., 1996
Nonylphenol-ethoxylate/octyl-phenol-ethoxylate	*Pimephales promelas*	J	76			R							Bistodeau et al., 2006
4-tert-pentylphenol	*Pimephales promelas*	J	180		x		R					Undifferentiated fish	Panter et al., 2006
	Cyprinus carpio	J	36–320		x	R		D			RR	Reduced diameter of seminiferous tubules	Gimeno et al., 1996
	Danio rerio	A	100		x			D					OECD, 2006
	Pimephales promelas	A	100					D					OECD, 2006
	Oryzias latipes	A	1000					D					OECD, 2006
Bisphenol A	*Pimephales promelas*	J	16			R	R	D					Sohoni et al., 2001
	Oryzias latipes	J	50–1820		x			DD	S			Abnormal connective tissue in males (1); loss of testicular structure and increase in interstitial spaces, increase in fibrotic tissue (1)	Yokota et al., 2000; Metcalfe et al., 2001
	Cyprinus carpio	A	1		x					x		Loss of typical lobular structure	Mandich et al., 2007
β-HCH	*Oryzias latipes*	J	100–180	f	x							X adipose tissue in testis	Wester and Canton, 1986; Scholz and Gutzeit, 2001
Flutamide	*Danio rerio*	A	500					D				Sertoli cell hypertrophy	OECD, 2006
	Danio rerio	J	10	m				D				Increased number of interstitial cells, Sertoli cell hypertrophy, increased size of early spermatogonia	Wester et al., 2003
	Oryzias latipes	A	500									Decreased number of postovulatory follicles	OECD, 2006
	Pimephales promelas	A	100				R	D		x			OECD, 2006; Filby et al., 2007
Vinclozoline	*Pimephales promelas*	A	250–700			I	R		D	x		Increased ovarian stages score	Makynen et al., 2000; Martinovic et al., 2008
	Oryzias latipes	A	2500		x			D		x		Increased testicular fibrosis	Kiparissis et al., 2003
Benzo-phenone-2	*Pimephales promelas*	A	1200					D	D	x			Weisbrod et al., 2007
4-tert-butylphenol	*Cyprinus carpio*	A	2300			R						Histo-architectural changes	Barse et al., 2006
p-dichloro-benzene	*Danio rerio*	A	10000				R						Versonnen 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 (μg/l)	Sex ratio	IS	TSI	OSI	Sp	Oo	AF	Other effects (number of studies) and notes	Reference
Tributyltin	*Danio rerio*	J	0.0001	m							Flagella lacking sperm (1)	McAllister and Kime, 2003
	Fundulus heteroclitus	J	2.1			R		S			Epithelial cells of seminal ducts were shrunken	Mochida et al., 2007
Trenbolone	*Danio rerio*	J	0.05	m				S				Örn et al., 2006b
	Oryzias latipes	J	0.05					S				Örn et al., 2006b
	Gambusia affinis	J	1		x			S				
	Danio rerio	A	0.05				R					Seki et al., 2006
	Pimephales promelas	A	0.5					S	D	x		Ankley et al., 2003
	Danio rerio	J	0.1	m (f)				S			Sertoli cell hypertrophy and hyperplasia; in females accumulation of vitellogenic oocytes; at higher concentrations estrogenic effects	Wester et al., 2003
	Pimephales promelas	J	0.1	m	x			S	D		Dysplasia in testis	Bogers et al., 2006
17-α-methyl-testosterone	*Danio rerio*	J	0.1		x			S				Örn et al., 2003
	Gasterosteus aculeatus	J	1	m	x				D		Severe testis abnormalities: large branched cavities	Hahlbeck et al., 2004
	Kryptolebias marmoratus	J	5	m								Kanamori et al., 2006
	Pimephales promelas	A	0.1				R			x		Pawlowski et al., 2004a
Ketoconazole	*Pimephales promelas*	A	6			I	I				Increased proliferation of Leydig cells	Ankley et al., 2007
Fadrozole	*Danio rerio*	J	10	m								Andersen et al., 2004
	Pimephales promelas	J	24.8			I	R					OECD, 2006
	Danio rerio	A	100					S	D		Interstitial fibrosis; oocyte membrane folding affected	OECD, 2006
	Pimephales promelas	A	50–100			I	R	S	D		Sertoli cell hypertrophy and increased number of interstitial cells (2)	OECD, 2006; Panter et al., 2004
	Oryzias latipes	A	20						D	x	Increased number of interstitial cells	OECD, 2006
Tamoxifen	*Danio rerio*	J	32						D	x	Increased number of Leydig cells and granulosa cell height; in males asynchronic meiotic maturation	Wester et al., 2003
Diazonin	*Lepomis macrochirus*	A	60							x	Paralleled by reduced estradiol serum levels	Maxwell and Dutta, 2005
ZM 189,156	*Danio rerio*	J	100	m							Undifferentiated fish	Andersen et al., 2004
Letrozole	*Oryzias latipes*	A	125			I	I	S	D		Enlarged lumen of seminiferous tubules	Sun et al., 2007
Prochloraz	*Oryzias latipes*	A	20						D		Increased number of interstitial cells	OECD, 2006
	Pimephales promelas	A	300							x	Increased number of late and post-vitellogenic oocytes	OECD, 2006
	Danio rerio	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 bipotentiality [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 μg/l, but could reach more than 100 μg/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 μg/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.

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