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Steroid catabolism in marine and freshwater fish

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ABSTRACT

Steroids play important roles in regulating many physiological functions in marine and freshwater fish. Levels of active steroid in blood and tissues are determined by the balance between synthetic and catabolic processes. This review examines what is known about pathways of catabolism of steroids, primarily sex steroids, in marine and freshwater fish. Cytochrome P450 (P450) isoforms present in hepatic microsomes catalyze steroid hydroxylation to metabolites with lower or no activity at estrogen or androgen receptors. Important pathways of steroid catabolism to readily excreted metabolites are glucuronidation and sulfonation of hydroxyl groups. Estradiol, testosterone, DHEA and hydroxylated metabolites of these and other steroids readily form glucuronide and sulfate conjugates in those fish species where these pathways have been examined. Little is known, however, of the structure and function of the UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) enzymes involved in steroid conjugation in fish. Glucuronide and sulfate conjugates of steroids may be transported into and out of cells by organic anion transporter proteins and multi-drug resistance proteins, and there is growing evidence that these proteins play important roles in steroid conjugate transport and elimination. Induction or inhibition of any of these pathways by environmental chemicals can result in alteration of the natural balance of steroid hormones and could lead to disruption of the endocrine system. Recent studies in this area are presented, with particular focus on phase II (conjugative) pathways.

1. Introduction

Steroid hormones play important roles in fish physiology and reproduction. Levels of these hormones are controlled in part through catabolism by one or more pathways. Disturbances in steroid catabolism can result in disruption of the balance of hormones needed for optimal fish health, and in particular to support reproduction. Several environmental chemicals are associated with disturbances in fish reproduction [1–4]. Some of these chemicals have been shown to interact with steroid catabolic pathways, as discussed below, although often such chemicals also interact directly with estrogen or androgen receptors, or affect steroid synthetic pathways.

As well as being required for steroid hormone synthesis, members of the cytochrome P450 (P450) superfamily of enzymes play a part in catabolizing steroids to inactive hydroxylated metabolites [5]. Different forms of P450, at different sites in the body, catalyze steroid catabolism compared with biosynthesis. These P450 enzymes are products of CYP genes. The main excreted forms of steroid hormones are the glucuronide and sulfate conjugates, formed respectively by UDP-glucuronosyltransferase (UGT) and PAPS-sulfotransferase (SULT) enzymes. Another pathway that has been implicated in steroid inactivation is esterification of the hydroxyl groups with endogenous fatty acids, however the ester metabolites are not excreted but are possibly storage forms of the steroids.

In fish, important androgens are testosterone and 11-ketotestosterone, the latter produced by oxidation of 11-hydroxytestosterone through the action of 11-hydroxysteroid dehydrogenase [6]. As in other vertebrates, the female sex hormones include 17-β-estradiol as the most active estrogen [7] and progesterone as the most active progestin [8]. This review will summarize recent studies of the catabolism of these steroids and their precursor dehydroepiandrosterone (DHEA), whose structures are depicted in Fig. 1, with emphasis on conjugation pathways.

2. Phase I metabolism

Active steroids are typically considerably more lipid-soluble than they are water-soluble. For several steroids the first step in their catabolism is through the phase I pathway of monooxygenation. The main site in the fish body of steroid catabolism is the liver, with some contribution from other excretory organs, the intestine, the kidney and the gills.

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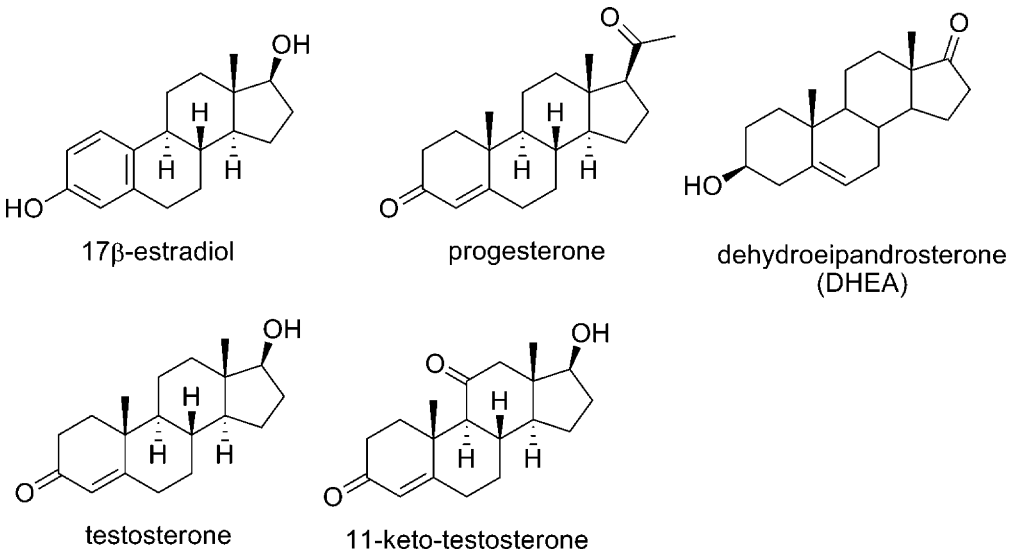

Fig. 1. Structures of steroid hormones associated with reproductive effects in fish.

2.1. Cytochrome P450-dependent hydroxylation

Introduction of hydroxyl groups into one or more of the steroid rings adds polar functional groups and somewhat increases the water solubility of the molecule. This is especially true for progesterone, which does not contain a hydroxyl group and therefore requires phase I metabolism (hydroxylation or keto reduction) in order to undergo conjugation and elimination. Studies in fish with 17-β-estradiol, progesterone and testosterone show that steroid catabolism through hydroxylation is mainly carried out by microsomal P450s that are normally expressed in liver [9–18]. This is similar to findings with mammalian species, which have been studied more extensively [5,19]. No studies of the further cytochrome P450-dependent hydroxylation of 11-keto-testosterone have yet been reported in fish, however it may be expected that this will occur. Compared with mammalian species, much less is known about the P450 forms in fish that catalyze specific positions of hydroxylation of steroids. Some similarities between fish and mammals have been found in positional metabolism of testosterone, where the 6β position is hydroxylated by CYP3A family P450s in those fish and mammals that have been studied to date [11,14–17], and of 17-β-estradiol, where the 2- and 4- positions are hydroxylated by CYP1 family members [10,12,18]. In contrast, while human CYP3A4 readily hydroxylated 17-β-estradiol at the 2-position, zebrafish CYP3A65 produced only small amounts of 2-hydroxy-17-β-estradiol, compared with other zebrafish CYPs [12]. In humans and rats, P450s in the 2 family play important roles in steroid hydroxylation, however there have been few studies of steroid hydroxylation by fish P450s in the 2 family [11]. The major positions of 17β-estradiol, testosterone and progesterone hydroxylation by fish P450s, and the associated P450 isoforms where known, are shown in Table 1. Other positions of hydroxylation of testosterone and progesterone have been reported in some fish species and in organs other than liver, however their rates of formation are generally lower [11,13].

In fish, as in mammals, up- or down-regulation of P450 protein content in liver or other tissues through exposure to naturally occurring or xenobiotic agents that affect P450 expression can result in changes in the rates of hydroxylation of steroids. For example, exposure of fish to benzo(a)pyrene or other aryl hydrocarbon (Ah) receptor agonists results in increased expression of CYP1 family enzymes [20,21] and increased rates of 2- and 4-hydroxylation

Table 1
Hydroxylation of steroids by cytochrome P450 isoforms in fish.

Steroid	Position of hydroxylation	Species	Reported major P450	Other P450s	Reference
Estradiol	2-	Winter flounder, *Pseudopleuronectes americanus*		Hepatic microsomal	[9]
		Scup, *Stenotomus chrysops*		Hepatic microsomal	[9]
		Channel catfish, *Ictalurus punctatus*	Induced by BaP (CYP1)	Hepatic microsomal	[10]
		Rainbow trout, *Oncorhyncus mykiss*	CYP2K1	CYP2M1, CYP3A27, hepatic microsomal	[11]
		Zebrafish, *Danio rerio*	CYP1A, CYP1C1	CYP1B1, CYP1C2, CYP1D1, CYP3A65	[12]
		Japanese eel, *Anguilla japonica*	CYP1A9		[18]
	4-	Channel catfish	Induced by BaP (CYP1)	Hepatic microsomal	[10]
		Zebrafish	CYP1C1, CYP1A	CYP1B1, CYP1C2, CYP3A65	[12]
Progesterone	6β-	Rainbow trout	CYP3A27		[11]
		Channel catfish		Intestinal and hepatic microsomes	[13]
	16α-	Rainbow trout	CYP2K1		[11]
Testosterone	6β-	Rainbow trout	CYP3A27		[14]
		Channel catfish	CYP3A		[15]
		Coho salmon, *Oncorhyncus kisutch*	CYP3A27		[16]
		Sea bass, *Dicentrarchus labrax*	CYP3A79		[17]
	16β-	Rainbow trout	CYP2K1		[11]
		Coho salmon	CYP2K1		[16]

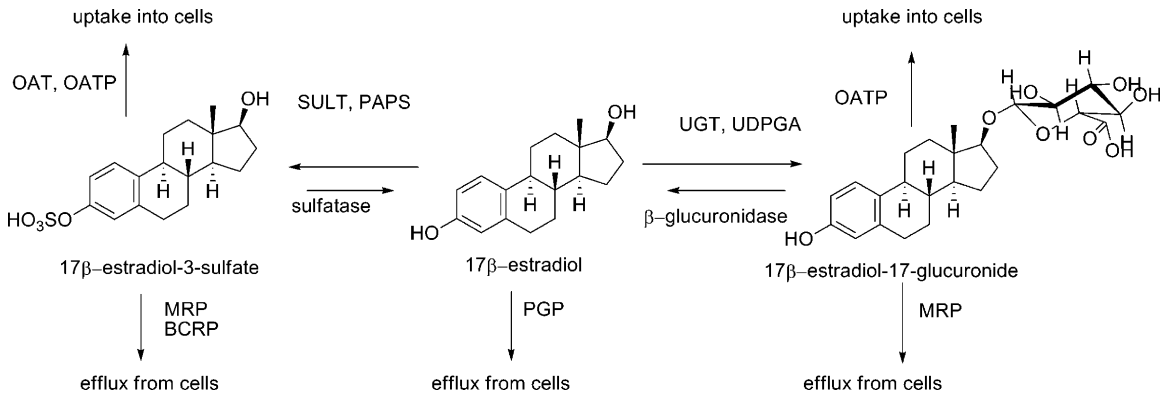

Fig. 2. Representative pathways of elimination of a steroid hormone, showing 17β-estradiol as an example. Shown are pathways of sulfonation or glucuronidation of 17β-estradiol and possible transport pathways of the parent steroid and its conjugates. Abbreviations are defined in the text.

of 17-β-estradiol [10]. Studies in mammalian and fish species have shown that introduction of additional hydroxyl groups in steroids usually reduces or abolishes activity at estrogen or androgen receptors [22], thus increasing hydroxylation through up-regulation of P450s can result in disruption of reproduction and other processes regulated by steroid hormones. Despite having generally lower endocrine receptor-mediated activity, some hydroxylated steroid metabolites, such as 4-hydroxy-estradiol (a catechol estrogen), are associated with toxicity in other vertebrates [23].

2.2. Esterification

Esters of steroids with fatty acids have been demonstrated in a number of vertebrate and invertebrate species [24]. In particular esters of testosterone and 17-β-estradiol with fatty acids have been suggested as storage forms of these steroids. Although esterification results in an inactive steroid, the active steroid can be released by esterase, thus it may not be accurate to describe this pathway as catabolism. Steroid esterification has not been reported in fish species, but is known to be important in invertebrates such as mollusks, echinoderms and crustaceans [25–27].

3. Phase II metabolism

Glucuronidation and sulfonation pathways play important roles in catabolism of steroids or their phase I metabolites that contain hydroxyl groups, and in their ultimate elimination from the body. An illustration of these pathways is shown with 17β-estradiol as an example in Fig. 2. Note that each of the hydroxyl groups in 17β-estradiol is potentially glucuronidated or sulfonated, and 3- and 17- sulfates and glucuronides have been identified in fish species [28,29]. Both sulfate and glucuronide metabolites are commonly formed in fish from a particular steroid and its hydroxylated metabolites [28,30]. The predominant pathway will depend upon the presence and properties of the UDP-glucuronosyl transferase (UGT) and sulfotransferase (SULT) enzymes that catalyze formation of these metabolites and can be expected to vary with tissue, availability of the cosubstrates UDP-glucuronic acid (UDPGA) and 3′phosphoadenosine-5′-phosphosulfate (PAPS), seasonal changes, exposure to xenobiotics that affect UGT or SULT expression or activity, and for reasons not yet fully understood. In human liver, glucuronidation of 17β-estradiol is a high capacity, relatively low affinity pathway and sulfonation a low capacity, high affinity pathway. The 17β-estradiol K_m values with individual human UGT isoforms range from 4 to 250 μM for 3-glucuronidation (15 μM in human liver microsomes) and from 2 to 73 μM for 17-glucuronidation (8 μM in human liver microsomes) [31,32]. V_{max} values for 3- and 17-glucuronidation were respectively 2 and 0.3 nmol/min/mg protein in human liver microsomes [32]. Sulfonation of 17β-estradiol by individual human SULT isoforms have K_m values between 0.01 and 1.5 μM for 3-sulfonation and 3 μM for 17-sulfonation: V_{max} values are 2–500 pmol/min/mg protein for 3-sulfation and 56 pmol/min/mg for 17-sulfonation [33]. In human hepatic S9 fraction 17β-estradiol sulfonation had K_m of 11.5 nM, and V_{max} of 3.8 pmol/min/mg [34]. It is not yet clear if fish enzymes show similar differences in affinity and capacity for sulfonation and glucuronidation.

The excretion of steroids and their phase II metabolites from cells can be facilitated by efflux transporter proteins, as shown in Fig. 2. Estradiol itself is a substrate for p-glycoprotein (PGP), a member of the ATP-binding cassette (ABC) transporter superfamily, in mammals [35] and in a fish species, the channel catfish [36]. Although shown in Fig. 2 in the unionized forms, glucuronide and sulfate metabolites exist largely as anions at physiological pH and are much more water-soluble than the parent steroids. The conjugates are readily eliminated from cells, probably with the aid of ABC-transporter proteins with specificity for anions, such as the multi-drug resistance protein (MRP) and breast cancer resistance protein (BCRP), as has been demonstrated in mammals [37–39]. Efflux transporters in the ABC superfamily have been found in marine and freshwater fish [40–43], though in fish, their function with steroid conjugates has not been extensively investigated. Formation of steroid glucuronides and sulfates and subsequent removal from the cell reduces the concentration of the biologically active steroid and is therefore important in modulating steroid action. In mammalian systems, glucuronidation and sulfonation reduce the biological activity of steroids at the estrogen or androgen receptors compared with the unconjugated steroid [44]. It is thought that sulfate conjugates of steroids, such as estrone sulfate and DHEA sulfate, are storage or transport forms of steroids that are secreted from sites of synthesis into the blood with the aid of efflux transporters, then circulated in the blood to a target organ where uptake transporters such as the organic anion transporters (OAT) or organic anion transporting polypeptide (OATP) facilitate cellular uptake from the circulation [45,46], and steroid sulfatase converts the conjugate back to the active steroid [47]. Transporter proteins with functions in conjugate uptake (OAT, OATP) have been identified in skate (*Raja erinacea*), flounder (*Pseudopleuronectes americanus*) and zebrafish (*Danio rerio*) [48–52]. The skate transporter was shown to be active with estrone sulfate [50].

3.1. Glucuronidation

Introduction of a glucuronic acid moiety into the hydroxyl group of a steroid has long been known to be an important pathway in fish [53,54]. Glucuronides have considerably lower

Table 2
Seasonal changes in mean glucuronosyl transferase activities with estradiol, testosterone and bisphenol A in microsomes from hepatopancreas or liver.[a]

Species, sex	Steroid	Spring	Summer	Autumn nmol/min/mg	Winter	Reference
Carp, *Cyprinus carpio*, male	17β-Estradiol (3 position)	0.06[b]	0.06[b]	0.19	0.19	[62]
	17β-Estradiol (17 position)	0.01[b]	0.025	0.04	0.04	[62]
	Testosterone	0.03[b]	0.05	0.06	0.7	[62]
	Bisphenol A	0.24[b]	0.13	0.17	0.1	[62]
Red mullet, *Mullus barbatus*, mixed sex	17β-Estradiol (position not specified)	0.65	–	0.5	–	[69]
	Testosterone	1.15	–	0.8	–	[69]

[a] Each study used 4–6 individual fish and the original article reported mean ± SEM.
[b] Significantly different from winter, $p < 0.05$.

potency than the unconjugated steroids at androgen or estrogen receptors, however they are not biologically inert. There is good evidence that in some fish species, androgen glucuronides excreted in urine of males attract female fish by acting as pheromones [55,56]. A study with cytosolic estrogen receptors from livers of rainbow trout (*Oncorhyncus mykiss*) and Atlantic salmon (*Salmo salar*) showed that 17β-estradiol-17-glucuronide could displace ^{3}H-estradiol from the hepatic estrogen receptors [57]. Although it was a low-potency ligand of these fish estrogen receptors, 10,000 times less potent that free 17β-estradiol, the 17β-estradiol-17-glucuronide was of similar potency to some synthetic estrogens such as nonyl-phenol and octyl-phenol [57]. Addition of 17β-estradiol-3-glucuronide to hepatocytes isolated from rainbow trout or sturgeon (*Acipenser baeri*) resulted in production of vitellogenin, a biomarker of estrogenicity [58]. The glucuronide was reported to be 3–10 times less potent than 17β-estradiol however it was not established that it was the added glucuronide and not 17-β-estradiol formed in hepatocytes from the action of β-glucuronidase that stimulated vitellogenin synthesis.

Steroid glucuronidation has been observed in liver and other tissues of several fish species. Activity with 17β-estradiol and testosterone has been found in microsomes isolated from liver of carp (*Cyprinus carpio*), turbot (*Scophthalmus maximus*), plaice (*Pleuronectes platessa*) and rainbow trout [59–64] and from carp intestine [62] as well as in liver and kidney slices of lake trout (*Salvelinus namaycush*) [28], zebrafish minced testes [56] and rainbow trout blood cells [59]. These studies have employed fixed concentrations of 17β-estradiol or testosterone, usually 0.1–0.5 mM, and so far the enzyme kinetics of glucuronidation of these steroids have not been reported in any fish species. The specific isoforms of UGT that catalyze biosynthesis of steroid glucuronides are not known in any fish species. As is the case with mammals, there is evidence for several separate UGT enzymes in those fish that have been studied, however fish differ from mammals and from each other in the number and organization of UGT genes [65,66]. Very little is known about substrate selectivity of the different UGT proteins in fish, since there are very few studies of expressed UGT proteins from fish. Expressed plaice UGT1B was shown to glucuronidate 17β-estradiol and testosterone, albeit slowly, and Leaver et al. suggested that these steroids are likely to be preferentially metabolized by other UGT isoforms [65]. Several human UGT enzymes including UGT1A1, UGT1A8, UGT1A10, and UGT2B7 catalyze the 3- and 17-glucuronidation of 17β-estradiol: formation of the 3-glucuronide usually proceeds more rapidly than the 17-isomer [31]. Testosterone glucuronidation in humans is preferentially though not exclusively catalyzed by UGT2A1 and 2B17 [67]. If fish UGT enzymes show similar versatility, it is likely that more than one UGT enzyme can catalyze glucuronidation of a particular steroid.

Since glucuronidation reduces or abolishes the interaction of 17β-estradiol and testosterone with their respective receptors, and it is well-documented that seasonal changes in these steroids are related to reproduction in annual breeding fish [8,68], it can be postulated that changes in glucuronidation activity may in part regulate the amount of active hormone. Hepatic microsomal steroid glucuronidation was studied in two fish species that typically spawn in the late spring following a rise in water temperature, the red mullet and the carp. As summarized in Table 2, a study with mixed sex but predominantly female red mullet from non-polluted sites showed no change in hepatic microsomal glucuronidation of testosterone or 17β-estradiol (position of glucuronidation not specified) between spring and autumn [69], whereas in male carp, glucuronidation of testosterone and of 17β-estradiol at the 3- and 17-positions were lower in microsomes from hepatopancreas and intestine in the spring than in the winter [62], consistent with the higher plasma steroid levels in spring [70]. In both studies, a constant assay temperature was used at the different seasons, 30 °C for the red mullet and 25 °C for the carp, and the concentration of the co-substrate, UDPGA, was saturating (3 mM). Interestingly, in the carp study, the glucuronidation of bisphenol A was higher in spring than winter, suggesting that a different form or forms of UGT catalyze glucuronidation of this environmental compound, compared with 17β-estradiol. The differences in hepatic microsomal activity with 17β-estradiol and testosterone between the red mullet and the carp are striking and suggest there may be other important species differences in steroid catabolism, such as differences in major organ site of catabolism, differences in enzyme kinetics (both studies used fixed concentrations, not specified for red mullet and 0.5 mM for carp) or differences in rates of steroid catabolism by other pathways such as sulfonation. In the carp, microsomes prepared from the intestine had higher activity with both 17β-estradiol and testosterone, compared with liver [62].

Although it is common to employ saturating concentrations of substrate and co-substrate in enzyme assays, it is noteworthy that the concentration of the co-substrate UDPGA usually used in assays of glucuronidation, 3 mM, is substantially higher than hepatic concentrations in the few fish that have been studied, where UDPGA concentrations of 0.02 mM (carp) and 0.12 mM (trout) were reported [71]. In the channel catfish, which spawn in late spring, there were seasonal differences in hepatic content of UDPGA, with average concentrations of 0.36 ± 0.07 nmol UDPGA/g liver (mean ± S.D., $n = 7$) in November/December and 0.13 ± 0.03 ($n = 8$) in May/June [72,73]. These concentrations approximate 0.36 and 0.13 mM and are lower than needed for maximal rates of glucuronidation of those substrates that have been studied in catfish liver, where values for UDPGA range from 0.28 to 0.7 mM [72,74]. While these findings suggest that *in vivo* rates of glucuronidation may be lower than reported from *in vitro* assays conducted under saturating conditions, and lower in catfish in May/June than November/December, no *in vivo* studies have been reported that could shed light on the effect of hepatic UDPGA concentration on rates of steroid glucuronidation in the intact fish.

Exposure of fish to xenobiotics can affect the expression of UGTs or their activity or both parameters. Exposure of flounder to a PCB mixture or to the polycyclic aromatic hydrocarbon benzo(a)pyrene resulted in 4- to 10-fold increased expression of UGT1B, an iso-

form that readily metabolized 1-naphthol and had low activity with estradiol and testosterone [65]. In contrast, CYP1A was induced 50–80-fold in the same experiment [65]. Exposure of juvenile turbot to fuel oil from the Prestige oil spill resulted in 2- to 3-fold increases in the glucuronidation of 4-nitrophenol, but had no effect on testosterone glucuronidation [63]. The fuel oil caused a 6- to 8-fold increase in EROD activity, a marker of CYP1A [63]. Channel catfish or mummichog (*Fundulus heteroclitus*) exposed to creosote exhibited 2-fold higher UGT activity with 9-hydroxy-benzo(a)pyrene than unexposed fish [75], however activity with steroids was not studied. These studies suggest that UGTs, particularly those that catalyze steroid glucuronidation, can be induced but are not as sensitive to induction by planar polycyclic aromatic compounds as the P450 isoforms in the CYP1 family. Studies have shown that exposure to polycyclic aromatic hydrocarbons is associated with altered steroid levels in fish [76,77], and it is possible that changes in the content and activity of steroid-metabolizing UGT could contribute to the observed effects, however further studies are needed to draw this conclusion.

Studies have shown that glucuronidation of steroids is subject to inhibition in the presence of common environmental pollutants. 17β-Estradiol glucuronidation in carp hepatic microsomes was inhibited by addition of 1 mM triphenyltin, nonylphenol or fenarimol, while testosterone glucuronidation was inhibited by these compounds as well as dicofol, tributyltin and dibutyltin [60]. Fenamirol was the most potent inhibitor, with IC_{50} values of 92 μM for estradiol glucuronidation and 71 μM for testosterone glucuronidation, and some inhibition observed at concentrations as low as 10 μM. Several 4-hydroxylated PCBs were shown to inhibit UGT activity with 3-hydroxy-benzo(a)pyrene in channel catfish intestinal and hepatic microsomes with IC_{50} values ranging from 1 to 40 μM [78], however the effect on steroid glucuronidation was not studied. Total metabolism of 17β-estradiol, 100 μM, was inhibited up to 85% in liver or kidney tissues of the lake trout in the presence of 100 μM concentrations of 4,4′-dihydroxy-3,3′,5,5′-tetrachlorobiphenyl or tetrabromobisphenol A or tetrachlorobisphenol A, and was inhibited between 75 and 80% by 100 μM 2,4,6-trichlorophenol, 2,4,6-tribromophenol or bisphenol A [28]. Water-soluble glucuronide and sulfate metabolites of 17β-estradiol were the targets of inhibition by these hydroxylated xenobiotics [28]. Taken together, these studies suggest the possibility that fish exposed to pollutants could exhibit lowered steroid glucuronidation, however it is not known if common environmental concentrations of the inhibitors would result in sufficiently high concentrations in liver (or other tissues where glucuronidation takes place) to substantially affect steroid glucuronidation. It is also not known if mixtures of such inhibitors, as would be encountered in the riverine, estuarine or coastal environment would result in additive effects, synergistic effects or other interactions. There is evidence that exposure of fish to nonylphenol affects reproduction [79], as does exposure to triphenyltin [80], however it is not known if these effects are related to changes in steroid glucuronidation.

Further studies in this area should pay careful attention to the substrate concentrations of both steroid and inhibitor, and their interaction in order to determine how relevant inhibition of glucuronidation is to endocrine disruption.

3.2. Sulfonation

Formation of sulfate esters of steroids is a major pathway of steroid catabolism in fish [81–83], as it is in mammals [84,85], and reduces or abolishes direct binding to estrogen or androgen receptors [85,86]. The sulfate esters of some steroids do exhibit biological activity. In mammals, sulfated steroids have been shown to interact with the olfactory system [87] and estrone sulfate inhibits glucose-6-phosphatase [88]. As is the case for steroid glucuronides, steroid sulfates have been shown to act as pheromones in several fish species [55,89,90]. One study showed that addition of 17β-estradiol 3-sulfate to rainbow trout or sturgeon hepatocytes resulted in production of vitellogenin, a biomarker of estrogenicity, with a similar potency to 17β-estradiol itself [58], however since the hepatocytes are likely to contain the hydrolytic enzyme sulfatase, free 17β-estradiol released from 17β-estradiol-3-sulfate could have been responsible for stimulating the vitellogenin synthesis.

Sulfonation of steroids has been demonstrated in tissues of several fish species. Sulfonation occurs in steroidogenic tissues, such as testis [82] and ovary [91], as well as in tissues commonly associated with elimination, such as liver [29,60,63,69,92] and intestine [92]. The position of 17β-estradiol sulfonation has been studied in hepatic cytosol from catfish and red mullet [29,69]. Both the 3- and 17-sulfates were found at estradiol concentrations above 10 nM (catfish) and 200 nM (red mullet) but only the 3-sulfate at low nM concentrations. Investigation of the kinetics of 17β-estradiol 3- and 17-sulfonation in catfish showed that 3-sulfonation was subject to substrate inhibition at 17β-estradiol concentrations above 0.8 μM [29], as is common for SULT1 family enzymes [93,94]. In contrast, 17-sulfonation was not inhibited at concentrations up to 6 μM. Although this has not been studied in fish, there is evidence in mammals that 17β-estradiol-3-sulfate but not 17-sulfate is subject to hydrolysis by sulfatase back to 17β-estradiol [95], thus the position of sulfation could be significant in determining the likelihood of rapid elimination of the conjugate compared with conversion back to the active steroid. SULT2A1 is the only human sulfotransferase known to form the 17-sulfate of 17β-estradiol [33], although several sulfotransferases (SULT1E1, SULT1A1, SULT1A3, SULT2A1) can form 17β-estradiol-3-sulfate [33], with SULT1E1 (estrogen sulfotransferase) having the highest affinity for estradiol [93]. There have been no studies of the position of sulfonation of 17β-estradiol by the fish sulfotransferases that have been isolated.

Several zebrafish SULT isoforms have been identified, expressed and studied with different steroid and non-steroid substrates [96–101]. SULTs with similarity to mammalian 1, 2 and 3 families are present in zebrafish [102]. Table 3 summarizes kinetic properties of several zebrafish sulfotransferases with steroid substrates. Of note is that none of the enzymes studied so far exhibit low nM K_m values, as has been reported for human SULT1E1 [93]. The lowest K_m was found with the isoform designated as SULT3 ST2 which had a K_m for overall 17β-estradiol sulfonation of 2.1 μM [97]. A zebrafish SULT2 isoform had good activity with DHEA and related hydroxysteroids with alcohol-type hydroxyl groups, however the pH optimum of the enzyme was 9.5 and the enzyme had much lower activity in the physiological pH range [101]. Another zebrafish enzyme, SULT3 ST3, exhibited lower K_m and V_{max}, and was optimally active in the physiological pH range (Table 3) [102]. A SULT2 family isoform known to be expressed in catfish liver metabolized DHEA but no activity was detected with testosterone or 17β-estradiol (K. Merritt and M.O. James, unpublished work). The kinetics of 17β-estradiol sulfonation have been studied with hepatic cytosol from three fish species (Table 3). In the catfish, 17β-estradiol-3-sulfation followed Michaelis–Menten kinetics at estradiol concentrations between 20 and 800 nM and the K_m was 400 nM. Total 17β-estradiol sulfonation (position not examined), when studied with substrate concentrations in the nM range, gave K_m values of 28 and 75 nM for 17β-estradiol in liver cytosol from the red mullet and four spotted megrim, respectively (Table 3). V_{max} values were very low, especially compared with rates of microsomal glucuronidation of 17-β-estradiol (Table 2).

Studies have examined the effect of possible inhibitors of steroid sulfonation in fish. Several metals were shown to inhibit DHEA sulfonation by zebrafish SULT2 and SULT2 ST3 enzymes (Table 4). Concentration dependence of the inhibition was not studied, but some metals, notably mercury, cadmium, copper and lead strongly

Table 3
Properties of steroid-metabolizing sulfotransferases in marine and freshwater species.

Species enzyme source	Steroid substrate	K_m, μM	V_{max}, nmol/min/mg protein	Activity at 50 μM	Reference
Expressed enzymes					
Zebrafish, *Danio rerio*					
SULT1 ST2	17β-Estradiol	13.0	217	79	[98,99]
	Estrone	12.5	366	80	
SULT 1 isoform 4	Estrone			1.5	[100]
	17β-Estradiol			Not detected	
SULT 2	DHEA	134	92	24	[101]
	17β-Estradiol			0.6	
SULT 3 ST1	17β-Estradiol	55.7	0.88		[97]
	DHEA	29.2	0.16		
SULT 3 ST2	17β-Estradiol	2.1	1.2		[98]
	DHEA	2.9	9.9		
SULT 3 ST3	17β-Estradiol			0.9	[102]
	DHEA	1.44	16.1	9.4	
	Pregnenolone	0.78	11.2	11.2	
Channel catfish, *Ictalurus punctatus*					
SULT 2	DHEA	43.7	0.52		[a]
Hepatic cytosol					
Channel catfish,	17β-Estradiol-3-sulfation	0.4	0.09	0.002	[29]
Ictalurus punctatus	17β-Estradiol-17-sulfation	1.07	0.026	0.025	
Red mullet, *Mullus barbatus*	17β-Estradiol	0.075	0.001		[69]
Four spotted megrim, *Lepidorhombus boscii*	17β-Estradiol	0.028	0.0002		

[a] K. Merritt and M.O. James, unpublished work.

Table 4
Inhibition of steroid sulfotransferase by environmental chemicals.

Species, enzyme source	Steroid substrate	Inhibitor	Concentration, effect	Reference
Zebrafish	DHEA	$HgCl_2$	5 mM, 75% inhibition	[101]
SULT2		$CuCl_2$	5 mM, 65% inhibition	
Zebrafish	DHEA	$HgCl_2$	1 mM, 98% inhibition	[102]
SULT3 ST3		$CuCl_2$	1 mM, 98% inhibition	
		$CdCl_2$	1 mM, 90% inhibition	
		$ZnCl_2$	1 mM, 90% inhibition	
		$FeCl_2$	1 mM, 75% inhibition	
		Lead diacetate	1 mM, 90% inhibition	
Zebrafish SULT1 ST2	17-β-Estradiol	Bisphenol A	90 μM IC_{50}	[99]
		4-*n*-Octylphenol	5 μM IC_{50}	
		4-*n*-Nonylphenol	17 μM IC_{50}	
Channel catfish	17-β-Estradiol	4′OH-CB35	0.25 μM IC_{50}	[29]
hepatic cytosol		4′OH-CB79	0.09 μM IC_{50}	
		4′OH-CB106	0.55 μM IC_{50}	
		Celecoxib	45 μM IC_{50}	
Red mullet hepatic	17-β-	4-Nonylphenol	62 μM IC_{50}	[69]
cytosol	Estradiol	Triphenyltin	65 μM IC_{50}	
Four spotted megrim	17-β-	4-Nonylphenol	73 μM IC_{50}	
hepatic cytosol	Estradiol	Tributyltin	40 μM IC_{50}	
		Triphenyltin	31 μM IC_{50}	
Carp hepatic cytosol	17-β-	4-Nonylphenol	41 μM IC_{50}	[60]
	Estradiol	Tributyltin	17 μM IC_{50}	
		Triphenyltin	18 μM IC_{50}	

4′OH-CB35 is 4-hydroxy-3,3′,4′-trichlorobiphenyl; 4′OH-CB79 is 4-hydroxy-3,3′,4′5-tetrachlorobiphenyl; 4′OH-CB106 is 4-hydroxy-3,3′,4′,5′,6′-pentachlorobiphenyl.

inhibited activity at 1 mM concentration, raising the possibility that these metals, which are sometimes found in coastal and estuarine environments, could affect steroid metabolism and perhaps fish reproduction [103]. Two organometallics, triphenyltin and tributyltin inhibited hepatic cytosolic sulfotransferase in the low μM range when assayed with 10 nM 17β-estradiol. Phenolic compounds released from detergents, nonylphenol and octylphenol, which have been found to be estrogenic in fish and to disrupt fish reproduction [79,103], inhibited estrogen sulfonation in the low μM range. Hydroxylated metabolites of some polychlorinated biphenyls (PCBs) were more potent inhibitors, with IC_{50} concentrations in the high nM range (Table 4). Some of the phenolic compounds are directly estrogenic, albeit with low potency [104,105], however their *in vivo* effects could be due to a combination of direct action at estrogen receptors and inhibition of estrogen conjugation.

4. Conclusion

Studies of steroid catabolism in marine and freshwater fish have shown that pathways employed are broadly similar to those identified in mammals, however there are important differences in specificity, selectivity, seasonal changes and response to environmental chemicals for individual fish species and routes of catabolism. Exposure to polycyclic aromatic hydrocarbons can cause induction of CYP1 enzymes and in some species induction of UGT and SULT enzymes, resulting in increased formation of potentially toxic catechol estrogens and increased steroid elimination, changes that could affect steroid homeostasis in affected fish species. Exposure of fish to certain metals, and to phenolic compounds such as nonylphenol and hydroxylated PCBs that inhibit sulfonation and glucuronidation could disrupt the normal metabolism of steroids, however it is not known if these

interactions occur in any fish species at environmentally relevant concentrations. Further research is needed to ascertain if environmental chemicals affect fish health through changes in steroid catabolism.

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