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Topic 4.5
Endocrine disruption in marine fish*

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Abstract: This topic reviews the whole field of endocrine disruption (ED) in marine fish and compares this with our knowledge of the situation in freshwater species. In broad terms, similar types of ED have been observed in the two groups, although effects in the marine environment tend to be less marked, presumably due to dispersion and dilution. There are, however, some data which suggest that marine fish that are top-predators can experience ED due to biomagnification of organochlorines. Processes such as smoltification, metamorphosis, and hermaphroditism, which are common in some marine species, may be particularly susceptible to ED, but have as yet been scarcely studied. As with freshwater fish, firm links to population-level effects have not yet been demonstrated, although it is not unreasonable to suppose that they are occurring in some locations. The topic concludes with some recommendations for future research.

INTRODUCTION

Knowledge about the causes and effects of endocrine disruption (ED) is probably more comprehensive for fish than for any other animal group. A useful general review of the subject has been published by Kime [1], but additional material can be found in several publications [2–22]. Possible reasons for this focus on fish include their early identification as a group showing ED effects in the field, their sexual lability (exploited for many years by fish farmers), their marked exposure to contaminants via both oral and branchial routes, their relatively well-understood endocrinology, and their amenability to experimental manipulation. The breadth of such studies has meant that many different types of ED have been observed in fish, including interference with hormone receptor-mediated processes, with normal hormone titres via metabolic and excretory mechanisms, and with gonadal development.

The overwhelming preponderance of studies has been concerned with the sex steroids and their "downstream" actions, in particular the impacts of exogenous estrogens and their mimics. However, there is also more limited information available on disturbances of the thyroid and adrenocorticoid systems in fish, as well as a number of syndromes that may or may not be attributable to ED. A recent review of the subject that also highlights the many unique aspects of fish endocrinology, general biology, and evolution, has been produced by the International Program on Chemical Safety [23]. Despite their uniqueness and long evolutionary history (at least 400–500 million years), it is nevertheless important to bear in mind that fish possess endocrine systems which mirror in many respects those of more recently evolved vertebrates (all vertebrates have similar adrenal and sex steroid receptors, e.g., [24]). Fish, therefore, have the potential to act as sentinels for possible effects on other vertebrates, including

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humans, although the differential exposure of various vertebrate groups is likely to be of major importance in determining the scale of such effects.

Although the literature is growing fast, the majority of it is related to freshwater fish, the group in which a variety of reproductive effects were first recognized in the field as types of ED [22,25–28]. There have been relatively few studies of marine fish, perhaps because contaminated discharges generally receive much greater dilution in salt waters than in rivers and lakes, a fact that probably discouraged an early search for effects in the sea. For a number of reasons, it is nevertheless timely to review progress in this field. For example, the diversity of marine fish is high by comparison with most freshwater assemblages (with some almost uniquely marine groups such as the 3000-strong elasmobranchs and chimaeras), and as we already know that there is considerable interspecific variation of response in fish to endocrine disruptors, this raises the possibility of new modes of action in marine species.

Secondly, marine fish other than the cyclostomes and elasmobranchs exhibit the unique ability to maintain their hypotonic body fluids by drinking, a behavior that probably exposes them to dissolved contaminants to a greater degree than freshwater fish. Their somewhat divergent physiology may also have a bearing on their responses to endocrine disruptors, although they generally possess similar endocrine systems to their freshwater relatives.

Thirdly, the well-known sexual plasticity of fish is particularly marked in some marine groups. Although the sexes are usually separate (gonochoristic), there are some functional hermaphrodites (e.g., some marine Serranidae and Sparidae), a feature which is rare in the vertebrates. Furthermore, in some other marine groups (e.g., the cyclostomes), the eggs and sperm can begin development in a single individual, with one gamete usually coming to dominate (although some individual hagfish may remain as sterile intersexes for life). In some coral reef fishes, individuals can change functional sex in response to social and environmental cues. There are examples of both protoandrous (sperm produced first) and protogynous (eggs first) genera. The ways in which sex reversal in fish is brought about are essentially unknown, although the involvement of steroid hormones is suspected [29]. It is interesting to note that there are usually germ cells of both sexes present in an individual fish of this type, although only one type of gamete is mature at a given time. Although these types of natural sex reversal are relatively rare among fish species, the ease with which fish farmers are able to change the phenotypic sex of some stocks through judicious application of exogenous steroids [30,31] is consequently not surprising. It should be noted that the performance of hormonally sex-reversed male and female fish is not comparable with normal ones [32]. The implications for endocrine disruption are clear.

The purpose of this paper is, therefore, to assess the nature and extent of ED in marine and estuarine fish, and to identify any substantive differences from the well-documented position in some fresh waters. The paper will focus on effects reported from the field, using laboratory studies where appropriate to help explain the observations.

VITELLOGENIN AND ZONA RADIATA PROTEIN INDUCTION AS BIOMARKERS FOR EXOGENOUS ESTROGENS, AND SPIGGIN INDUCTION AS A BIOMARKER OF ANDROGEN EXPOSURE

The processes of vitellogenesis and zonagenesis in normal female teleost livers are described in [33] and [34], respectively. They are both driven by endogenous estradiol (E2) secreted by the ovaries in response to environmental cues acting via the hypothalamus and pituitary. Vitellogenin (VTG) induction in male fish (which only contain very low endogenous E2, but whose livers nevertheless are able to synthesize VTG and zona radiata protein (ZRP) in response to exogenous E2) was the first effect of estrogen contamination to be reported from the freshwater environment [27], and a number of studies have now measured this biomarker in marine fish. It is worth bearing in mind that although VTG induction in males is an excellent biomarker of exposure to exogenous estrogens and their mimics acting via the hepatic estrogen receptors (demonstrated in many laboratory experiments, e.g., [35–37]), it can also be associated with liver enlargement and kidney damage (caused by its production and attempted excre-

tion, respectively), and is generally accompanied by various degrees of reproductive interference at similar or lower ambient estrogen concentrations [38–43]. In other words, VTG induction in male fish is also a marker for a number of adverse effects.

Lye et al. [44,45] were the first to report VTG induction (and accompanying testicular abnormalities) in a male marine fish, the euryhaline flounder (*Platichthys flesus*). These were caught near a sewage treatment works (STW) discharge on the Tyne estuary (NE England) and later work suggested that alkylphenols may have been partly responsible [46]. More detailed surveys of *P. flesus* in 13 UK estuaries [35,36,47,48] showed that VTG induction is widespread in the males of this species, at titres of up to 20 mg VTG/ml plasma in the worst cases (Tees, Tyne, Clyde, and Mersey estuaries). Elevated VTG titres have even been observed in males migrating offshore to breed, although it is thought that this derives from earlier estuarine exposure. At some stations on the Mersey and Clyde, VTG titres appear to have declined over the period 1996–2001 during which the standard of sewage treatment in these areas has been improving, but the existence of real temporal trends remains to be confirmed. Males in other estuaries (e.g., Thames, Forth, Humber, and Dee) show medium or negligible amounts of VTG induction, despite large inputs of sewage effluent in some of these areas. Sediments in the Tees and Tyne are much more contaminated with estrogens than the overlying water [49], and there are indications that the flounder's benthic food organisms may be an important source of estrogen exposure [48]. However, the main causative substances have yet to be fully identified [49].

Male flatfish elsewhere have also been reported to show VTG induction, although not to the degree seen in UK fish. For example, Madsen et al. [50] report up to 86 μg VTG /ml in male *P. flesus* from 7 Danish coastal and estuarine stations, up to 2.2 μg VTG /ml was found in male *Pleuronectes yokohamae* from Tokyo Bay [51], and a maximum of only 0.06 μg VTG /ml was recorded in *P. yokohamae* from the Strait of Tsugaru [52]. In addition, VTG synthesis has been detected in more than 50 % of male English sole (*Pleuronectes vetulus*) from some organochlorine-contaminated areas of Puget Sound in the western United States [53], in male grey mullet (*Mugil cephalus*) from Osaka Bay in Japan [54], and in a few male *P. flesus* caught in a Dutch industrial harbor zone and in offshore spawning areas [55]. It is interesting to note that when larval sunshine bass (*Morone saxatilis* x *M. chrysops*) were experimentally exposed for 4 days to New York City sewage effluent, not only VTG but also estrogen receptor (ER) expression was induced [56]. This upregulation of ER has also been observed in the laboratory in trout exposed to octylphenol or E2 [57], and suggests that early exposure to estrogens may predispose fish to later hypersensitivity to estrogens. This has indeed been shown experimentally in male Japanese medaka, which gave a larger VTG response to E2 if they had been exposed as larvae to the estrogen mimic *o,p′*-DDT [58].

The studies reviewed above suggest that UK estuarine waters may be particularly contaminated with estrogens, and VTG mRNA has indeed been detected in males of another UK estuarine species, the viviparous blenny (*Zoarces viviparus*), caught at stations on the Forth, Tees, Clyde, and Tyne [48]. However, other studies have failed to find VTG in male eel (*Anguilla anguilla*) from some of the same UK estuaries (e.g., Tyne and Tees) in which male flounder are strongly vitellogenic [59,60], and VTG mRNA has been undetectable in male sand goby (*Pomatoschistus minutus*) from these estuaries [48,61]. In both these species, VTG can be induced by exposure in the laboratory to rather high estrogen doses, so it is assumed that the absence of VTG induction under estrogen-contaminated field conditions is due either to lower exposure than that experienced by flounder and blenny, or to lower sensitivity. Data discussed below suggest that wild UK gobies *are* exposed to estrogens, so it is possible that differential sensitivity is the main explanatory factor.

With the exception of some UK and Dutch flounder migrating offshore from contaminated estuaries to breed, none of the examples described above indicate that fish in the open ocean are experiencing significant estrogenic exposure. However, recent data on the Mediterranean swordfish (*Xiphias gladius*) a top-predator caught in the Straits of Messina near Sicily and in the open Atlantic around the Azores show that some males from the Straits are experiencing VTG and ZRP induction several times higher than Atlantic fish [62]. The data are based on rather few samples, but it appears that VTG and

ZRP induction increases with age, suggesting that it is related to the long-term bioaccumulation of lipophilic estrogenic substances. This is in contrast to UK flounder [47] where even immature fish show strong VTG induction at some sites, and known lipophilic estrogen-mimics are only bioaccumulated to a moderate extent in adults (e.g., maximum pooled hepatic ΣPAH = 0.36 mg/kg wet wt. in Tees and Mersey fish; max. pooled hepatic ΣPCB = 1.3 mg/kg wet wt. in Thames and Mersey fish; max. pooled hepatic ΣDDT = 0.49 mg/kg in Mersey fish). Note, however, that these levels of PCB contamination have been associated with reduced viable hatch of Baltic flounder fry [63]. It would, therefore, be worth investigating whether other predatory fish are experiencing significant estrogenic exposure under open-sea conditions.

It should be borne in mind that female fish can also be affected by exogenous estrogens. For example, Matthiessen et al. [47] reported elevated VTG titres in juvenile female flounder, but the already high natural levels in adult females were not further increased. The full implications of this require investigation, but it is possible that females may mature too early in estrogen-contaminated locations. It has been reported [64,65] that *P. flesus* exposed to harbor sediment showed premature vitellogenesis, although this could have been due to elevated endogenous estradiol titres rather than exogenous estrogens. Cadmium (not an estrogen mimic) has also been shown to cause increased vitellogenesis in female Atlantic croaker (*Micropogonias undulatus*), but this is thought to be caused by direct toxic action on the pituitary, leading to altered secretion of gonadotropin hormone (GtH) [66,67].

Finally, it is worth noting that *reduced* VTG titres have occasionally been reported in female fish [68–70] from contaminated marine harbors, etc., an effect which might be attributable to antiestrogen or androgen exposure, or to generalized stress [71–74], leading to lower VTG synthesis, which could have implications for normal egg development.

In summary, the available data show that demersal fish in some estuarine and coastal waters, and those pelagic top predators containing biomagnified contaminants, can be exposed to sufficient exogenous estrogen to cause VTG and/or ZRP induction in males, and/or premature induction in females. Unlike in rivers, where VTG induction seems to be associated primarily with natural and synthetic estrogenic hormones in STW discharges [75], the similar effects in estuaries (at least, those in the United Kingdom) appear to be linked to present or past industrialization, and to largely unknown contaminants adsorbed to sediments. Experimental data show that strong VTG induction in males can be accompanied by a range of other abnormalities [e.g., 40] and the following sections will demonstrate that some of these have also been observed in the field.

An analogous biomarker to VTG, but sensitive to androgens rather than estrogens, has been developed in the three-spine stickleback (*Gasterosteus aculeatus*) [76,77]. This is the protein spiggin, which is produced in the male kidney under the control of 11-ketotestosterone (11-KT), and is secreted via the bladder for use as a glue during nest construction. It has been shown [77] that spiggin is produced in females exposed to exogenous androgens, and that it is also induced during experimental exposure to an androgenic Baltic pulp-mill effluent. However, only a few STW discharges to UK estuaries appear to show androgenic activity in in vitro assays, the activity is solely attributable to natural androgens [78], and female sticklebacks caged in the vicinity of such discharges only show small spiggin responses [48,77]. Although these data are rather preliminary, it appears at present that androgens are relatively unimportant contaminants of sewage-contaminated estuaries by comparison with estrogens. The reverse, however, is probably true near pulp-mill discharges in the Baltic and elsewhere.

ALTERED SEX STEROID TITRES

Based on work in freshwater fish, impacts of contaminants on sex steroid titres might be expected in marine fish, but few have been reported to date. In principle, altered levels of sex steroids in plasma might be due to interferences with the control of steroid synthesis via the pituitary-gonadal axis, or to effects on steroid metabolism and excretion. For example, experimental exposure to pulp-mill effluent fractions containing low-molecular-weight phenolics (but not plant sterols) can cause depressions in

testosterone (T) in the estuarine killifish (*Fundulus heteroclitus*) [79,80], probably via one or more independent mechanisms including action at the pituitary on GtH secretion, reductions in cholesterol availability, and inhibition of enzymes which catalyse sex steroid synthesis. Similar depression of plasma androgens has also been observed in perch (*Perca fluviatilis*) caught near a pulp-mill discharge in the Baltic Sea [81], and this effect probably explains the demasculinization of these fish (see below). In vitro laboratory experiments with *P. flesus* ovarian tissue exposed to high doses of polycyclic aromatic hydrocarbons (PAHs) have shown that they can inhibit the steroidogenic enzyme P450 17,20-lyase, leading to reduced secretion of androstenedione and E2 [82]. This may in turn explain the reduced VTG synthesis in some female marine fish living in contaminated environments (see above).

On the other hand, even less is known about the effects of estrogen exposure on steroid titres. One of the few observations made in marine fish [83] showed that male *P. flesus* from the estrogenically contaminated Tyne and Mersey estuaries contained up to 5 times more plasma E2 than fish caught in the open North Sea (but much less than seen in normal females). Furthermore, Dutch experiments in which female *P. flesus* were exposed to PAH- and PCB-contaminated harbor sediments for 3 years revealed elevated E2 and T titres, which were tentatively attributed to decreased steroid clearance via the cytochrome P450 system [65]. However, it has also been shown in the laboratory that maturing Atlantic croaker (*M. undulatus*) females exposed to E2 or the estrogen mimic *o,p′*-DDT experience increased GtH release and consequent ovarian growth, which may in turn lead to elevated E2 titres [84]. On the other hand, wild female kelp bass (*Paralabrax clathratus*) from the South California Bight showed *depressed* plasma GtH and E2, but elevated ovarian T production, and these effects were correlated with increased body-burdens of organochlorines [85]. Depressed plasma E2 has also been observed in flatfish (*Pleuronectes vetulus* and *P. bilineatus*) from areas of Puget Sound that are highly contaminated with organochlorines and PAHs [86,87].

The data described above show that endocrine disruptors can cause either increases or decreases in steroid titres of marine fish, depending on sex and contaminant type. These changes can in turn produce a cascade of effects, not the least of which are alterations in secondary sexual characteristics which are under sex steroid control.

ABNORMAL GONAD DEVELOPMENT OR SECONDARY SEXUAL CHARACTERISTICS

A number of instances of abnormal gonad development in marine fish have been attributed to pollutants, but these may of course not have been mediated by endocrine disrupting mechanisms. For example, the *Amoco Cadiz* oil spill off the coast of Brittany caused delayed ovarian development in plaice [88], and although this may have been an example of the antiestrogenic action of some PAHs, such effects could equally well be explained by generalized stress, or by reproductive toxins which do not act directly on the endocrine system.

The most frequently reported form of abnormal gonad development in wild marine fish which in some cases is almost certainly a form of endocrine disruption linked to estrogen exposure is the presence of intersex or ovotestis, i.e., primary or secondary oocytes in the testicular tissue of species which normally have completely separate sexes. The precise mechanism whereby this occurs is unknown, but the phenomenon can easily be induced experimentally through larval exposure (e.g., in sheepshead minnows [89]) but not when fish are already adult. In some of the very estrogenically contaminated UK estuaries (Mersey, Tyne, Clyde, and Forth), up to 15–20 % of male *P. flesus* and *Z. viviparus* at some stations show ovotestis (primary and occasionally secondary oocytes), and occasional examples of ovotestis have also been seen in *P. flesus* from less-contaminated situations [35,36,47,48,90]. However, ovotestis has never been observed in these species when caught from a relatively uncontaminated reference estuary (the Alde). It is noteworthy that the prevalence of ovotestis is rather low compared with that reported (up to 100 %) in freshwater roach from several UK rivers [22], but it is not known if this is due to interspecific differences in response, or to differential exposure.

There are clear interspecific differences, however, between *P. flesus* and *Z. viviparus* on the one hand, and the sand gobies *P. minutus*/*lozanoi* on the other [48]. Despite an intense sampling program in the United Kingdom, ovotestis has never been observed in the sand gobies, even though they inhabit similar areas to flounder and blenny. This mirrors the lack of VTG induction in gobies discussed above. However, recent data [48,61] show that many male gobies (up to 75 %) from estrogenic UK estuaries, while free of VTG and ovotestis, are exhibiting a condition known as morphologically intermediate papilla syndrome (MIPS) in which the urogenital papilla (used for depositing urine and sperm) takes on some of the characteristics of the equivalent female organ (used in oviposition). This condition has been induced in exposure experiments with E2 (10–1000 ng/l) in which juvenile fish were ambiently exposed for up to 32 weeks until adulthood, so it appears to be an effect of estrogen exposure on the expression of a secondary sexual characteristic. This suggests that the absence of VTG and ovotestis induction in wild gobies is more likely to be due to differential sensitivity than decreased exposure.

Examples of apparently abnormal, but relatively mild, ovotestis in marine fish have also been observed from a range of other locations, including the Seine estuary in France (*P. flesus* [91]), Tokyo Bay in Japan (*P. yokohamae* [51]), the southern Baltic in Germany (*Z. viviparus* [92]), and the Mediterranean (*Xiphias gladius* [115]). These cases generally consist of a few primary oocytes scattered throughout otherwise apparently normal testicular tissue. Indeed, other testicular changes such as abnormal spermatogenesis have not frequently been reported, although such abnormalities have been found in Tyne flounder [45], and the testicular tissue in some intersex flounder [47] appeared severely abnormal. Early exposure to estrogens may also cause precocious sexual development in females, and examples of this have been reported in marine flatfish [93,94], although it is not known whether the associated elevations in tissue concentrations of organochlorines were the causative factor.

As it is known that larval estrogen exposure of sufficient intensity can produce a completely female phenotype in genetically male fish [31], the cases of ovotestis cited above may well be incomplete examples of this phenomenon. This implies that some apparently normal females seen in contaminated estuaries etc. may be genetic males. This has not yet been tested in marine fish (although Nagler et al. [95] have observed this phenomenon in phenotypic female Chinook salmon from the Columbia River), due to an absence of suitable genetic sex markers, but it suggests that apparent sex ratios of fish populations exposed to estrogens may become biased toward females. Matthiessen et al. [47] did not observe sex ratios in UK flounder which differed substantially from 50:50, although it is extremely difficult to establish this with certainty under field conditions. More recently, it has been reported [48] that broods of *Z. viviparus* fry from the Forth, Clyde, and Tyne contain 52–60 % females, and although sufficient broods could not be caught from the reference estuary, other work [96,97] suggests that the sex ratio in uncontaminated broods of this species in the Baltic is 50:50. Furthermore, it has been shown [96,97] that broods from *Z. viviparus* females living near a large pulp-mill discharge on the Baltic are biased towards males (mean proportion of males up to 58 %), presumably in response to the known androgenic properties of pulp-mill effluent. It is interesting to note that this bias temporarily disappeared after a 17-day shutdown of the mill in 1999, which coincided with the period of embryonic gonadal differentiation [98].

REDUCED REPRODUCTIVE SUCCESS AND POPULATION-LEVEL EFFECTS

The observations discussed above raise the question of whether marine fish which have experienced disturbances including estrogen exposure, VTG induction, perturbed steroid titres, and ovotestis, etc. have been reproductively compromised, and whether populations are potentially at risk. This is not an easy question to answer because quantitative observations are hard to make in the field. Furthermore, any causative mechanisms for altered reproductive parameters or population declines in marine fish are likely to be multifactorial and include nonendocrine modes of action, as well as factors such as overfishing, habitat loss, and climate change [99]. Experimental work referred to above, however, certainly shows that the induction of VTG and ovotestis in males tends to be accompanied by a range of repro-

ductive abnormalities in breeding groups, including reduced egg production, fertilization success, and fry survival. Whether any of these are likely to cause population declines in the field will depend on their magnitude, and on factors such as density-dependent compensatory effects, which tend to counteract population-level responses to relatively small changes in reproductive output.

There are some field data that suggest that high levels of lipophilic contaminants in marine fish can be associated with reduced reproductive success. For example, it has been shown [100] that a DDT-contaminated population of white croaker (*Genyonemus lineatus*) in California had decreased fecundity and/or spawning inducibility when individual levels of ovarian total DDT residues exceeded about 4000 ng/g wet wt. Similarly, a variety of flatfish species (e.g., *P. bilineatus* and *P. vetulus*) from contaminated areas of Puget Sound are showing precocious sexual maturation, retarded gonadal development, reduced egg weight, and reduced overall spawning success [87,94]. These fish are contaminated inter alia with a variety of aromatic hydrocarbons and PCBs, which are potentially implicated as causative agents, either as antiestrogens (some PAHs) or estrogen-mimics (some PCBs).

A number of marine field studies have linked reduced hatching success and fry survival to increased levels of lipophilic contaminants in eggs. For example, it has been shown [63] that elevated levels (>120 ng/g wet wt.) of PCBs in Baltic flounder (*P. flesus*) ovaries were correlated with impaired egg development and fry survival, and a similar study of Baltic herring (*Clupea harengus*) found that ovarian DDE residues of >18 ng/g wet wt. or PCB residues >120 ng/g wet wt. were significantly associated with reduced viable hatch [101]. High larval mortality and reduced hatching success in Baltic cod (*Gadus morhua*) have also been associated with organochlorines [102], but an early mortality syndrome known as M74 which is widespread in Baltic salmon (*Salmo salar*) seems to be caused by thiamine deficiency, although an indirect link to contaminants has not been ruled out [7,103].

In most of these species, there is massive redundancy in the numbers of fry produced, so it is unclear whether the effects described have significance for populations. One possible exception to this concerns the contamination of some Canadian rivers with the estrogen-mimic nonylphenol (sprayed as a pesticide coformulant during the control of forest pests). This was associated with subsequent major catch declines in returning salmon (*S. salar*) and blueback herring (*Alosa aestivalis*), and it has been speculated that the observations may have resulted from the known antagonism which gonadal steroids exert on the smoltification process [104]. In general, it remains to be established whether the causes of effects described above are indeed connected with endocrine disruption or some other effect. It should also be noted that the high levels of organochlorines and PAHs with which various observations have been associated are not necessarily causal because most study sites are generally contaminated with a complex of co-occurring substances. All these uncertainties imply the need for more laboratory-based reproductive studies with environmentally realistic dosing. Despite the difficulties, it is also necessary to conduct semifield experiments with free-breeding fish populations in order to establish true thresholds of environmental effect.

OTHER POSSIBLE EXAMPLES OF ENDOCRINE DISRUPTION IN MARINE FISH

At least two other major endocrine systems, the adrenal (located in fish in the interrenal and chromaffin tissue) and the thyroid, in addition to the estrogen signalling pathways, are subject to chemical interference in fish (see Topic 4.10 [8,105]), but such effects have not been widely studied in marine species. The adrenal system plays a vital part in responses to stress, while thyroid hormones influence a number of processes in fish, including neural development, metabolism, smoltification in salmonids, and metamorphosis in flatfish. It is worth noting, furthermore, that there is significant "cross-talk" between the different systems, so interfering with one is likely to cause knock-on effects in others. One example of this is the synergism between the thyroid hormones and the actions of gonadotropin hormone during early ovarian development [106]. Reciprocally, elevated plasma E2 can lead to depressed thyroid activity and reduced plasma levels of 3,5,3′-triiodo-L-thyronine (T3).

The only published example of interference with the thyroid system in marine fish concerns mummichogs (*Fundulus heteroclitus*) living in Piles Creek, New Jersey, an estuarine environment contaminated inter alia with heavy metals, PCBs and DDTs [107,108]. Compared with a clean reference site, these fish are sluggish with poor prey capture and avoidance of predators [109], and this has been attributed to interference with the thyroid system, perhaps through its involvement in neurological development. The fish from Piles Creek have larger thyroid follicles and follicular cell heights, and contain elevated titres of plasma thyroxine (T4), but not plasma or tissue T3. Furthermore, clean fish held in conditions simulating Piles Creek also develop elevated T4 titres. Interestingly, the Piles Creek fish do *not* show reproductive abnormalities, their gametes and embryos having developed some contaminant-resistance [110]. Mechanisms of action are still not fully understood, but "cross-talk" from the adrenal system may be partly involved [107]. Normally, the stress hormone cortisol stimulates conversion of T4 to T3, but chronic organic and metallic pollution can nonspecifically "exhaust" the cortisol-secreting interrenal cells [111,112], possibly leading in turn to a build-up of T4. However, there are several alternative (but not necessarily mutually exclusive) explanations, including heavy metal inhibition of the enzyme (5′-monodeiodinase) which catalyses T4 to T3 conversion. Pollution has been frequently shown to cause a reduced cortisol response to stress in freshwater fish, but the only known example at present from the marine environment concerns marbled sole (*Pleuronectes yokohamae*) caught in the contaminated Tokyo Bay [113]. This effect was associated with impaired cortisol release from the head kidney in response to adrenocorticotrophic hormone (ACTH) challenge in vitro.

The example of thyroid disturbance in *F. heteroclitus* serves well to illustrate the potential complexity of endocrine-disrupting effects in fish, and the extent of our ignorance of this subject outside the restricted (but important) field of estrogenic impacts.

CONCLUSIONS AND RECOMMENDATIONS

The main conclusion that arises from this brief review is that marine fish are no less susceptible to endocrine disruption than their freshwater relatives, and display as wide a range of effects as those seen in the latter group. Not surprisingly, most of the reported phenomena have been observed in estuarine and coastal fish, probably because they are more frequently studied, and generally exposed to higher levels of contaminants, than open-sea or oceanic species. Having said that, it is noteworthy that there may be a relationship between the bioaccumulation of lipophilic contaminants and endocrine disruption in large pelagic fish, which suggests that species living in the open sea should not be ignored in this respect. An analogy might be made with the large marine mammals, which are well known to accumulate lipophilic materials.

Marine fish have broadly similar endocrinology and physiology to freshwater species, so the lack of major differences is not unexpected. There are, however, some indications from this review that interference with the hormone systems controlling osmoregulation and smoltification may be an important factor in some euryhaline or marine species. In particular, the discovery that pollutant-induced disruption of the thyroid system can occur in marine fish opens up the possibility not only of impaired neural development, etc., but also of disrupted ionic balance in euryhaline species, and of potential interference with the metamorphosis of flatfish larvae. To the author's knowledge, these subjects have as yet received scant attention.

Protoandrous and protogynous species (which are relatively common in the sea) may also be at risk from endocrine disruptors that could trigger sex changes at inappropriate times. This possibility has not been investigated under natural conditions, but preliminary experimental work with the protogynous cuckoo wrasse (*Labrus bimaculatus/mixtus*) (Hylland in [74]) has shown that high doses of methyl testosterone (MT) were able to prevent the color changes associated with natural masculinization. This apparently paradoxical result was probably caused by the aromatization of MT to estrogen, but it suggests that further work on such species under polluted conditions might be worthwhile. Similar com-

ments apply to marine protoandrous species such as the black porgy (*Acanthopagrus schlegeli*), which can be reversibly sex-changed by exposure to exogenous estrogens [114].

None of the available marine data have yet made a credible connection between the plethora of changes in individual fish caused by endocrine disruptors, and potential impacts on fish populations. If for no other reason than that marine fish form the basis of many important fisheries, this is a serious information gap. In particular, there is a need for experimental studies of free-breeding fish populations to establish the degree to which they are stable to a variety of endocrine-induced disturbances at the individual level. This endeavor would be assisted by the development of suitable population models, although their value would be limited by the availability of species-specific life history parameters.

In summary, the following research recommendations flow from this review:

- Experimental studies of free-breeding fish populations are required to investigate their stability to perturbation by endocrine disruptors.
- The potential endocrine-disrupting effects of bioaccumulated lipophilic substances, especially in long-lived pelagic fish species, need further research.
- More field studies of marine fish are needed to discover the importance of disruption of the adrenal and thyroid systems.
- The potential for interference with the endocrine control of osmoregulation and smoltification is worthy of further study.
- The likely impact of sex steroids and their mimics on naturally hermaphrodite, protogynous, and protoandrous marine fish species should be investigated in the field.

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