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Topic 4.3
Endocrine disruption in wild freshwater fish*

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Abstract: Endocrine disruption has been reported in freshwater fish populations around the world. This phenomenon ranges from subtle changes in the physiology and sexual behavior of fish to permanently altered sexual differentiation and impairment of fertility. Despite widespread reports of endocrine disruption in fish (and this is well characterized at the individual level), few studies have demonstrated population-level consequences as a result of exposure to endocrine-disrupting chemicals (EDCs). An exception to this is in Lake Ontario Lake trout where precipitous declines in the population have been linked with periods of high exposure to organochlorine chemicals (known EDCs). Recently, it has been established that roach (*Rutilus rutilus*) exposed to treated sewage effluent (that contains complex mixtures of EDCs) in UK rivers, have a reduced reproductive capacity. This, in turn, *may* have population-level consequences.

Evidence for a link between exposure to effluents from kraft mill (BKME) and sewage treatment works (STW) and altered reproductive function in freshwater fish is compelling. In most cases, however, a causal link between a specific chemical and a physiological effect has not been established. Indeed, identifying specific chemical(s) responsible for adverse effects observed in the wild is difficult, given that tens of thousands of man-made chemicals enter the aquatic environment and that mixtures of chemicals can have combination (e.g., additive) effects. Some EDCs are known to act at a number of different body targets to affect a variety of physiological processes, further complicating the identification of the causative agent(s).

Endocrine disruption appears to be particularly widespread in freshwater fish populations. There is little evidence, however, to suggest fish are more susceptible to EDCs relative to other wildlife. Notwithstanding this, there are some features of the endocrine physiology of fish that may be particularly susceptible to the effects of EDCs, including the processes of sex-determination and smoltification (in salmonids). Furthermore, their aquatic existence means that fish can be bathed constantly in a solution containing pollutants. In addition, uptake of chemicals readily occurs via the gills and skin, as well as via the diet (the major exposure route for most EDCs in terrestrial animals). The exposure of fish early life stages to the cocktail of EDCs present in some aquatic environments may be of particular concern, given that this is an especially vulnerable period in their development.

The challenge, from the point of view of ecological risk assessment, is to determine effects of EDCs on freshwater fish populations and freshwater ecosystems. In order to meet this challenge, high-quality data are required on the population biology of freshwater fish, on the effects of EDCs on their various life history characteristics, and comprehensive and ap-

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propriate population models. Basic information on the population biology of most species of wild freshwater fish is, however, extremely limited, and needs significant improvement for use in deriving a sound understanding of how EDCs affect fish population sustainability. Notwithstanding this, we need to start to undertake possible/probable predictions of population level effects of EDCs using data derived from the effects found in individual fish. Furthermore, information on the geographical extent of endocrine disruption in freshwater fish is vital for understanding the impact of EDCs in fish populations. This can be derived using published statistical associations between endocrine disruption in individual fish and pollutant concentration in receiving waters. Simplistic population models, based on the effects of EDCs on the reproductive success of individual fish can also used to model the likely population responses to EDCs. Wherever there is sufficient evidence for endocrine disruption in freshwater fish and the need for remediation has been established, then there is a need to focus on how these problems can be alleviated. Where industrial chemicals are identified as causative agents, a practical program of tighter regulation for their discharge and/or a switch to alternative chemicals (which do not act as EDCs) is needed. There are recent examples where such strategies have been adopted, and these have been successful in reducing the impacts of EDCs from point source discharges on freshwater fish. Where EDCs are of natural origin (e.g., sex steroid hormones from human and animal waste), however, remediation is a more difficult task. Regulation of the release of these chemicals can probably be achieved only by improvements in treatment processes and/or the implementation of systems that specifically remove and degrade them before their discharge into the aquatic environment.

INTRODUCTION

Endocrine disruption has been reported in freshwater fish populations in various parts of the world [reviewed in 1,2]. This phenomenon ranges from subtle changes in the physiology and sexual behavior of fish, to permanently altered sexual differentiation and impairment of fertility. Most of the data comes from studies in Europe and America, although evidence for endocrine disruption in freshwater fish has also been reported in Australia [3] and Japan [4]. Biological effects in wild freshwater fish that have been attributed to the effects of endocrine disruptors include the inappropriate production of the blood protein vitellogenin (VTG; the female-specific and estrogen-dependent egg yolk protein precursor) in male and juvenile fish, inhibited ovarian or testicular development, abnormal blood steroid concentrations, intersexuality and/or masculinization or feminization of the internal or external genitalia, impaired reproductive output, precocious male and/or female maturation, increased ovarian atresia (in female fish), reduced spawning success, reduced hatching success and/or larval survival, altered growth and development (thyroid hormone-like effects) and alterations in early development (altered rate or pattern) [2]. These effects may arise due to disruption of a range of endocrine-mediated mechanisms (including receptor-mediated processes, and/or interference with steroid metabolism and/or excretion), although nonendocrine toxicity could also explain some of these effects. Overall, current scientific evidence strongly suggests that certain effects observed in freshwater fish can be attributed to cocktails of chemicals that mimic and/or disrupt hormone function/balance. In most cases, however, the evidence of a causal link between a specific physiological disruptor and a specific effect is weak, largely due to the fact that freshwater fish and, indeed, all other wildlife, are exposed to a wide range of chemicals, that act at a number of different body targets, to affect a variety of physiological processes. Sewage treatment works (STWs), for example, (which often receive domestic, industrial and/or agricultural waste) release a complex (and ill-defined) mixture of natural and synthetic chemicals into the aquatic environment, following their partial or complete biodegradation during the treatment process. It is estimated that 60 000 man-made chemicals are in routine use worldwide and most of these enter the aquatic environment [5]. Identifying specific chemical(s) responsible for adverse effects observed in the wild is, thus, difficult and requires extensive laboratory studies to support the hypotheses drawn from field stud-

ies. Moreover, very recent studies investigating the interactive effects of mixtures of estrogenic chemicals in fish, using vitellogenin induction as an endpoint, have shown that combinations of steroid estrogens, alkylphenolic chemicals and a pesticide (methoxychlor) are additive in their effect [6]. This highlights the fact that even chemicals that are have slight effects on the endocrine system should be taken into consideration when assessing the effects of chemical mixtures in freshwater fish. A weak link in establishing whether observed adverse effects in freshwater fish are caused by exposure to EDCs is the lack of data documenting what freshwater fish are actually exposed to and what they take up into their bodies. Moreover, there is often a large discrepancy between the relatively high levels of pollutants generally used in laboratory studies and the low levels of these pollutants that actually occur in the aquatic environment. Exposures of fish to environmentally relevant concentrations of EDCs (and at the relevant life stages) are essential to adequately evaluate exposure/response relationships in field studies and produce credible risk assessments.

CURRENT EVIDENCE FOR ENDOCRINE DISRUPTION IN FRESHWATER FISH

Although there is a considerable amount of evidence for endocrine disruption in wild freshwater fish, only in a very few cases has a causal link between the presence of EDCs in freshwaters and altered endocrine function in exposed fish populations been demonstrated. In order to determine causality between an EDC and a particular perturbation, clearly, a relationship between exposure to the putative stressor and the effect of concern needs to be firmly established (e.g., decline in the population or reduced fertility). For a chemical to be designated an endocrine disrupter, exposure to the stressor has to result in an endocrine-mediated event (and at the relevant exposure concentration that occurs in the environment) that ultimately results in an effect of concern. In the following section of the review, documented examples of endocrine disruption in wild freshwater fish are described. Those examples for which there is considerable evidence for a link between exposure and effect are described first, followed by cases where the evidence is less convincing and/or where further research is much needed in order to provide a definitive association.

Reproductive abnormalities in freshwater fish living downstream of pulp- and paper-mill effluents

Over the last 10 years, a number of species of freshwater fish in Canada (white sucker, *Catostomus commersoni*; longnose sucker, *Catostomus catostomus*; lake whitefish, *Coregonus clupea formis* [7–15]) and Europe (perch, *Perca fluviatilis*; roach, *Rutilus rutilus*; [16,17]) living downstream of pulp- and paper-mill effluents have been found to exhibit an array of altered features in their reproductive development, including reductions in gonadal growth, inhibition of spermatogenesis, depressed sex steroids, reduced pituitary hormone concentrations, and delayed sexual maturity. In the studies on perch (but not for the suckers) viability of the developing larvae was also affected [18]. Lowered egg production and delayed reproduction have also been induced in fathead minnows in life-long exposures to bleached kraft mill effluents (BKMEs) [19]. Furthermore, the endocrine changes seen in wild fish are less severe during periods of reduced effluent discharge [20] and decrease with increasing distance from the effluent outfalls into the rivers. There is, thus, very strong evidence to suggest that something in the BKME is causing the adverse effects seen. The causative agents responsible for these reproductive effects in fish in Canada and Europe have, however, not been identified [21], although in a very recent in vivo study, using a toxicity identification and evaluation approach, Hewitt et al. [22] were able to provide the first evidence that at least one of the effects (the depression in steroid hormone concentrations) seen in wild fish in the vicinity of pulp mills may be due to products of the degradation of lignin. The authors showed that these chemicals were present in active fractions of the effluent that caused depressions in serum testosterone concentrations in mummichogs both in vitro and in vivo. Moreover, although not proven, other studies have suggested that the reproductive effects may (at least in part) be mediated

through disruption of the process of steroidogenesis, by affecting the availability of cholesterol and pregnenolone and thus impairing steroid production by the gonads [23,24]. Still other in vitro studies suggest that mixtures of both estrogenic (e.g., β-sitosterol, lignans, stilbenes, and resin acids [25,26]) and androgenic chemicals (e.g., stigmastanol and a β-sitosterol degradation products [27]), together with Ah-receptor agonists (e.g., polychlorinated dibenzofurans and thianthrenes, dibenzothiphenes, and diphenyl sulfides), are found in these effluents, and these studies are supported by in vivo studies that show that white suckers, living in the vicinity of BKME discharges, rapidly accumulate chemicals that bind to the estrogen receptor, androgen receptor, and sex steroid binding protein [28]. Another study showed that during the spawning migration of white sucker in Jackfish Bay in Canada, returning fish were found to have altered pituitary function, as determined by depressed levels of luteinizing hormone (LH) in males and females compared with control fish from a reference location [29]. When taken together, the evidence shows clearly that the endocrine disrupters within BKME act at many targets in the hypothalamic-pituitary-gonad axis. Although it has not (thus far) been possible to link endocrine disruption (leading to deleterious effects on reproduction and development) in these various species of fish to a specific chemical or group of chemicals, it is clear that the endocrine effects are clearly linked to the constituents of pulp-mill effluents.

Interestingly, the multiplicity of androgenic-, estrogenic-, and steroidogenesis-inhibiting chemicals in paper-mill effluents reported for BKMEs in Canada has not been reported for BKMEs in Florida, USA. Instead, in Florida, only androgenic effects have been identified. In these studies, development of a male gonopodium was observed in female mosquito fish exposed to BKME (an androgenic effect [30,31]), but no apparent feminizing effects were seen in males. A recent in vitro study by Parks et al. [32] determined that the pulp-mill effluent from a Florida mill exhibited androgenic activity (determined by transcriptional activity of the androgen receptor) at levels sufficient to account for the masculinization of the female mosquitofish. It is not yet known whether the differences in effects of BKME on fish in Florida and Canada are due to differences in species sensitivities, or to different substances discharged into the BKME in Canada compared with that in Florida. Further characterization of the effluents is needed to more fully understand causation. The ecological significance of the physiological effects of BKME are not known, but could be hypothesized to result in the gradual impairment and eventual loss of reproductive function after continued BKME exposure. These seemingly intuitive population-level predictions have not, however, been observed directly in any wild population of fish exposed to BKME. Indeed, some recent evidence suggests the contrary, LeBlanc et al. [33], for example, recently observed a reduction in the intensity and duration of the spawning period in *Fundulus heteroclitus* exposed to BKME in the Mirichami Estuary, New Brunswick, Canada, but they also reported a simultaneous marked *increase* in reproductive investment and increased fecundity in these individuals.

Reproductive abnormalities in freshwater fish living downstream of sewage treatment works discharges

There is considerable (and increasing) evidence for endocrine disruption in freshwater fish populations living in stretches of river downstream of treated sewage effluent discharges in Europe [34–40], Canada and America [41–43] as well as more recent evidence of endocrine disruption in riverine carp in Osaka in Japan [4]. In the original work on freshwater fish, conducted in the United Kingdom, it was established that effluents from treated sewage effluents were estrogenic, inducing the production of vitellogenin, in male fish [44]. Vitellogenin is normally synthesized by the liver in female oviparous (egg-laying) vertebrates in response to estrogen and is sequestered by developing oocytes and stored as yolk to act as a nutrient reserve for the subsequent development of the embryo [45]. The production of VTG, therefore, is usually restricted to females. Male fish however, do contain the VTG gene(s), and exposure to both natural and synthetic estrogens can trigger its expression, resulting in the secretion of VTG in the blood plasma [46]. Vitellogenin is now one of the most widely used biomarkers for exposure to estrogen(s) in fish in freshwaters and it has been detected in the blood of both male and juvenile fish in

rivers, lakes and streams contaminated by effluents from STWs and/or mixtures of estrogens [reviewed in 47]. Although almost all effluents tested in the United Kingdom have been shown to be estrogenic, causing induction of VTG in exposed male fish, there are some STW effluents in the United States that do not appear to be estrogenic to fish (they do not induce VTG [48,49]), probably due to the large dilution that occurs when the effluent reaches the receiving river and/or to the more extensive sewage treatment processes that are in place at these sites.

In addition to VTG production, exposure to treated sewage effluents has also been associated with deleterious effects on gonad differentiation and development [3,4,34–43,50] in various species of fish and with the abnormal development (feminization) of secondary sexual characteristics in male mosquitofish (*Gambusia affinis*) in Australia [3]. The most thoroughly studied effects are concerned with the widespread incidence of intersex reported in some species of freshwater fish in the United Kingdom, parts of Continental Europe, and the United States. Freshwater fish species in which an occurrence of intersex has been reported and deemed to be abnormal include the roach (*R. rutilus* [37]), bream (*Abramis abramis* [34,35]), the chub [38], gudgeon (*Gobio gobio* [36]), the barbel (*Barbus plebejus* [39]), the perch (*Perca fluviatilis*), the stickleback (*Gasterosteus aculeatus* [40]), and the shovelnose sturgeon (*Scaphirhynchus platyorynchus* [41]). Intersex as a consequence to exposure to effluent has been most intensively studied in the roach, a cyprinid fish common throughout lowland rivers in the United Kingdom and Europe. At some river sites downstream from large STW discharges in the United Kingdom, all of the "male" roach population has been reported to be intersex [37]. Intersex roach often have both male and female reproductive ducts, and many also have female germ cells (oocytes) within a predominantly male "testis". The number, pattern, and developmental stage of oocytes within testicular tissue in intersex roach vary greatly; the condition ranges from the presence of single primary oocytes scattered randomly throughout testicular tissue in a mosaic fashion, to a condition in the more severely feminized fish, where large areas of ovarian tissue occur that are clearly separated from testicular tissue [50]. Intersex roach also often have an altered endocrine status (altered plasma sex steroid hormone concentrations), and an elevated concentration of plasma VTG relative to normal male fish [51] and gonadal growth is often inhibited in severely intersex roach. More recent studies suggest that intersex roach (*R. rutilus*) also have impaired fertility relative to normal male fish from reference sites. Small numbers of wild roach in UK rivers were found that could not produce any gametes at all due to the presence of severely disrupted gonadal ducts [52]. Fertilization and hatchability studies have further shown that intersex roach (even with a low level of gonadal disruption—"mildly intersex") are compromised in their reproductive capacity and produce fewer offspring than fish from uncontaminated sites. In these studies, an inverse correlation was demonstrated between reproductive performance (defined as the ability to produce viable offspring) in intersex roach and severity of gonadal intersex. This, in turn suggests that the intersex condition is quite likely to have population level consequences, although further studies on wild populations are necessary to confirm or refute this.

In contrast to the effects observed in male roach, effects in female roach living in rivers contaminated by treated sewage effluents in UK rivers were less obvious [52]: There was a higher incidence of oocyte atresia and a slight, but statistically significant, lower fecundity in effluent-exposed fish compared with females from the reference sites. Interestingly, at some river sites, small proportions (up to 14 %) of the adult female fish (aged between 3 and 7 years) were sexually immature or sexually indifferent, and, although not proven, it is possible that these effects are also due to endocrine disruption.

There is substantive evidence (principally from lab-based studies, see below) to support that hypothesis that gonadal disruption in wild freshwater fish, inhabiting rivers that receive treated sewage effluents, is caused by estrogenic substances contained within these effluents. Moreover, the statistical associations between the various gonadal abnormalities that occur in wild freshwater fish and plasma VTG concentrations [34,37,51], adds further weight to the evidence that suggests these effects are all caused by estrogenic factors within the effluent.

Analyses of treated sewage effluents using toxicity, identification, and evaluation (TIE) approaches have shown that estrogens and their mimics are present in most, if not all, treated sewage ef-

fluents [53–55]. Studies in the United Kingdom have indicated that alkylphenolic compounds (e.g., nonylphenol, NP) and low levels of natural and synthetic steroidal estrogens (estradiol-17β estrone and 17α ethynylestradiol) are the primary estrogenic constituents of sewage effluents [53]. Moreover, laboratory studies have shown that the concentrations of the 17α ethynylestradiol, estradiol-17β, and estrone [6,44,55–58] or (in some industrial effluents) alkylphenolic chemicals [6,52], present in STW effluent in England are sufficient to explain the induction of vitellogenin synthesis in caged fish placed close to effluent discharges. Many rivers (in which the fish live) contain more dilute STW effluent, and thus the concentrations of estrogens in these rivers may not be high enough to induce plasma VTG that is seen in wild fish (based on short-term exposures). Longer-term exposures of freshwater fish to effluents have, however, been shown to reduce the threshold level for effect; a study by Rodgers-Gray et al. [59] found that exposure of roach to a STW effluent for 1 month induced a vitellogenic response at an effluent concentration of 37.9 +/– 2.3 %, but at an effluent concentration of only 9.4 +/– 0.9 %, after a 4-month exposure. The abnormal occurrence of VTG in wild freshwater fish is thus likely to occur, in many cases, as a result of long-term exposure to mixtures of estrogens present in effluents. It is probable that natural and synthetic estrogens, and in some instances, alkylphenolic chemicals found in STW effluents, also cause the effects on gonadal development and differentiation, and play a part in the evolution of intersexuality in wild fish; both groups of chemicals have been shown to do this under laboratory conditions. Concentrations of steroid estrogens and/or xenoestrogens required to induce these effect on the gonad, however, are higher than found in most effluents [e.g., 60–62], with the exception of some highly polluted rivers, and/or in times of drought (when river flow is low and the contribution made by effluent is high). Few studies have investigated whether environmentally relevant concentrations of estrogens within effluents, or indeed, the effluents themselves can cause the effects on the gonad duct seen in wild freshwater fish populations. In a study in which juvenile roach were exposed to a treated sewage effluent, it was proven that feminization of the development of the gonadal duct (prevalent in wild roach in UK and European rivers) occurs as a consequence of exposure to treated sewage effluent during the period of sexual differentiation [63]. Furthermore, a lab-based study has shown that gonad duct disruption can be induced in fish exposed to ethinyloestradiol at a concentration found in some treated effluents, when the exposure occurs during early life [64]. Although it is theoretically possible to produce an intersex or sex-reversed fish by exposure to sex steroid hormones or alkylphenols (usually during early life), in relatively short-term exposures, even higher concentrations are required to do so than for those inducing duct disruption. Furthermore, induction of altered sex cell development has not been shown in fish exposed to sewage effluents in controlled experiments. The reasons for this might be that the effluents used for the exposures [63] did not contain a sufficient concentration of the causative agent(s) and/or that the appropriate life stages have not been exposed and/or that the fish were not exposed long enough to cause this effect (the maximum duration of these exposures was 4 months). In our own unpublished studies on wild roach, we have found a positive correlation between the age of the fish (length of the exposure) and the severity of the intersex condition. This suggests that in real exposure scenarios (such as roach living in an effluent contaminated river in the United Kingdom), the longevity of the exposure might be of greater importance for disruptions in sex cell development (inducing oocytes in the testis), than the window in development during which the exposure occurs. In support of this hypothesis, NP has been shown to induce ovo-testes in the medaka, at a concentration of only 17 μg/l in the water when the exposure was life-long [65].

In summary, it seems that exposure of freshwater fish in the wild to natural steroidal and synthetic estrogens and, in some instances, alkylphenols cause inappropriate VTG induction and disruptions in the development of the reproductive ducts. Although not yet proven, it seems likely that these chemicals are also responsible for (or at least significantly contribute to) the occurrence of oocytes in the testes of male fish, for retarded testicular and ovarian development and delayed maturation.

Early life stage mortality syndrome and blue sac disease

There are very few studies that have demonstrated that freshwater fish are being impacted at the population level by exposure to a specific chemical (including EDCs). One such case, however, is for lake trout (*Salvelinus namaycush*) living in Lake Ontario where exposure to tetrachlorodibenzodifuran (TCDD) and coplanar polychlorinated biphenyls (PCBs) caused population declines because of negative impacts on reproductive success and early life survival [reviewed in 66]. The organochlorines induced a condition called blue sac disease, which is characterized by yolk sac edema, hemorrhaging, cranofacial abnormalities, and mortality in early larval development. Lake trout populations in Ontario declined precipitously during the 1950s when environmental concentrations of organochlorine chemicals were the at their highest. Subsequent, retrospective studies (based on measured PCB, PCDF, and PCDD residues in dated sediment cores) have established a strong relationship between the concentrations of TCDD, PCDDs, and PCDFs and the observed historical trends in lake trout reproduction, including the more recent signs of successful reproduction [67,68]. Laboratory studies have also shown that exposure to Ah (aryl-hydrocarbon) receptor agonists, including TCDD and coplanar PCBs, induces blue sac disease [69], but there is no evidence to show that these effects occur through an endocrine-mediated mechanism.

Reduced hatching success, low embryo survival, and slower rates of development in fry have also been reported in lake trout in the Great Lakes [70,71] and in Arctic char (*Salvelinus alpinus*) in Lake Geneva [72] and causally linked with exposure to coplanar PCBs, TCDDs, and PCDFs. Other conditions found in the Great Lakes fish during the 1960–1980s including early mortality syndrome and (in Baltic salmon) M74, resulting from thiamine deficiency, were thought to have a chemical etiology [73]. Like blue sac disease, M74 affects fry and is characterized by a loss of equilibrium, spiral swimming, lethargy, hemorrhaging, and death. There are data correlating incidences of M74 in Baltic salmon to elevated body burdens of PCDFs and coplanar PCBs and DDT [e.g., 74,75]. In none of these examples on fish in the Great Lakes, however, is there sufficient evidence to link the effects seen to a specific endocrine disruptor and/or their mixtures. Furthermore, the mechanisms via which these effects occur are generally unknown, and thus ascribing these effects to endocrine disruption at this time would be inappropriate.

Thyroid dysfunction in Great Lakes fish

Alterations in thyroid function have been reported in several wild populations of fish as a consequence of disruptions in their endocrine systems. Epizootics of thyroid hyperplasia and hypertrophy (affecting the whole population) have been reported in various species of salmonids in heavily polluted regions of the Great Lakes in the United States [76–79]. Although enlargement of the thyroid gland can occur as a result of iodine deficiency in the diet, this has been ruled out as a causative factor in the case of these salmonids. It was originally hypothesized that organochlorine contaminants, functioning as EDCs might be responsible for these effects [79]. Studies in the laboratory have shown that goiters and depressed thyroid hormone concentrations can be induced in rodents fed with contaminated fish from the Great Lakes, although fish fed with the same contaminated fish did not develop thyroid lesions [80,81]. Laboratory-based studies, however, have failed to identify the causative chemicals of these thyroid disruptions in the wild fish [reviewed in 82]. In summary, more than 40 years after the discovery of the thyroid dysfunctions in salmonids in the Great Lakes, although a chemical etiology has been established, the mechanism (endocrine, or otherwise) via which these effects occur is still uncertain. Very recently, thyroid abnormalities were also reported in mummichogs (*Fundulus heteroclitus*) from a polluted site (Piles Creek, New Jersey, USA) in the United States [83]. These effects have been loosely associated with exposure to a range of contaminants, especially mercury and petroleum hydrocarbons. When taken together, these studies suggest that thyroid function in fish appears to be sensitive to contaminant exposure generally.

Disruptions in adrenal physiology

There is a limited amount of evidence to suggest that environmental contaminants chronically stress fish, resulting in a compromised responsiveness of the HPI axis [84–89]. For example, Hontela et al. [84–86] demonstrated that yellow perch (*Perca flavescens*) and Northern pike (*Esox lucius*) from sites in Canada contaminated with heavy metals, PCBs, and PAHs were unable to produce cortisol in response to acute handling stress. Moreover, the adrenocorticotrophic hormone (ACTH)-producing cells (corticotrophs) in these fish were severely atrophied. Other studies by Hontela [87] have shown that both corticotrophs and the interrenal steroid producing cells undergo atrophy when fish are exposed to PAHs, PCBs, and heavy metals. It was speculated by the authors that the atrophy of the cells was a result of prolonged secretory hyperactivity of the cells. This hypothesis was later supported by studies on brown trout living in metal-contaminated waters that were shown to be hyper secreting ACTH and corticotrophin-releasing hormone [88,89]. More research is necessary to establish if the effects seen on the interrenal axis during exposure to specific contaminants have consequences to the health of affected fish populations.

ARE FRESHWATER FISH MORE SUSCEPTIBLE TO ENDOCRINE DISRUPTORS THAN OTHER ANIMALS?

Endocrine disruption appears to be particularly widespread in freshwater fish populations. There is little evidence, however, to suggest that fish are more susceptible to EDCs relative to other wildlife. Indeed, the evidence available on receptor binding affinities for chemicals that mimic sex steroid hormone, thyroid, and retinoic acid receptors suggests that vertebrates are likely to be similarly sensitive to environmental EDCs. Furthermore, there are many more similarities between the endocrine systems of fish and other higher vertebrates, notably with respect to the nature of the hormones, their receptors, and in the regulatory control of their endocrine system [7]. Notwithstanding this, there are more than 10 000 species of freshwater fish worldwide, displaying a high degree of heterogeneity in their physiology, anatomy, behavior, and ecology, and there are some features of the endocrine physiology of freshwater fish that may be particularly susceptible to the effects of EDCs, including those that determine sex (sex determination in fish has been shown to be especially sensitive to steroid hormones) and the process of smoltification in salmonids.

Living in the aquatic environment, fish can be bathed constantly in a solution of chemical pollutants. Furthermore, uptake of chemicals into fish can readily occur via the gills and skin, as well as via the diet (the major route of exposure to EDCs in terrestrial animals) [90]. Features of the gills including thin epithelial membranes and a large surface area coupled with the relatively high ventilation rates that occur in fish, facilitate the uptake of compounds from the water and their transfer into the blood stream. Some freshwater fish species are also top-predators and thus, are likely to bioconcentrate EDCs to a greater degree than other organisms at lower trophic levels. Freshwater fish are hypo-osmotic with their surroundings and thus a considerable movement of water into their bodies occurs down an osmotic gradient (taking chemicals with it). A major route of exposure to EDCs in fish during early life is from contaminants that have accumulated in lipid reserves within the egg as a consequence of maternal transfer during ovary development. These contaminants that have accumulated in the egg are mobilized when the lipid reserves are metabolized to fuel embryo development, exposing early life stages to especially high concentrations of EDCs at a time of greatest vulnerability to disruptions in their developing endocrine system. Furthermore, early life stages of fish have a limited capacity to metabolize and excrete contaminants, including EDCs. In situ exposures of fish have been used to assess both the bioavailability of EDCs, contained within complex mixtures, such as treated sewage effluents, and to determine non-point sources of pollution (agricultural run-off) and their biological effects. In such studies on rainbow trout, Larsson et al. [91] reported significant bioconcentration factors (in bile) for natural and synthetic sex steroid hormones (17β-estradiol, estrone, 17α ethinylestradiol) of up to 10 000-

fold after a 3-week exposure, whereas xenoestrogens (e.g., nonylphenol and bisphenol) bioconcentrate by several hundred to 1000-fold. Hewitt et al. [28] similarly obtained evidence for a very rapid uptake of EDCs in fish exposed to BKME, but here they also demonstrated that a rapid depuration of these chemicals occurs too. Apart from these two studies, however, there is very little information on the bioavailabilty of EDCs in wild fish or caged fish exposed to effluent discharges.

Another important issue that complicates determination of cause–effect relationships for EDCs, that is sometimes overlooked, is the possible time lag between the time of exposure and the biological response. Fish for example, living in the vicinity of sewage effluent outfalls will accumulate harmful contaminants in their tissues which may not cause any immediate deleterious effects, but which might affect the embryo development of their subsequent offspring. The biological responses in freshwater fish are very often especially influenced by physical environmental features, and concentrations of contaminants in the aquatic environment can vary widely temporally, and hence responses and effects may vary with season. All of these considerations are rarely taken into account in the analysis and interpretation of field-simulated exposures.

SCALE OF THE PROBLEM OF ENDOCRINE DISRUPTION IN FRESHWATER FISH

Endocrine disruption has only been studied in a small proportion of freshwater fish species, and data on cyprinids and salmonids dominate the literature. The differences in the sensitivities of different fish species to the effects of EDCs has not been comprehensively examined, although studies on the effects of pulp mill [92] and STW effluents [93], respectively, suggest that inter-species differences in sensitivity are likely to exist, between some fish species. Moreover, given the fact that endocrine disruption is commonly associated with exposure to effluents from domestic or industrial processes that enter rivers and streams, it seems likely that endocrine disruption in freshwater fish is more widespread than is currently documented. In the United Kingdom, for example, there are more than 70 000 consented discharges and 6500 of these are STW. Worldwide, each year, more than 5000 km^3 of water are used [94], and this figure is increasing every year. Furthermore, in some rivers, at times of low flow, up to 80 % of the river volume is made up of STW effluent discharge and this figure can be even higher in periods of drought. Using established statistical associations between endocrine disruption in freshwater fish and effluent dilution in receiving waters, theoretical predictions of the geographical extent of endocrine disruption can be estimated. In the United Kingdom, our own unpublished predictions, based on a statistical association between the concentration and dilution of the sewage effluent and the degree of feminization in wild fish exposed to the effluent, indicate that intersex fish are likely to exist at more than 50 % of 464 river sites that have effluent discharges with a population equivalent of more than 10 000. These predictions can be made using simple associative data from surveys of endocrine disruption in fish from a limited number of rivers (eight, in this case). Obviously, the more rivers from which one collects data, the better the predictions are likely to be. In our studies on roach in eight UK rivers, the following equation has been derived linking intersex in roach with effluent concentration in the rivers from where they were sampled: $\log (y + 1) = (0.000\,002\,88 * x) + 0.203$ where y = the intersex index and x = the concentration of effluent in the river (calculated by dividing the population equivalent of the effluent by the dilution factor for that effluent upon its entry into the river). The intersex index is a numerical index used to describe the degree of feminization of the gonads, based on their histological appearance [37]. The results from the UK analyses in roach for predicting intersexuality are illustrated in Fig. 1. This predictive map is now being validated, through determining the actual (observed) incidence of intersex at 46 of these study sites.

Additional studies in the roach have shown that the intersex index is correlated with fertility of intersex fish and hence, a predictive map of gamete quality can be constructed using the information on the intersex index and its relationship with fertility. Using this approach, we have estimated that at approximately 13 % of the 464 river sites selected for study in the United Kingdom (that receive effluent discharges with a PE of more than 10 000), the degree of intersexuality is estimated to be severe enough

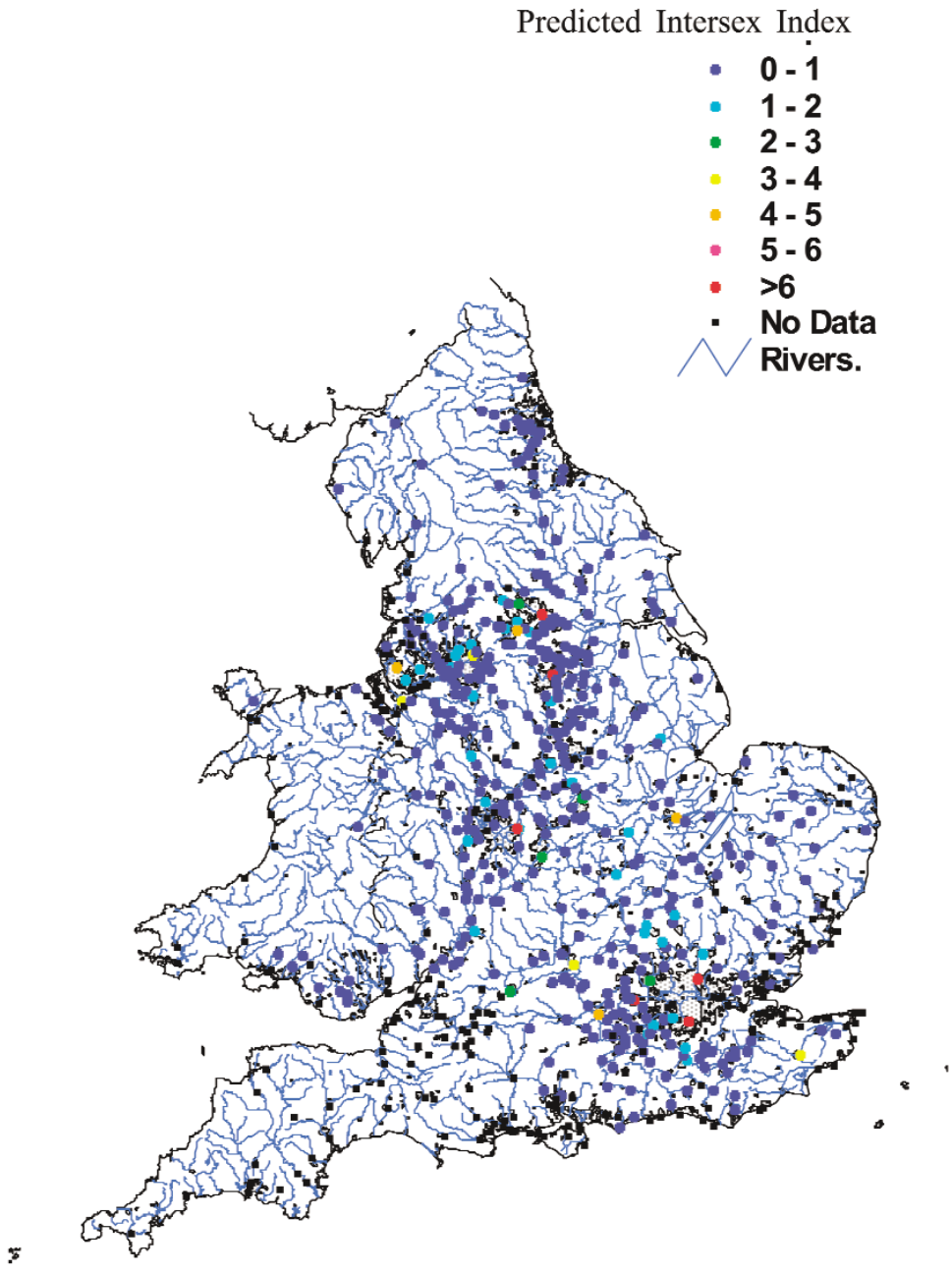

Fig. 1 Predicted intersex indices for populations of roach living downstream of sewage treatment works in selected rivers throughout the UK. The results were obtained using the following equation that links intersex in roach with effluent concentration in the rivers from where they were sampled: $\log (y + 1) = (0.000\ 002\ 88 * x) + 0.203$ where y = the intersex index and x = the concentration of effluent in the river (calculated by dividing the population equivalent of the effluent by the dilution factor for that effluent upon its entry into the river). The correlation between the intersex index and sperm quantity and quality predicts the following effects on fertilization success. Intersex index: 0–1 = little effect, 1–3 = slight effects, 3–4 = moderate effects, 4–>6: severe effects.

to have deleterious effects on gamete quality (fertilization success of intersex individuals would be predicted to be less than 60 %). At a further 16 % of the sites, the gamete quality of intersex and/or male fish would be predicted to be impaired, relative to male fish from reference sites. This system could be extended to other fish populations exposed to sewage and other types of effluent in order to provide a predictive map of the reproductive effects of endocrine disruption in freshwater fish populations worldwide.

Notwithstanding this, in any predictive study of ED in freshwater fish, it is important to establish the influence of age or longevity of exposure upon the effect that one is measuring. Our own investigations into the inter-relationships between age and intersexuality, for example, suggest that the intra-site variability in the degree of feminization that one observes in wild intersex roach is severely influenced by the ages of the fish collected, as older fish are more feminized than their younger counterparts (Jobling, unpublished data). Consequently, a perceived difference in the incidence and severity of intersex in fish collected from several different sites may be due to the differential age distributions of the fish sampled, rather than to differences in the endocrine-disrupting potencies of the various waters from whence the fish were collected.

POPULATION-LEVEL EFFECTS OF ENDOCRINE DISRUPTION

Much of the research on the effects of EDCs in freshwater fish has focused on effects at the individual level. A major challenge, from the point of view of ecological risk assessment, is to determine effects of endocrine disruption on populations and ecosystems. In order to meet this challenge, high-quality data on the population biology of freshwater fish, effects of EDCs on their various life history characteristics, and comprehensive and appropriate population models are needed. Basic information on the population biology of most species of wild freshwater fish is, however, extremely limited and it needs significant improvement for use in deriving a sound understanding of how EDCs affect fish population sustainability. Population growth rate in fish is determined by the balance between birth rate and the mortality rate. Collective fecundity and mortality thus predict the population's fate. In a large population with a stable age structure and sex ratio, future population size can be predicted from life table and fecundity data. This assumes, however, that each individual has an equal chance of contributing genes to the next generation, and this rarely happens in practice due to unequal sex ratios, differences in individual fertility, nonrandom mating, and variation in age structure. All of these factors influence the number of breeding individuals and hence, variation in the effective population size. In fish, juveniles are often not recruited to the adult population until they are 2–4 years old, and hence juvenile mortality and the rate of sexual maturity have a major bearing on the number of breeding individuals. Various external factors enhance population growth and others limit, and even prevent, population growth, and many of these are dependent on the density of the population. The most common density-dependent factors that limit population growth are food supply, space, predators, disease, and parasitism. Population-limiting factors in fish that are independent of population density include abiotic factors such as drought or floods.

Many of the parameters affecting fish population growth rate and sustainability are difficult to measure accurately in the field and are consequently, poorly understood. Whilst endocrine disrupters are known to affect factors such as individual fertility, and rate of sexual maturation and fecundity, a thorough assessment of these effects would require very extensive studies on the general life history and population biology of the exposed species compared with a reference population. Moreover, population declines are not usually caused by only one factor alone, but occur because of the effects of a multitude of factors.

Notwithstanding this, there are some examples in freshwater fish where there is substantial information on the consequences of endocrine disruption—on key reproductive parameters at the *individual* level, either from studies of wild populations, or from laboratory studies in which fish have been exposed to concentrations of EDCs known to be present in freshwaters. In the roach, for example, UK

studies have provided sufficient evidence to show that widespread intersexuality, as a result of exposure to estrogenic sewage effluents, results in reduced fertility; there is a negative correlation between the degree of feminization of the intersex fish and their fertilization success ($r = –0.603$; $p < 0.001$). This information could be used albeit simplistically, to model the likely population responses to endocrine disruptors in the wild (in the absence of other factors that might also affect the population). Basic population models are already available, for this purpose. In a recent paper, Gleason and Nacci [95] attempted to model the effects of exposure to 17β-estradiol on populations of fathead minnow based (based on laboratory studies that showed egg production by females was negatively correlated with plasma VTG concentrations in the exposed males). The model predicted a negative linear correlation between the population growth rate of populations of fathead minnow and plasma vitellogenin production in males. Although these predictions are based on simple density-independent population models that require verification in realistic settings, they nevertheless provide a starting place for projecting population responses to EDCs from lab-based studies.

RECOMMENDATIONS

This account illustrates that there is very good evidence for endocrine disruption in freshwater fish. In order to more comprehensively assess the importance of endocrine disruption in freshwater fish, however, it will be necessary to put endocrine disruption into context with other environmental pressures that face freshwater fish populations. In our opinion, this will require research to define the global extent of the problem by expanding studies of endocrine disruption to other parts of the world and studies to extrapolate effects on individual fish to predict effects on populations and higher levels of biological organization.

Assessment of the extent of the problem of endocrine disruption in freshwater fish requires a more widespread sampling of a variety of wild populations of fish, ideally using nondestructive sampling methodologies and biomarkers. For biomarkers to be meaningful in this regard, efforts need to be directed at determining how they are related to the health of both individuals and populations. The biomarkers available for monitoring endocrine disruption are rather limited, and development of novel biomarkers should be encouraged to extend beyond those for estrogenic effects, with an emphasis on biomarkers that are indicative of reproductive and/or developmental effects and/or population responses. The presence of VTG in male fish, for example, is known to be negatively correlated with testicular growth and maturation [96]. In intersex fish, VTG is positively correlated with the degree of gonadal feminization [51], and hence also with their perceived reproductive success (which declines with increased degree of feminization [52]). A widespread assessment of VTG concentrations or of the degree of gonadal feminization in freshwater fish in a particular catchment could thus provide predictive information on the likely state of the testes or of their likely reproductive success, respectively. Vitellogenin concentration, however, could not (on its own) be used to directly predict the perceived fertility of a population of fish because even male fish, when exposed to estrogen for short periods of time, exhibit elevated plasma VTG concentrations, and little is known about the relationship between the timing and longevity of exposure to estrogen and the manifestation of gonadal feminization. Predictive maps and models thus need to be interrogated (by conducting both field and lab studies) to establish their validity. Information on what extent freshwater fish are exposed to EDCs in the wild is lacking, and hence more information is needed on what EDCs (and their concentrations) are present in the environment and to what extent they are absorbed and metabolized by freshwater fish. Moreover, the responses of fish to environmentally relevant mixtures of chemicals (containing EDCs) require further study and understanding.

Even with more widespread field data, research on endocrine disruption in wild freshwater fish is likely to be limited to those species in which large numbers of individuals are easily obtainable. A more global assessment of endocrine disruption in freshwater fish should ideally include the more rare and vulnerable species, although this is less practical. Current research on endocrine disruption in freshwa-

ter fish is limited to studies on a very few species, and there has been little or no attention given to the comparative sensitivities of different species of fish to EDCs, or indeed, of other animal species, on which freshwater fish may be dependent (e.g., many invertebrate species). Furthermore, current risk assessment strategies for endocrine disruption in freshwater fish are based on the responses of laboratory fish species and are unlikely to represent the full range of fish species that may be at risk in the wild. Laboratory studies will, therefore, need to be targeted at species with different life histories and different reproductive strategies, in order to compare the sensitivities of different fish species to EDCs and their mixtures. Moreover, an assessment of the implications of endocrine disruption on wild freshwater fish will require a comprehensive understanding of their physiology, endocrinology, and population biology, and thus a further recommendation for future research is to develop this information for sentinel species.

Wherever there is substantive evidence for endocrine disruption in freshwater fish, and the need for remediation has been established, there is a requirement to focus on how these problems can be alleviated. Where high quantities of industrial chemicals are used that are known to cause/contribute to endocrine disruption in freshwater fish, a program of tighter regulation for their discharge and/or a switch to alternative greener chemicals (which do not impact the endocrine system) is needed. The success of such programs can be illustrated by schemes implemented in both the United States and United Kingdom that have reduced the concentrations of EDCs discharged (either as a consequence of changes in industrial processes [97–98], or due to closure of a treatment plant [30]), which subsequently resulted in concomitant decreases in endocrine disruption in the exposed fish. Many known EDCs cannot, however, easily be eliminated at source, because they are of natural origin (e.g., sex steroid hormones from human and animal waste). For these types of contaminants, regulation of their release is likely to be achieved by improvements in treatment processes and/or the implementation of systems that specifically remove and degrade them. EDCs also enter the freshwater environment through non-point sources, but there has been very little study to assess the risk posed by these sources to freshwater fish. Studies of this nature are also needed.

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