Endocrine disruption, parasites and pollutants in wild freshwater fish

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SUMMARY

Disruption of the endocrine system has been shown to occur in wild freshwater fish populations across the globe. Effects range from subtle changes in the physiology and sexual behaviour of fish to permanently altered sexual differentiation, impairment of gonad development and/or altered fertility. A wide variety of adverse environmental conditions may induce endocrine disruption, including sub-optimal temperatures, restricted food supply, low pH, environmental pollutants, and/or parasites. Furthermore, it is conceivable that any/all of these factors could act simultaneously to cause a range of disparate or inter-related effects. Some of the strongest evidence for a link between an adverse health effect, as a consequence of endocrine disruption, and a causative agent(s) is between the condition of intersex in wild roach (*Rutlius rutilus*) in UK rivers and exposure to effluents from sewage treatment works. The evidence to indicate that intersex in roach (and other cyprinid fish living in these rivers) is caused by chemicals that mimic and/or disrupt hormone function/balance in treated sewage effluent is substantial. There are a few parasites that affect the endocrine system directly in fish, including the tape worm *Ligula intestinalis* and a few parasites from the micropsora phylum. *L. intestinalis* acts at the level of the hypothalamus restricting GnRH secretion (resulting in poorly developed gonads) and is one of the very few examples where an endocrine disrupting event has been shown to result in a population-level effect (reducing it). It is well established that many parasites affect the immune system and thus the most common effect of parasites on the endocrine system in fish is likely to be an indirect one.

Key words: parasite, pollutant, endocrine, fish, disruption, intersex.

INTRODUCTION

Disruption of the endocrine system of fish has become an increasingly important area of research over the past decade. Effects that have been reported in wild fish populations around the globe include compromised growth, disruptions in reproduction and altered sexual development (Kime, 1998; Vos *et al*. 2000). The most thoroughly documented examples of endocrine disruption in wild fish are in roach, *Rutilus rutilus*, and gudgeon, *Gobio gobio*, living in rivers in the UK, where the presence of vitellogenin (VTG) in the blood of male fish (VTG is an oestrogen–dependent, and normally female-specific blood protein – see below) and intersexuality (ovotestis) are widespread (Jobling *et al*. 1998; van Aerle *et al*. 2001). These effects are not restricted to freshwater fish, but have also been documented in estuarine fish such as the euryhaline flounder (*Platichthys flesus*; Allen *et al*. 1999) and the sand goby (*Pomatoschistus minutus*; Matthiessen *et al*. 2002). Studies throughout mainland Europe (Flammarion *et al*. 2000; Vigano *et al*. 2001; Gercken & Sordyl, 2002; Hecker *et al*. 2002), the USA (Folmar *et al*. 1996, 2001; Harshbarger, Coffey & Young, 2000) and in Japan (Hashimoto *et al*. 2000) have similarly documented evidence for feminization of male fish, albeit that the extent and severity generally appears to be less than in the UK. In the USA and Canada, and in Australia, masculinization of wild fish has also been reported (Howell, Black & Bortone, 1980; Munkitterick *et al*. 1991, 1998; McMaster *et al*. 1991; Van Der Kraak, 1992; Batty & Lim, 1999; Bortone & Cody, 1999); in these cases female fish develop secondary sex features characteristic of males. Few studies have shown that the endocrine changes reported in wild fish populations have resulted in adverse reproductive and/or developmental consequences. Furthermore, the effects of alterations in the endocrine system in fish on immunity, longevity or population growth and stability are little understood. Recent studies have shown that wild intersex fish have altered sex steroid hormone profiles (roach and bream, *Abramis brama*; Jobling *et al*. 1998; Hecker *et al*. 2002), and altered spawning time and reduced sperm production (roach; Jobling *et al*. 2002*a*). Furthermore, studies in the roach have shown that these intersex fish are reproductively compromised, producing sperm with poorer motility and with a lower fertilisation success than normal male fish (Jobling *et al*. 2002*b*). This suggests that endocrine disruption in individual fish, potentially, has consequences to fish populations as a whole.

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Non-reproductive alterations in physiological function have also been reported in several wild populations of fish as a consequence of disruptions in their endocrine systems. Epizootics of thyroid hyperplasia and hypertrophy (affecting 100% of the population) have been reported in various species of salmonids in heavily polluted regions of the Great lakes in the USA (see Leatherland & Sonstegard, 1982; Leatherland *et al*. 1989; Leatherland, 1993). Similarly, long-term exposure of flounder in mesocosms to sediments heavily polluted with cocktails of contaminants has been shown to cause reduced retinoid levels in both the liver and the plasma (Besselink *et al*. 1998). Very recently, thyroid abnormalities were reported in mummichogs from a polluted site in the USA (Carletta, Weis & Weis, 2002). When taken together, these studies suggest that thyroid function in fish appears to be sensitive to contaminant exposure.

There are significant gaps in our understanding of the causes of endocrine disruption in wild fish and this has hindered the process of hazard identification. Furthermore, it is now clearly established that cocktails of chemicals can have interactive effects in mediating these endocrine changes in fish. Endocrine disruption in fish, however, does not always have a chemical aetiology and can also arise as a result of exposure to a variety of other adverse environmental conditions and/or factors, including sub-optimal temperatures, restricted food supply, low pH, environmental pollutants and/or parasites. Furthermore, it is conceivable that any/all of these factors could act simultaneously to cause a range of disparate or inter-related effects. This short review is focused on the effects of pollutants and parasites (and the interactions between them) on the endocrine system of fish.

POLLUTANTS AS CAUSATIVE AGENTS OF ENDOCRINE DISRUPTION IN FISH

Some of the major causative agents of endocrine disruption seen in fish are thought to be endocrine disrupting chemicals (EDCS). These are chemicals (including both natural and man-made chemicals) that can interfere with the normal functioning of the endocrine systems in humans and wildlife (Vos *et al*. 2000). Endocrine-disrupting chemicals can act by selectively binding to hormone receptors to generate (agonists) or block (antagonists) hormone-mediated responses. Agonists for the oestrogen receptor, for example, include natural oestrogens such as coumesterol and genistein, oestradiol and oestrone, and pharmaceuticals such as ethinyloestradiol, and industrial chemicals such as DDT, bisphenol-A and nonylphenol. Antagonists of the androgen receptor include metabolites of the fungicide vinclozolin and the DDT metabolite p,p'DDE, and thus they block testosterone-induced cellular responses. EDCs can also act by interfering with hormone synthesis, metabolism or excretion. Compounds that have been shown to alter oestrogen biosynthesis include cyanoketone, ketoconazole, the herbicide atrazine (Sanderson *et al*. 2001), and the fungicide fenarimol (Hirsch *et al*. 1987). Most of the known endocrine disruptors studied to date appear to affect the reproductive system, although there are other environmental agents that are known to alter the thyroid hormone system.

In UK freshwaters, it is thought that endocrine disrupters, present in treated sewage effluents, are responsible for the widespread occurrence of feminization in roach (Jobling *et al*. 1998). Male roach (and other species of fish) living in the proximity of sewage treatment works effluent discharges synthesise and secrete the female specific yolk protein precursor, vitellogenin, and in the more heavily impacted regions, develop ovo-testes. Vitellogenin is normally produced in the liver of female fish as a result of the binding of endogenous oestrogen to the oestrogen receptors found there. VTG is then carried by the blood to the ovary where it is sequestered by the growing oocytes and cleaved to form yolk. Male fish would not normally produce vitellogenin (as they have no ovaries), but will if they are exposed to an exogenous source of oestrogens. The presence of vitellogenin in the blood of male fish is, therefore, a reliable indicator that they have been exposed to an unnaturally high level of oestrogen (which may be derived from an endogenous and/or exogenous source). Chemical fractionation of sewage effluents, together with screening and identification of hormonally active constituents of the effluents using recombinant yeasts containing the human oestrogen receptor, has identified several natural oestrogens (oestrone and oestradiol), the synthetic contraceptive pill hormone ethinyloestradiol (from human and animal waste), as well as oestrogen-mimicking chemicals, such as 4-nonylphenol (derived from the manufacture and use of surfactants), in most of the effluents examined (Desbrow *et al*. 1998). Sex steroid oestrogens are extremely potent at low concentrations. Alkylphenols are comparably weak oestrogens, albeit they are more persistent, and bioaccumulate in animals. The fact that these chemicals are present in treated sewage effluent discharges that continually enter UK rivers means that fish are constantly exposed to them. Laboratory-based studies have shown that both steroid oestrogens and alkylphenols, such as 4NP, are present in effluents at concentrations sufficient to induce VTG synthesis in fish (Sheahan *et al*. 1994; Jobling *et al*. 1996; Routledge *et al*. 1998; Gray, Teather & Metcalfe, 1999) and, for steroid oestrogens, at concentrations close to those that will induce feminization of structural features in the testis (induction of an ovarian cavity; van Aerle *et al*. 2002). Furthermore, it is well established that exposure of fish eggs, embryos or

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young fry to pharmacological doses of steroidal oestrogens can cause partial or complete sex reversal. Similarly, very high concentrations of 4NP will induce gonadal sex reversal in fish (Yokota *et al*. 2001). Whilst this does not prove that there is a direct causal link between intersexuality, seen in wild fish and the presence of these particular chemicals, the weight of evidence is very strong indeed. It is likely, therefore, that oestrogenic chemicals and their mimics are at least partially responsible for the occurrence of hermaphrodite roach in rivers that receive sewage effluents (which almost all do). Notwithstanding this, there are no studies in which a specific endocrine disruption event in wild fish has been causally linked with exposure to a *specific chemical*. Furthermore, effluents are highly complex mixtures of chemicals and studies investigating the interactive effects of binary mixtures of oestrogenic chemicals in fish have shown that combinations of steroid oestrogens, alkylphenolic chemicals and pesticides are additive in their effects. This highlights the fact that even chemicals that are weakly active as hormone mimics need to be taken into consideration when assessing exposure of fish populations to complex mixtures of chemicals such as those found in effluents. Complicating the issue of sexual disruption in wild fish even further is the fact that recent studies have found that effluents from STWs also have androgenic activity (Kirk *et al*. 2002).

PARASITES AS CAUSATIVE AGENTS OF ENDOCRINE DISRUPTION IN FISH

The relationships between parasites and their hosts are complex, and neurohormones, growth factors and hormones play prominent roles in these relationships. In vertebrates, such as fish, parasites may secrete hormones, neuropeptides, or cytokine-like molecules that influence the hosts physiological and immunological responses. Alternatively, the parasites may secrete factors that directly alter the host's hormone levels (Beckage, 1993). Having said all of this, relatively few parasites are known to affect the endocrine system of fish and cause reproductive and/or developmental dysfunction. The best known of these is the tapeworm *Ligula intestinalis*. *Ligula* acts by interfering with the pituitary-gonadal axis of its fish host so that parasitised fish are unable to reproduce (Arme, 1997; Williams *et al*. 1998). During its development, *Ligula intestinalis* grows rapidly to a large size in the fish's body cavity, characteristically distending the abdomen. There are also recent reports of parasite-induced gigantism in a single population of ligulosed roach, although whether these growth effects represent an adaptive response by the host or a manipulation by *L. intestinalis* of the host, remains to be elucidated (Loot *et al*. 2002).

Two other parasites, from the phylum Microsporidia are known to infest the gonadal tissue of fish and can cause endocrine disruption. These parasites, *Pleisistophora mirandellae*, a parasite that infests the gonadal tissue, and Myxozoa (*Myxobolus* sp. a parasite that infests the connective tissue of the gonad and other tissues) are identified by their characteristic spores. *P. mirandellae* has been suggested to be the possible causative agent of intersex roach from Finnish brackish waters (Wiklund *et al*. 1996). These authors report the degeneration of the ovarian tissue and the appearance of testicular tissue in fish infected with the parasite. In our own extensive studies of roach in UK rivers, however, there is no evidence to suggest a link between intersexuality and *P. mirandellae* infection, as the two phenomena have never been found to occur simultaneously in sufficient numbers of fish to present a convincing argument. Infection with *P. mirandellae* is characterized by the appearance of cysts containing spores in both ovarian stroma and in the cytoplasm of the oocytes. The presence of parasitic stages in early development in primary oocytes and mature spores in secondary oocytes (those undergoing vitellogenesis – yolk uptake) provides evidence that the development of the parasite is synchronized with the development of the oocyte. In heavily infected individuals, the host or immune response is so intense that the degeneration of the ovarian tissue occurs causing a reduction in reproductive capacity. Although *P. mirandellae* is a highly specific parasite that normally targets oocytes (Pekkarinen, 1995), infections of testicular tissue have also been recorded. Although the infected testicular cysts did displace the testicular germ cells, the parasite does not infect the germ cells themselves.

In contrast with *P. mirandellae*, *Myobolus* sp. occurs scattered throughout the connective tissue of infected individuals. Indeed, *Myxobolus* sp. in general, display little specificity in the tissues they infect and can be found in the gill lamellae, kidney and other connective tissues within the peritoneal cavity. In addition, the host response to *Myxobolus* sp. is not as pronounced as seen in *P. mirandellae*. Notwithstanding this, there are reports of *Myxobolus* sp. inducing destruction of the ovaries leading to complete castration of parasitised individuals (Gbankoto *et al*. 2001).

When taken together, the various reports of parasitism and endocrine disruption suggest that parasitism can lead to disruption of reproduction and development in some species of fish, although the frequency with which these effects have been reported suggests that parasitism alone could not explain the widespread incidence of endocrine disruption in wild fish populations found in most parts of the world.

THE INTERACTION BETWEEN PARASITES AND POLLUTANTS

The interactions of the immune and endocrine systems means that endocrine disruption and immunotoxicity (and therefore susceptibility to disease/infection) can occur simultaneously, and probably do in many environments where fish are challenged with pollutants, parasites and other environmental stressors (Yada & Nakanishi, 2002). Pollution of the aquatic environment with industrial or agricultural sewage is known as an important immunosuppressing factor resulting in higher susceptibility to infectious diseases (Fatima *et al*. 2001; Lacroix *et al*. 2001; Kollner *et al*. 2002). Recent reports suggest that sewage-caused organic enrichment of sediments may lead to an increased prevalence of infection by parasites in the vicinity of sewage treatment works outfalls (Marcogliese & Cone, 2001). These data, however, are not substantial and were not supported by studies on the immune function of these animals. In conclusion, there are clearly some chemicals and some parasites that alone can cause physiological disturbances as a consequence of inducing disruptions in the hormone balance of fish, and some cases these interactions lead to adverse health effects. To develop a clearer understanding of how parasitism and exposure to pollutants act together to impact the endocrine system in fish needs carefully targeted studies to do so, and to date these have not been forthcoming.

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