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Estrogenic effects in fish in The Netherlands: some preliminary results

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Abstract

Recently, a large-scale field study in The Netherlands has focused on the effects of estrogenic contaminants on feral fish populations. The freshwater bream (*Abramis brama*) and the estuarine flounder (*Platichthys flesus*) were sampled at a large number of locations in the spring and autumn of 1999. Concentrations of the yolk protein vitellogenin (VTG) in blood plasma of male flounders were small at most sites. At two sites, however, moderately elevated concentrations were found in autumn. Both sites were situated in the same industrial harbour zone also receiving effluent from sewage treatment works. At many sites VTG levels in male bream were significantly greater than at the control site. The greatest concentrations were observed in individuals collected from a small stream, close to the discharge of a relatively large municipal waste water treatment plant. This was also the only site where considerable intersex occurred; 37% of male bream exhibited ovotestes. Ovotestis was not observed in any of the male flounder captured. The results from The Netherlands are briefly discussed and compared with the well-known case studies in the UK.

Keywords: Bream; Flounder; Estrogenic effects; Vitellogenin; Intersex; Field study

1. Introduction

In recent years, the presence of endocrine disrupting compounds in the environment has become a major issue of concern from the perspectives of both human health and ecosystem integrity. The Netherlands have a very high population density and support intensive industrial and agricultural activity. This potentially increases the discharge of estrogens and xeno-estrogens into the environment. Although there is some evidence that (xeno-)estrogens are active in the Dutch aquatic environment (Belfroid et al., 1999; Health

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Council of The Netherlands, 1999) relatively little is known about their patterns of occurrence and their effects. For this purpose a large-scale baseline study, the National Investigation into Estrogenic Compounds (Dutch acronym LOES), was set up. This paper summarises some of the findings of the 1999 LOES field survey. The aim was to determine the magnitude and extent of estrogenic effects in feral fish in surface waters, to highlight areas of specific concern and to validate the applied techniques for routine monitoring. The results will be published elsewhere in more detail.

The freshwater bream (*Abramis brama*, Cyprinidae) and the euryhaline flounder (*Platichthys flesus*, Pleuronectidae) were chosen as indicator species. Bream commonly occurs in large freshwater bodies and rivers. Flounder, a flatfish, is a common species in muddy shallow coastal waters, estuaries and large freshwater bodies. Plasma concentrations of the yolk protein vitellogenin (VTG) in male specimens (Sumpter and Jobling, 1995; Tyler et al., 1996) and the presence of gonadal abnormalities were the principal parameters investigated in both species.

2. Methods and materials

Mature male fish were captured in the spring (March 2–April 22) and autumn (September 9–November 1) of 1999. Some 300 bream and 400 flounder were captured at 21 sites. Blood samples were taken and plasma was prepared according to the methods described by AquaSense (2000). Gonads were removed and stored in a formaldehyde solution. VTG in male plasma was analysed using a competitive Enzyme-Linked Immunosorbent Assay (ELISA) in 96-well microtiter plates adapted from Smeets (1999) and Nichols et al. (2001). Details of the method are given by AquaSense (2000). After routine processing and embedding of the tissue samples in paraffin, male gonadal tissue was screened for presence of oocytes, a condition also termed ovotestis.

3. Results

From various studies it can be deduced that VTG concentrations in male fish greater than 1000 ng/ml may be abnormal (e.g. Allen et al., 1999; Harries et al., 1997, 1999; Tyler et al., 1996).

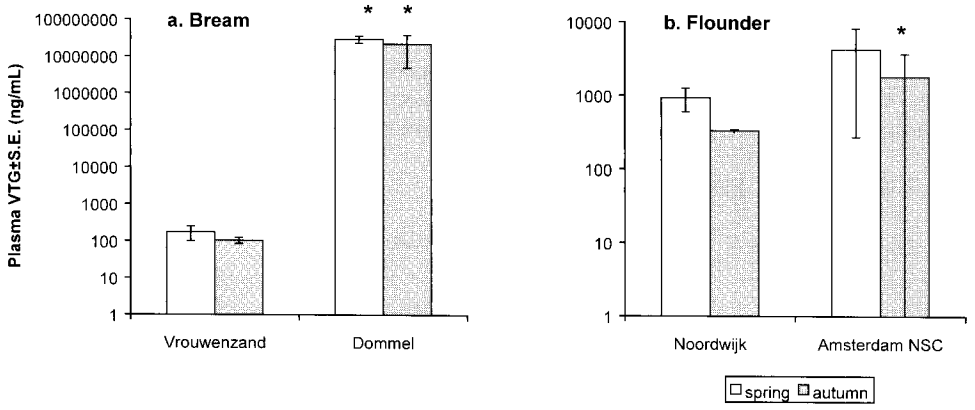

Fig. 1. Plasma vitellogenin concentrations in spring and autumn in (a) male bream from a clean reference site (Vrouwenzand) and a stream (Dommel) which receives discharges from a municipal sewage treatment works (STW), and (b) in male flounder from a clean coastal reference site (Noordwijk) and the industrialised harbour area of the North Sea Canal (NSC) near Amsterdam which also receives input of STW effluents and where sediments are polluted with persistent substances. Asterisks denote significant deviations from the reference sites ($P \leq 0.05$; one way ANOVA with Tukey's mean comparison test).

At most of the 14 sites where flounders were captured, average concentrations of VTG in blood plasma of males were less than 1000 ng/ml, for example in the North Sea near Noordwijk (Fig. 1b). At two sampling sites in the North Sea Canal (Port of Amsterdam), more elevated, but variable, plasma VTG concentrations were measured in male flounder in autumn (example in Fig. 1b). The North Sea Canal is polluted with persistent industrial chemicals but also receives discharges from sewage treatment works (STWs). In general, VTG concentrations measured in plasma of male bream were greater than in male flounder. VTG concentrations in male bream also tended to be greater in spring than in autumn at many sampling sites. Bream was captured at nine locations. Increased VTG levels, i.e. as great as 1 000 000 ng/ml blood plasma, occurred where the rivers Rhine and Meuse enter The Netherlands (at Lobith and at Eijsden respectively), in major river sedimentation areas, in some rural areas and again in the North Sea Canal. The greatest concentrations of VTG measured during LOES were observed in male bream from the small inland stream called Dommel (Fig. 1a). Both in spring and autumn, 80% of the male fish contained concentrations of 10 000 000 ng VTG/ml or greater.

In spring, the testes of six out of 14 (43%) male bream specimens from the Dommel clearly contained oocytes inside the seminiferous tubules (Fig. 2). In autumn, 33% (three out of nine) of the males presented this condition. Ovotestis was also observed in male bream specimens from two other locations, but at a lesser frequency (4 and 9%) and only in the spring. No gonadal deviations were found in flounders or in bream from other locations.

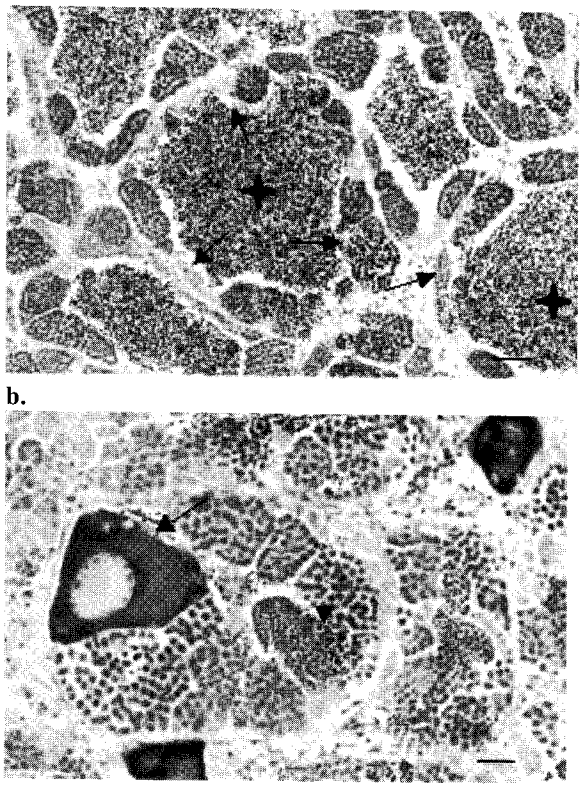

Fig. 2. Ovotestis in male bream (*A. brama*). Normal testicular tissue (a) tubules filled with spermatozoa (asterisks) and partially lined with pre-stages of spermatozoa, and sertoli cells (arrows) (bar = 67 μm). Testis of a hermaphroditic bream at a greater magnification (b) showing an oocyte in a testicular tubule (arrow) and testicular tubules containing (few) spermatocytes (arrow head) (bar = 34 μm). The follicular epithelium that differentiates to granulosa cells is absent and no vitellogenin is deposited in the oocyte. Tissues were stained with haematoxylin-eosin.

4. Discussion

The results of the VTG measurements suggest that, in The Netherlands, estrogenic effects occur in both bream and flounder, i.e. at sites where VTG concentrations greater than 1000 ng/ml plasma are measured in males. Most individuals with increased concentrations of plasma VTG were collected from inland waters that are known to be influenced by industrial, agricultural and/or domestic pollution sources. Male flounder from the open sea, coastal waters and major estuaries seem relatively devoid of estrogenic effects, even where anthropogenic influence is considered large as in the Scheldt, New Waterway (Port of Rotterdam) and Ems estuaries. In general, the results from flounder from Dutch estuaries revealed much lower concentrations of VTG than flounder captured in various estuaries in the United Kingdom (Allen et al., 1999). In addition, none of the

male flounders from the Dutch sites showed ovotestis while in the UK the condition was observed in the more contaminated estuaries of the Mersey and Tyne Rivers.

Very great concentrations of VTG in plasma were observed in male bream from the Dommel, a small stream, that should be regarded as a worst-case situation, i.e. heavily affected by human activities, including agriculture and industry. The fish were captured close to a major STW. The results of bream from our survey seem to compare well with those observed in the UK where significant effects in several other fish species were predominantly found at locations that receive STW effluents (e.g. Harries et al., 1997; Jobling et al., 1998).

Based on this first survey it may cautiously be concluded that the problem of estrogenic endocrine disruption in fish in The Netherlands seems not widespread in marine, estuarine and large inland waters. Thus, the situation in The Netherlands seems less alarming than, for instance, for the well-known examples from the UK. Further surveys in small inland waters are currently underway to assess if estrogenic effects are confined to the most contaminated sites, such as the Dommel, or if such types of water are more generally affected.

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