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Vitellogenin as biomarker for estrogenicity in flounder *Platichthys flesus* in the field and exposed to 17α-ethinylestradiol via food and water in the laboratory

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ABSTRACT

The ability of 17α-ethinylestradiol (EE2) to elevate vitellogenin levels were investigated in male flounder *Platichthys flesus* and vitellogenin concentrations in flounders from the Danish coastal environment were determined. Male flounders were exposed to 17α-ethinylestradiol (EE2) via food or water. Average vitellogenin concentrations in the control fish ranged between 25 and 100 ng mL^{-1}. Exposure to 5.1, 8.1 and 16.8 ng EE2 L^{-1} in water and 500 and 5000 ng EE2 kg^{-1} body weight (bw) every second day in the food increased the plasma vitellogenin concentration in a concentration and time dependent manner, whereas exposure to 2.7 ng EE2 L^{-1} in water for 21 d and 5 and 50 ng EE2 kg^{-1} bw for 12 days in the food did not. EE2 could be detected in liver and testes (but not in muscle) after exposure to 8.1 and 16.8 ng EE2 L^{-1} in the water and 5000 ng EE2 kg^{-1} bw in the food; the highest concentration was 6 ng g^{-1} wet weight in liver. The majority of the male flounders collected from nine coastal Danish sites from 1999 to 2004 had vitellogenin concentrations below 100 ng mL^{-1}, and only at two sites moderate estrogenic inputs were indicated.

1. Introduction

Wild populations of freshwater and marine fish have been shown to exhibit signs of endocrine disruption in the form of elevated vitellogenin levels, altered sex steroid levels, skewed sex ratios and abnormal gonad development (reviewed by Desforges et al., 2010; Jobling and Tyler, 2003; Matthiessen, 2003; Sumpter, 2005).

In the marine and estuarine environment, especially the flounder *Platichthys flesus* has been used as a biomonitoring organism in such studies (Allen et al., 1999a,b; Kleinkauf et al., 2004; Lye et al., 1997, 1998, 1999; Matthiessen et al., 2002; Matthiessen, 2003; Matthiessen and Law, 2002; Scott et al., 2006; Vethaak et al., 2002, 2005) and the synthesis of the egg yolk protein (vitellogenin) in juvenile and male fish has been used as one of the most well established endpoints for demonstration of estrogenic effects of environmental chemicals (Harries et al., 1997; Sumpter and Jobling, 1995).

Estrogenic constituents in effluents from sewage treatment plants are the main causative agents responsible for the induction of vitellogenin synthesis in male and juvenile fish (Desbrow et al., 1998). The compounds causing the majority of the estrogenic effects have been identified as the natural (17β-estradiol [E2] and estrone [E1]) and synthetic hormones (EE2) (Desbrow et al., 1998; Larsson et al., 1999). EE2 is the major component of oral contraceptives and is excreted mainly as glucoronide conjugates in the urine of women. When released into water these EE2 glucoronide conjugates are unconjugated and the EE2 enters the aquatic environment via untreated sewage and the effluent from sewage treatment plants (Lopez de Alda and Barcelo, 2001). EE2 has been detected in concentrations up to 15 ng L^{-1} in effluents from sewage treatment plants and up to 5.1 ng L^{-1} in surface water (Kuch and Ballschmiter, 2001; Langston et al., 2005; Ternes, 1998; Ternes et al., 1999). EE2 is a more potent inducer of vitellogenin synthesis in male and juvenile fish than either E2 and E1 (Bjerregaard et al., 2008; Holbech et al., 2006; Thorpe et al., 2003) and the half-life for EE2 in the aquatic environment is longer than that of E2 (Robinson and Hellou, 2009). EE2 will thus have a risk of exerting estrogenic effects in the coastal environment. Exposure to 14.5 ng EE2 L^{-1} for three weeks induced vitellogenin synthesis in male

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flounders whereas exposure to a nominal concentration of 1 ng EE2 L^{-1} did not (Allen et al., 1999b).

The most important exposure route for EE2 in fish is probably from water. However, EE2 has been shown to accumulate in sediments (de Alda and Barcelo, 2001) and with the benthic feeding habits of the flounder, uptake of EE2 from food might also add to the estrogenic effect as we have demonstrated in experiments with 4-*tert*-octylphenol (Madsen et al., 2003, 2006).

In laboratory experiments, EE2 has been shown to accumulate in rainbow trout (*Oncorhyncos mykiss*) upon water exposure (Skillman et al., 2006) and EE2 has been detected in redhorse suckers (*Moxostoma macrolepidotum*) living downstream discharges from waste water treatment plants (Al-Ansari et al., 2010), but EE2 uptake in flounders has never been investigated.

Elevated levels of plasma vitellogenin in male flounders were found at many sites in English estuaries and coastal marine areas (Allen et al., 1999a,b) and also in a similar Japanese study of vitellogenin in male flounders *Pleuronectes yokohamae* collected in Tokyo Bay (Hashimoto et al., 2000). In a large-scale field study in The Netherlands, the vitellogenin levels of *P. flesus* were low at most sites except at two sites where moderately elevated vitellogenin levels were found. These stations were situated in the same industrial harbour zone also receiving effluent from sewage treatment works (Vethaak et al., 2002). Danish waste water treatment plants generally quite efficiently remove estrogens from the waste water (Stuer-Lauridsen et al., 2005) but the potential occurrence of estrogenic effects in the Danish coastal environment has not been investigated.

The aim of this study was to produce dose-relationships for the estrogenic effect of EE2 administered to male flounders in food and water, to assess uptake of EE2 and to carry out a preliminary investigation on the presence of estrogenic effect in the Danish marine environment.

2. Materials and methods

2.1. Laboratory experiments

2.1.1. Experimental animals and experimental setup

Sexually mature male flounders *P. flesus* were caught in November at Gabet, Denmark (Fig. 1); fifty fish were used for both the feeding experiment (421.6 ± 10 g; mean ± SEM) and the water

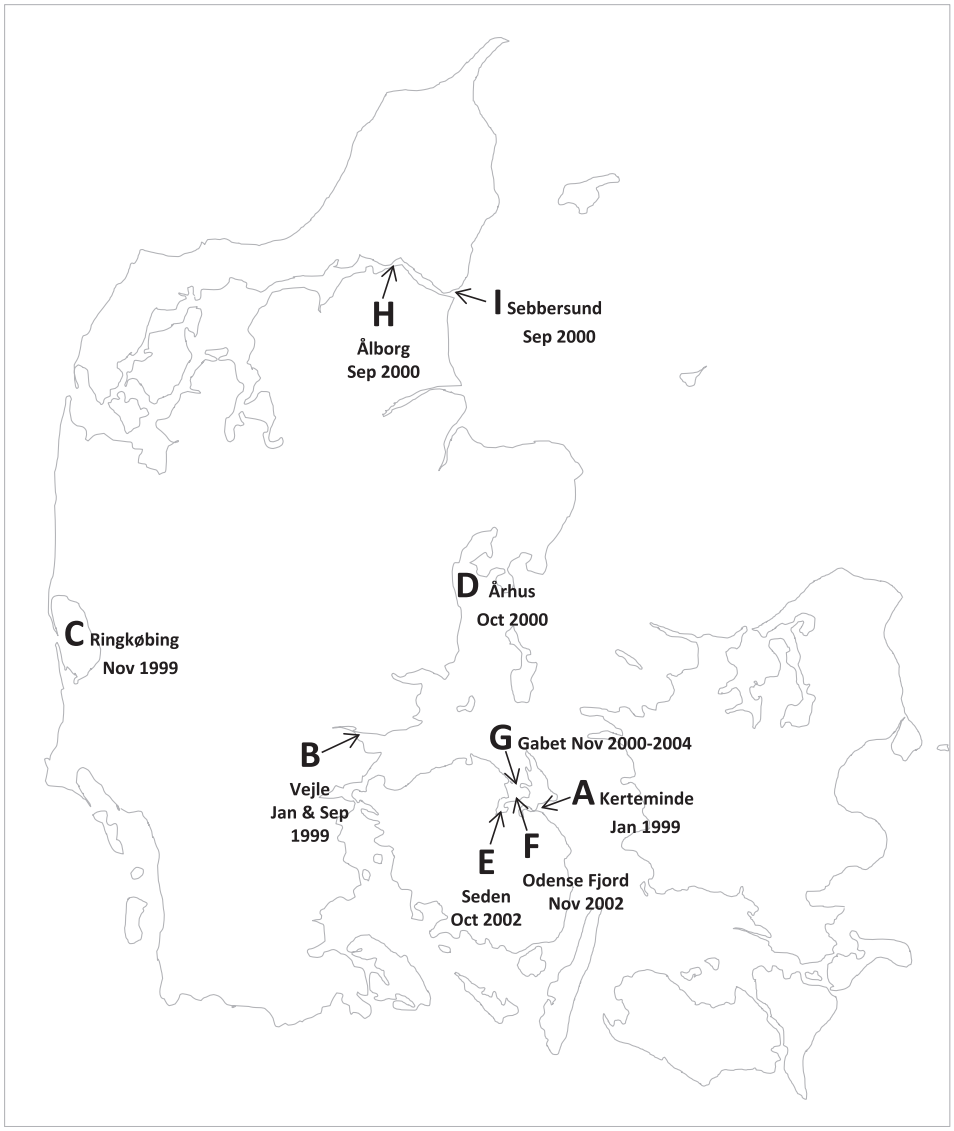

Fig. 1. Map showing the sampling locations, names of locations and sampling times in the field investigations.