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Seasonal reproductive health of flounder *Platichthys flesus* exposed to sewage effluent

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ABSTRACT: Indicators of environmental oestrogen exposure in male and female wild flounder *Platichthys flesus* were followed over the annual reproductive cycle. Fish were obtained from the Tyne estuary, UK, which receives effluent from a large sewage treatment works and several other anthropogenic sources and the Solway Firth, UK, which receives only low levels of sewage effluent. Vitellogenin (vtg), a female-specific protein, was present in the plasma of up to 94% of males from the Tyne and was accompanied by inhibited spermatogenesis and the incidence of pathological testicular abnormalities. The vtg levels in males are independent of the seasonal cycle. Female flounders from the Tyne exhibited increased vtg levels and a higher proportion of degenerating oocytes than Solway Firth females. This study provides limited evidence that wild populations of flounders showing oestrogenic responses are also suffering reproductive disturbances but the degree of alteration varies during the seasonal reproductive cycle.

KEY WORDS: *Platichthys flesus* · Vitellogenin · Sewage · Oestrogen · Testicular morphology · Ovarian development

INTRODUCTION

The disruption of hormonal and reproductive systems in humans and wildlife by anthropogenic and naturally occurring chemicals present in the environment has become a major topic of scientific research and the focus of considerable scientific attention in recent years (for reviews see McLachlan 1981, 1985, Colborn & Clement 1992, Harrison et al. 1995, Toppari et al. 1995, Kavlock et al. 1996, EPA 1997). Evidence for increasing levels of substances that can cause feminising or oestrogenic effects in the environment during the past half-century comes from several sources, notably an increasing consumption of oestrogens in human diet (Sharpe & Skakkebæk 1993, Carlsen et al. 1995) and the expanding use of many different groups of synthetic chemicals (e.g. some PCBs, DDT, phthalates, alkylphenols, and Bisphenol-A), many of which have been shown to exhibit oestrogenic activity and/or degrade to persistent metabolites which are oestrogenic (Brotons et al. 1995, Jobling et al. 1995, Soto et al. 1995, Harris et al. 1997).

An observable and quantifiable effect of exposure to oestrogens is the stimulation of vitellogenin (vtg) synthesis. Vtg is the major precursor of yolk proteins in all oviparous vertebrates and is synthesised by the liver in response to circulating oestrogens (Bromage & Cumaranatunga 1988). Although vtg is a female-specific protein it can be induced in males if they are treated with exogenous oestrogens and thus the presence of vtg in the plasma of males is a useful biomarker for oestrogenicity of chemicals (Heppel et al. 1995, Korach & McLachlan 1995, Sumpter & Jobling 1995). It is now becoming evident that most domestic sewage effluents entering rivers in the UK are strongly oestrogenic to caged male trout *Oncorhynchus mykiss* where formation of vtg in plasma has been widely observed (Purdom et al. 1994). These effects are also present in the receiving waters (Harries et al. 1996, 1997) and have been ascribed mainly to natural oestrogens, largely from human sources (Waldock et al. 1997) but also to oestrogen-mimicking substances (Harries et al. 1997).

Recently it has been demonstrated that wild marine flounder *Platichthys flesus* taken from an estuary receiving large inputs of sewage effluent showed ele-

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vated plasma levels of vtg and gross abnormalities in testis morphology (Lye et al. 1997). This observation, coupled with a reported high incidence of inter-sex gonads (e.g. the simultaneous presence of female and male characteristics) and increased levels of vtg, both effects linked to discharges of sewage effluent, in roach *Rutilus rutilus*, and to a certain degree also in bream *Abramis abrama*, gudgeon *Gobio gobio* and chub *Leuciscus cephalus* from a number of UK rivers (Jobling et al. 1997, Environment Agency UK 1998), suggests that wild fish exposed to oestrogenic substances are suffering oestrogenic effects which can result in reproductive disturbance. However, the physiological consequences of vtg in wild male fish and of additional exposure to oestrogens on females are as yet unclear and warrant further investigation.

The flounder *Platichthys flesus* is widely distributed in estuaries and coastal zones. Its demersal behaviour and resuspension of the substrate during swimming activity makes this species especially vulnerable to sediment-associated pollution normally occurring in high concentrations in the recipients of land-based point sources, river run-offs and urban areas (Goksøyr et al. 1996). Most oestrogenic substances are both strongly lipophilic and sorptive (Ahel et al. 1993) and are therefore likely to accumulate in the organically rich sediments of an estuary. Flounders are directly exposed to water-borne contaminants via their gills, skins and fins and through direct contact with contaminated sediment, for example through food (Berge & Brevik 1996). This study follows the changes in both male and female flounders over the annual reproductive cycle to establish the effect of exogenous oestrogens on the reproductive biology of this estuarine fish species.

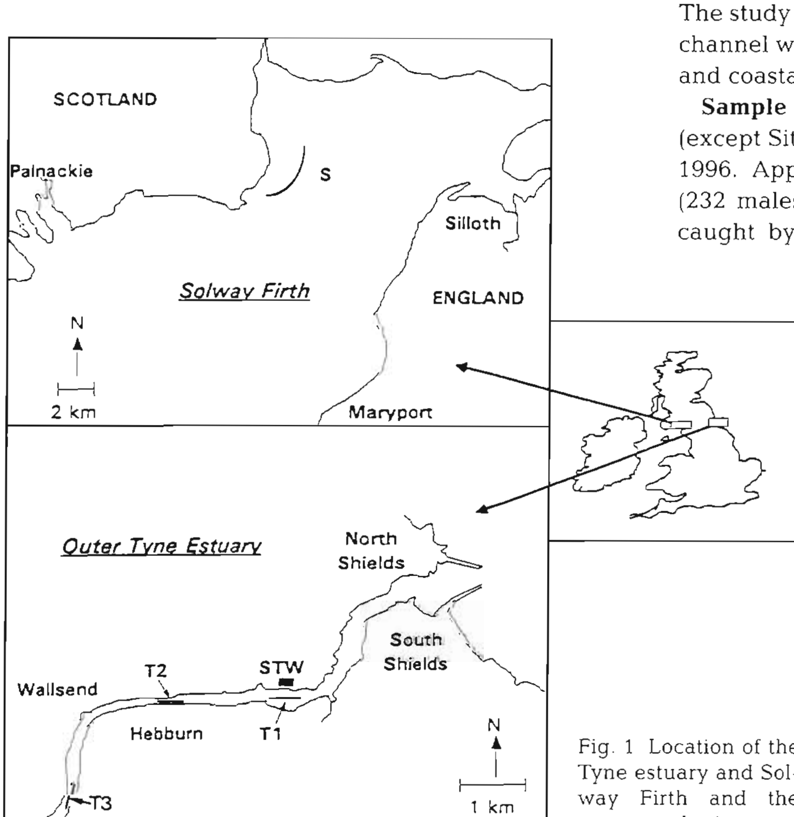

Fig. 1. Location of the Tyne estuary and Solway Firth and the sample sites

METHOD

Study sites. Flounders were collected adjacent to the outfall of the major sewage treatment works (STW) at Howdon in the Tyne estuary, UK (Site T1) and 2.5 and 5 km upstream of this site (T2, T3; Fig. 1). The STW in the Tyne provides primary treatment to sewage from a large catchment zone (2935 km^2) and a population of about 1.25 million people. The supernatant from the settlement tanks is discharged into the estuary, with an average dry weather flow exceeding 240000 m^3 d^{-1} (Environment Agency UK pers. comm.), and the settled sludge is disposed of at sea. For comparison, fish were taken from the Solway Firth, UK, which drains a catchment covering a sparsely populated region with only 1 large (i.e. serving a population equivalent of >100000) STW on its tributaries. There are also small treatment plants serving rural communities often comprising a few houses (Solway Firth Partnership 1996). The study site selected (Site S) was in the main estuary channel well away from inputs from tributary estuaries and coastal industrial discharges (Fig. 1).

Sample collection. Sampling took place in July (except Site T3), September, December 1995 and April 1996. Approximately 640 sexually mature flounders (232 males and 407 females, length >250 mm) were caught by beam trawl and blood-samples taken for subsequent serum analysis according to procedures described in Lye et al. (1997). In the laboratory individual fish lengths and weights were recorded after which the fish were dissected for examination of internal organs. Liver and gonad weights were recorded and the hepatosomatic and gonadosomatic index (e.g. HSI, GSI; organ weight [g]/body weight [g]) were calculated. Gonads were examined macroscopically for gross changes in morphology (see Lye et al. 1997). Gonad samples were also preserved for histological examinations (see below).

Fixation of fish tissue. Testicular tissues with suspected lesions (n = 30) were fixed in 10% formalin and in paraffin. Serial sections (10 µm) were

cut and stained with haematoxylin and eosin. From 4 samples showing eosinophilic amorphous material in testes by microscopic examination additional sections were cut and Crystal Violet was used to stain for the presence of amyloids, a group of diverse extra-cellular protein deposits associated with many different diseases (Kisilevsky 1994). Ovarian tissues with suspected lesions (n = 25) were fixed in formalin saline, processed and cut using a freezing microtome following standard techniques.

Serum analysis. Identification of vtg was carried out as described by Lye et al. (1997). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was routinely performed and the protein quantified by scanning densitometry.

Statistical analysis. Data were checked for normality and variance homogeneity and, where necessary (GSI, HSI), transformed ($\log_{10}$) prior to analysis. The Tukey multiple comparison test was used after initial 2-way analysis of variance (ANOVA, $p < 0.05$) using the General Linear Model (GLM) Procedure in the Minitab Statistical Package (Zar 1984). Chi-square tests were used for testing between site differences in frequencies. When there were no proven significant differences between the sites in the Tyne, data for these sites were pooled.

RESULTS

Serum analysis

The presence of vtg in serum was found in male flounder (n = 232) from both sites investigated, the highest prevalence (94%) in male flounder from the Tyne (April 1996) and the lowest prevalence (27%) in flounder from Solway Firth (September 1995) (Table 1). Quantification of vtg showed that there was a significant difference in plasma vtg levels of male flounders between the sites but not between sampling occasions (2-way ANOVA, site: $F = 20.7$, $p < 0.05$; date: $F = 1.8$, $p > 0.05$) (Fig 2). Thus, significantly higher plasma vtg

Table 1. Percentage of male flounders *Platichthys flesus* from sites in the Tyne (T1, T2, T3) and Solway Firth (S) with vitellogenin (vtg) present in their plasma in July, September, December 1995 and April 1996 (n = 232)

Site	Jul 1995	Sep 1995	Dec 1995	Apr 1996
T1	88%	85%	81%	88%
T2	82%	64%	85%	95%
T3	[a]	73%	75%	78%
S	40%	27%	37%	50%

[a]Not sampled in July 1995

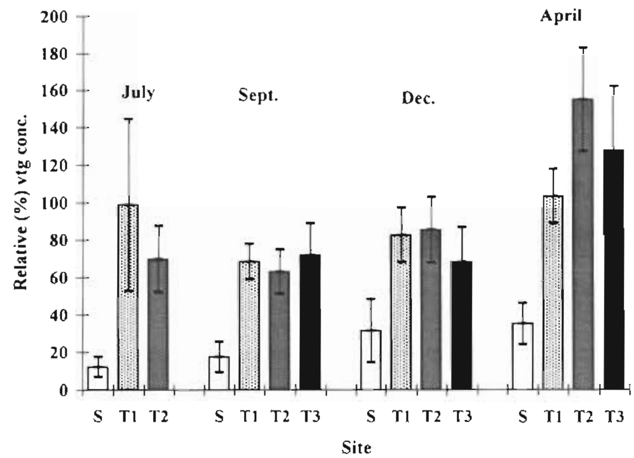

Fig. 2. Levels of vitellogenin (vtg) (mean ± SE) in plasma of male flounders *Platichthys flesus* from sites in the Tyne Estuary (T1, T2, T3) and the Solway Firth (S). Values are expressed as a percentage of the protein level in a control female

levels were found in male flounders from the Tyne compared to those from Solway Firth on all sampling occasions (Tukey's, $p < 0.05$) except for Site T3 in December 1995. Some individual flounder from the Tyne sites in April showed vtg plasma levels similar to those of gravid females (>300 units). Significantly higher levels were also found at Site T1 compared to Site T2 and T3 in the Tyne in April (Tukey's, $p > 0.05$) (Fig. 2).

In female flounders (n = 407) vtg plasma levels varied significantly both between sites and sampling occasions (2-way ANOVA, site: $F = 11.9$, $p < 0.05$; date: $F = 34.5$, $p < 0.05$) (Fig 3), the seasonal reproductive cycle effecting vtg levels in females from both sites, with peak vtg levels in April. Significantly higher plasma levels were found in female flounders from the Tyne compared to those in Solway Firth in September

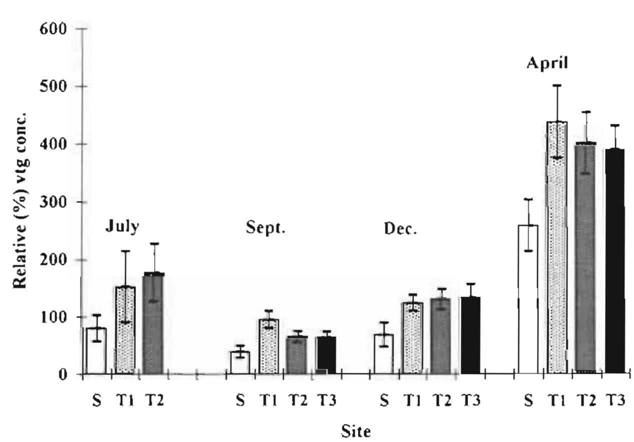

Fig. 3. Levels of vtg (mean ± SE) in plasma of female flounders *Platichthys flesus* from sites in the Tyne Estuary (T1, T2, T3) and the Solway Firth (S). Values are expressed as a percentage of the protein level in a control female

(T1), December and April (Tukey's, $p < 0.05$). Apart from significantly higher levels in female fish from Site T1 in the Tyne in September, no significant difference was found between sites within the Tyne (Tukey's, $p < 0.05$).

Testicular and ovarian gross morphology

Abnormalities of testis morphology were found in a substantial proportion (53 to 67%) of the examined flounders from sites in the Tyne (n = 172) whereas a significantly lower prevalence (maximum of 12%) was observed in testes from male flounders from the Solway Firth (n = 70; chi-square, $\chi^2 = 25.8$, $p < 0.05$) (Fig. 4). These differences were apparent on all sampling occasions. No differences were found between sampling occasions or between the sites within the Tyne (Fig. 4). The condition of the testis of flounder from the Tyne was characterised by truncated lobes, lack of lobular structure and a thickening of connective tissue surrounding the testis (see also Lye et al. 1997).

Abnormalities in ovarian morphology were observed in females from all sites investigated (Fig. 5). A significantly higher prevalence was found in females from the Tyne estuary (up to 36%, n = 292) compared to females from Solway Firth (maximum of 12%, n = 115) (chi-square, $\chi^2 = 18.9$, $p < 0.05$). This difference was apparent in September, December and April; gross ovarian abnormalities being absent in July (Fig. 5). The frequency of abnormal ovaries varied between sampling occasions and similar levels of incidence were recorded at all sites within the Tyne (Fig. 5). Gross morphological examinations of ovaries revealed that fish taken from the Tyne exhibited abnormal ovaries consisting of large masses of aggregated and deformed oocytes sometimes accompanied by serious degeneration of the ovarian tissue in one or both gonads, the most prominent characteristics of these ovaries being the distinct yellow to green colour of the oocytes. Abnormalities in ovaries in Solway Firth were restricted to occasional discoloured and deformed oocytes (1 to 10 oocytes per fish).

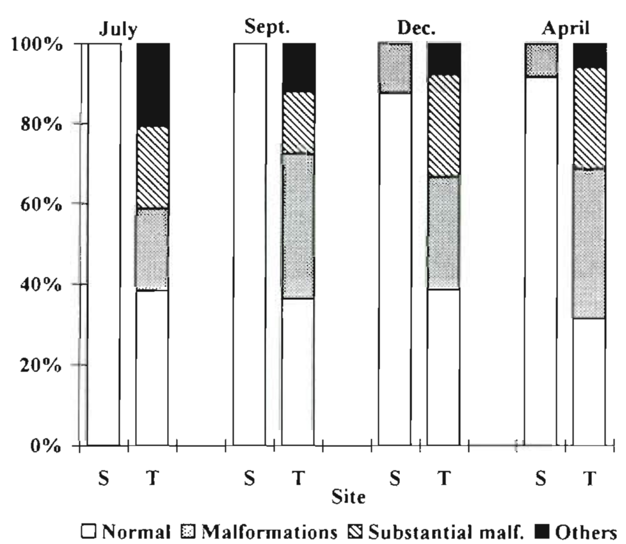

Fig. 4. Frequency (%) of testis malformations found in flounder *Platichthys flesus* (n = 232) from sites in the Tyne Estuary (T1, T2, T3) and the Solway Firth (S) in July, September, December (1995) and April (1996). (Normal: elongated testis; Malformed: altered shape; Others: appearance of cysts/growth; blood-filled sperm duct; see Lye et al. 1997)

Qualitative examination of testis histology

Abnormal testes (n = 30) from Tyne flounders, in all cases also showing detectable levels of vtg in their blood plasma, were examined from the 3 stages of spermatogenesis: proliferative cells (spermatogonia I, II), meiotic cells (spermatocytes I, II) and ripe cells (spermatids, spermatozoa). Normally, the histological picture of the testis of flounder presents a well-organised structure through all developmental stages, the interstitial tissue surrounding the testis lobules becoming distended and thin as the lobule lumen fills up with spermatogenetic elements (Janssen et al. 1996). In contrast, abnormal testes in proliferation (sampled in April/May), from flounder showing the highest levels of vtg, were generally characterised by disorganised lobules, a connective tissue thickening and vacuolation of the lobule wall, and the presence of spermatozoa remains in the lobular lumen (Figs. 6 & 7). The lobules were typically devoid of germ cells and contained few cysts of spermatogenetic elements. In some cases,

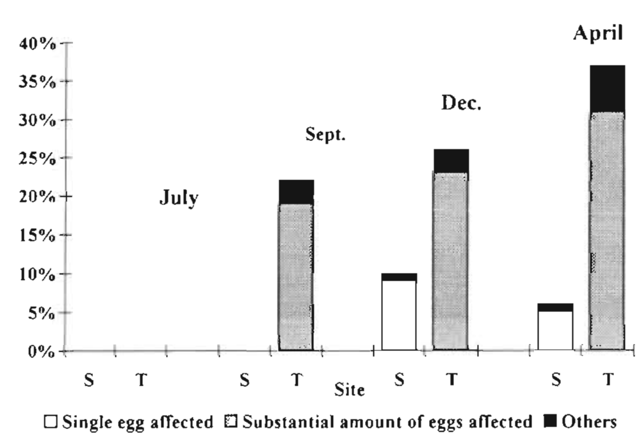

Fig. 5. Frequency (%) of oocyte malformations found in flounder *Platichthys flesus* (n = 407) from sites in the Tyne estuary (total T1, T2, T3) and the Solway Firth (S) in July, September, December (1995) and April (1996). (Others. lymphocystis, green, soft tissue, blood-filled ovaries, degeneration)

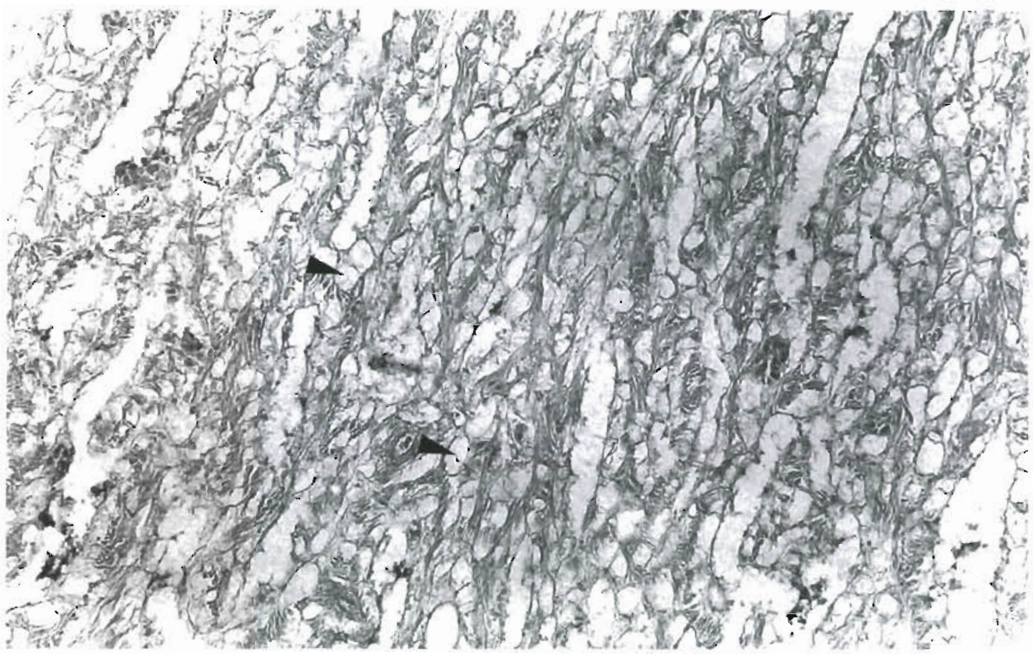

Fig. 6. Photomicrographs of testicular tissue from testes in proliferation obtained from flounders *Platichthys flesus*, showing high levels of vtg. Note poorly organised lobule structure, fibrosis, thickened and highly vacuolated lobule walls (arrowheads) accompanied by a reduced number of, or the absence of, germinal cells. Haematoxylin/eosin (×160)

lobules were fully converted into fibrous tissue resulting in the absence or reduction in germ cells (Fig 6). Frequently observed within or around the testis lobules was the accumulation of amorphous eosinophilic structures (Fig. 7). The presence of amyloids could not be confirmed using staining with Crystal Violet thus the significance of these eosinophilic structures is unknown.

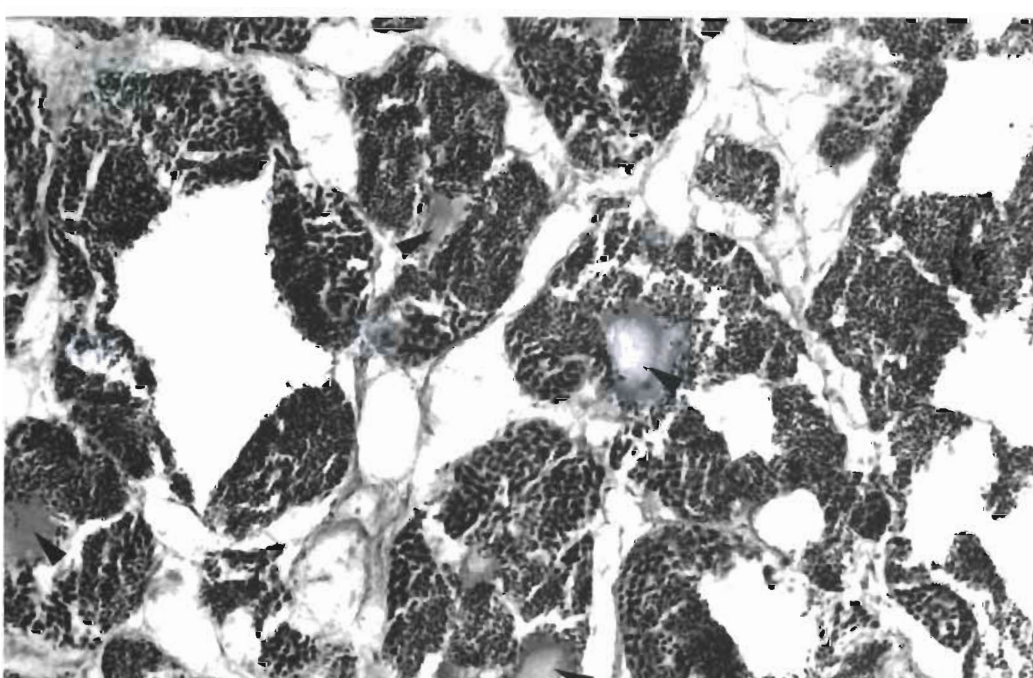

Fig. 7. Photomicrographs of testicular tissue from testes in proliferation obtained from flounders *Platichthys flesus*, showing high levels of vtg. Note fibrosis and deposition of amorphous eosinophilic extra-cellular material in the lumen (arrowheads). Haematoxylin/eosin (×250)

Spermatogenesis was well under way in Tyne fish testes examined at a later stage of the testicular cycle (sampled in October) and was characterised by the presence of spermatocytes. Despite the increase of germ cells, thickened lobule walls were still present and the lobules varied in size (Fig. 8). Vacuolation were also observed in these abnormal testes (Fig. 8). Testes containing mature sperm (sampled in December/January) revealed some changes in testicular morphology (e.g. partly thickened lobule walls and the presence of amorphous materials) whereas other parts of the testes showed normal testicular development.

Qualitative examination of ovarian histology

Ovaries of flounders examined from the Tyne (n = 25) consisted of oocytes in early and advanced vitellogenesis. Oocytes in the vitellogenic stage are normally characterised by densely packed yolk granules, the nucleus located at the centre of the oocyte and the oocyte surrounded by zona radiata and the follicle layers (Fig. 9). In contrast, the structure of oocytes in abnormal ovaries was atypical and breakdown of developing oocytes was observed along with their conversion to fully formed corpora atretica (e.g. degenerating oocytes) (Fig. 10). In these oocytes zona radiata was ruptured and the interior of oocytes invaded by phagocytic cells resulting in the breakdown of yolk globules. The oocyte nucleus had also broken down and become granular (Fig. 10a). Fibroblast-like cells and a vast number of phagocytic cells extensively infiltrated the ovary and yellow pigments characteristic of the late stage of the atretic process (Dodd 1983) were frequently observed amongst these cells. Degenerating oocytes were present in large aggregates encapsulated by a membrane of several cell layers (Fig. 10b). Development of new oocytes was not observed within these structures. Degenerated oocytes were more frequently observed in ovaries in advanced vitellogenesis (sampled in December/January) than ovaries in early vitellogenesis (sampled in September).

Gonadosomatic index

The mean GSI-values of female flounders varied significantly between sites and sampling occasions (2-way ANOVA, site: $F = 14.2$, $p > 0.05$; date: $F = 16.1$, $p < 0.05$). The latter reflects the annual reproductive cycle with gonad weight increasing to a maximum in April. Significantly larger mean GSI-values were found in female flounders from the Tyne in September and April compared to those in Solway Firth and in female flounders from Solway Firth in December compared to those in the Tyne (Fig. 11). In addition, there was a significant difference in September between the sites within the Tyne (Tukey's, $p < 0.05$). The mean GSI-values of male flounders varied significantly between site and sampling occasions (2-way ANOVA,

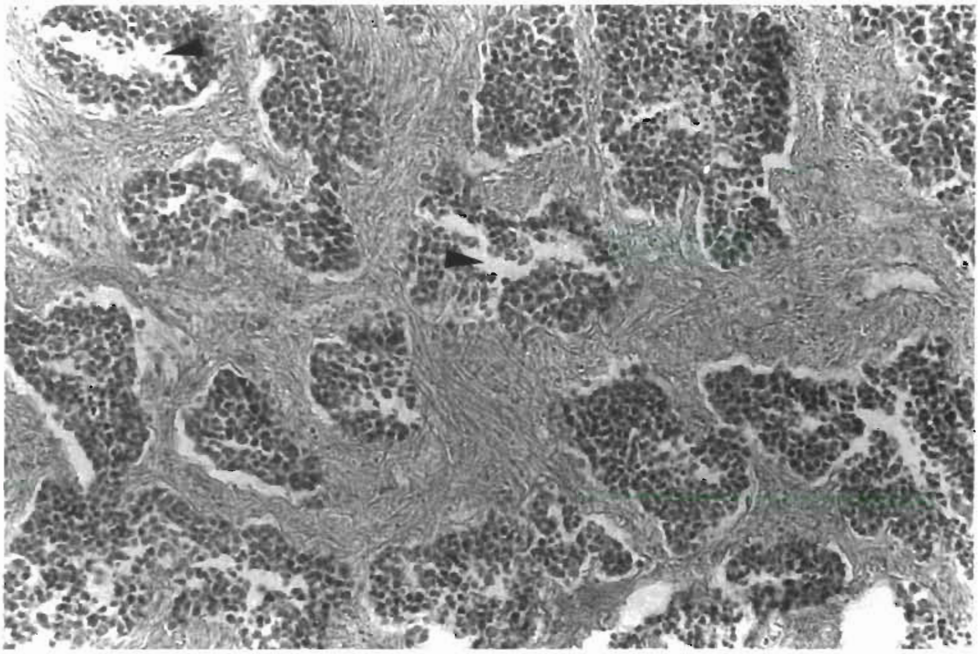

Fig. 8. Photomicrographs of testicular tissue from testes in meiosis, flounders *Platichthys flesus*, showing thickening of connective tissue in lobule walls, poorly organised lobule structure and varying lobule size and the presence of vacuolated cells (arrowheads). Haematoxylin/eosin (×160)

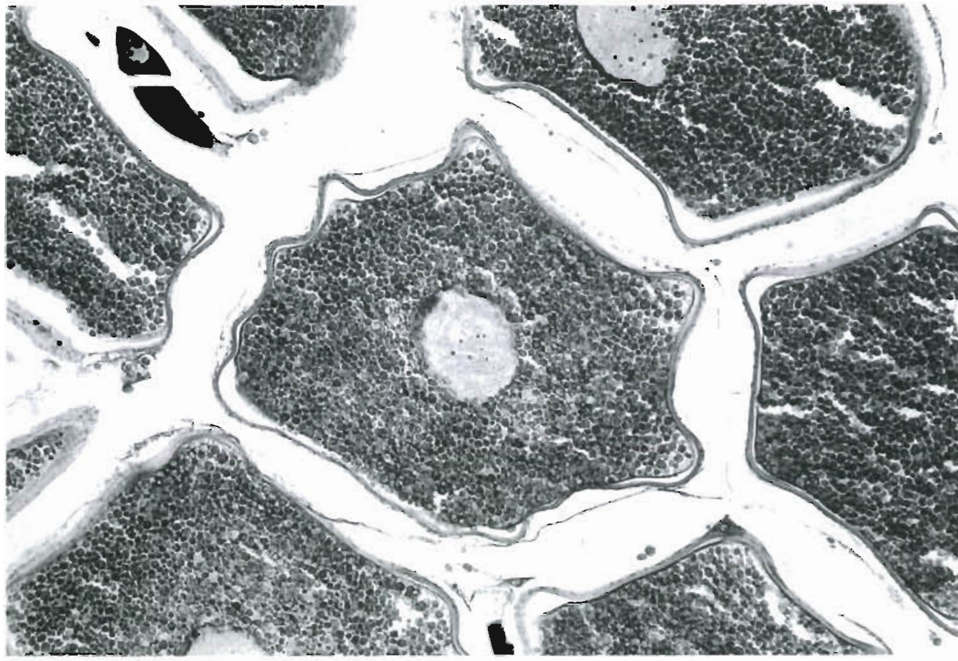

Fig. 9. Photomicrographs of ovarian tissue (*Platichthys flesus*) showing vitellogenic oocytes unaffected by gross morphological changes. The oocytes are densely packed with numerous yolk granules, the nucleus intact containing nucleoli and the cytoplasm surrounded by a marked zona radiata and follicular layers. Haematoxylin/eosin (×160)

site; $F = 21.3$, $p < 0.05$; date; $F = 80.1$, $p < 0.05$), the inter-site differences being restricted to significantly larger GSI-values in male flounders from the Tyne in April compared to those in Solway Firth fish (Tukey's, $p < 0.05$). A significant difference could also be found in December between the sites within the Tyne (Tukey's, $p < 0.05$).

Hepatosomatic index

The mean HSI-values of both female and male flounders varied significantly between site and sampling occasion (2-way ANOVA, site: $F = 47.5$ [female], 31.5 [male], $p < 0.05$; date: $F = 93.0$ [female], 14.6 [male], $p < 0.05$) (Fig. 12). Thus, female and male flounder from all sites in the Tyne and at all sampling occasions, except for females in July (Tukey's, $p > 0.05$), exhibited mean HSI-values significantly larger than those of Solway fish, indicating increased liver weights in fish from the Tyne (Fig. 12). Apart from significantly lower mean HSI-values in male fish from Site T3 in the Tyne in December (Tukey's, $p < 0.05$) there were no differences detected between either males or females taken from sites within the Tyne (Tukey's, $p > 0.05$).

DISCUSSION

This study provides further evidence that wild populations of flounder *Platichthys flesus* from the Tyne estuary, UK, which receives large inputs of sewage effluent, are showing elevated plasma concentrations of vtg, and are therefore exposed to oestrogenic substances (Sumpter & Jobling 1995). Effects in the Solway Firth are less severe and this can be related to the lower input of sewage and other anthropogenic effluents in the Solway Firth. The presence of vtg was recorded in up to 50% of male fish from the Solway Firth and up to 94% of male fish from the Tyne estuary, indicating that, at the more fundamental levels of biological organisation, e.g. at the molecular and cellular levels of individual organisms, effects of environmental exposure to oestrogens may be widespread. At this level, there tends to be a great deal of similarity among vertebrate classes due to the extreme phylogenetic conservation of hormones and hormone receptors binding characteristics (Folmar et al. 1995, Heppell et al. 1995, Sumpter & Jobling 1995, Kavlock et al. 1996). It is therefore reasonable to assume that other species, including both vertebrates and invertebrates, and in particular bottom-living, sediment-dwelling species, inhabiting waters receiving various amounts of oestrogens derived from sewage or other anthropogenic discharges, are also affected. The susceptibility of different species and/or sub-populations may make some more susceptible to the effects of environmental oestrogens than others (Petersen & Korsgaard 1989, Hylland & Braaten 1996, Steinmetz et al. 1997).

High levels of vtg in male rainbow trout have been shown to be accompanied by a decrease in testis growth and to affect spermatogenesis (Jobling et al. 1996). Although testis weight (e.g. GSI) was largely

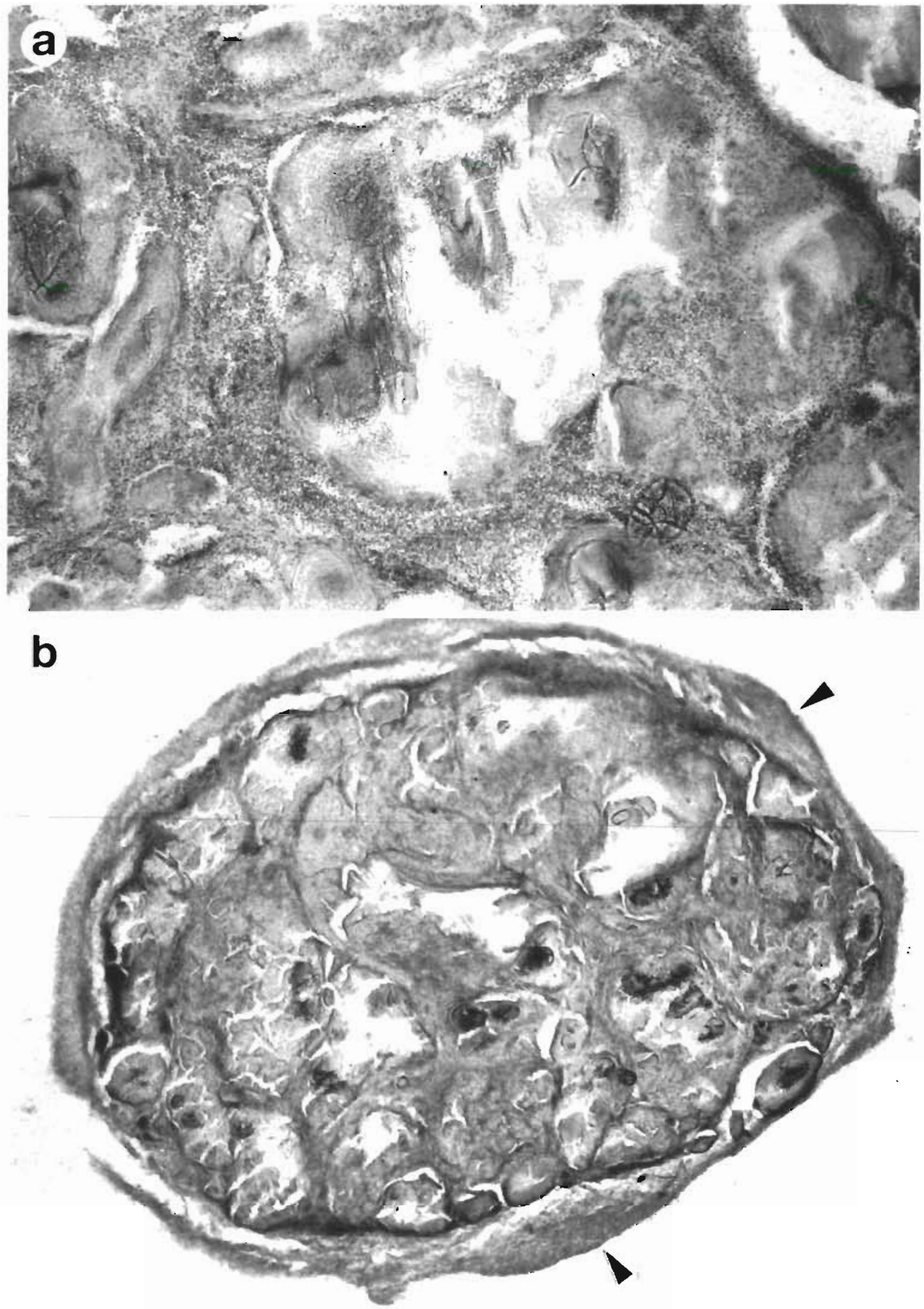

Fig. 10. Photomicrographs of ovarian tissue (*Platichthys flesus*) showing (a) degenerating oocytes (e.g. rupture of zona radiata, the breakdown of yolk granules and nucleus, and extensive infiltration of fibroblast-like cells) (×160). (b) Aggregate of atretic oocytes encapsulated by several cell layers (arrowheads). Haematoxylin/eosin (×25)

unaffected in fish from the Tyne in this study, histological examinations revealed testis morphology that does not correspond with the description of normal teleost testis morphology (Barr 1963a, Htun-Han 1978a, Janssen et al 1996). Examined testes from the Tyne flounder had poorly organised seminiferous tubules consisting largely of connective tissue that, in turn, reduced the number of germ cells markedly Additionally, these testes showed remnants of spermatozoa/luminal debris in the seminiferous tubules, disrupted lobule walls, vacuolation and the frequent presence of amorphous eosinophilic precipitates. These effects are consistent with the effects observed in several teleost and avian species experimentally exposed to a range of steroids and oestrogenic substances (e.g. some PCBs, β-HCH, chlordecane, 4-*tert*-pentylphenol) and some metals (Cohen 1946, Fagerlund & McBride 1975, Goetz et al. 1979, Billard et al. 1981, Sangalang

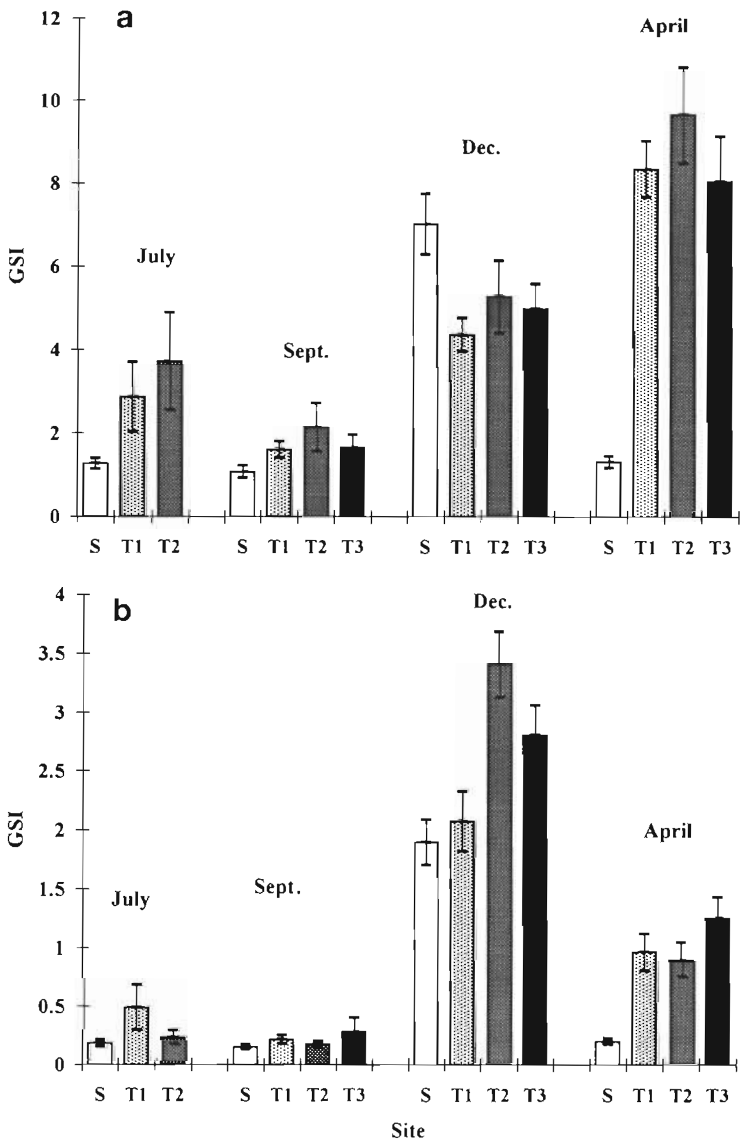

Fig. 11. Mean (± SE) gonadosomatic index (GSI: gonad weight [g]/body weight [g]) of (a) female and (b) male flounders *Platichthys flesus* from sites in the Tyne Estuary (T1, T2, T3) and the Solway Firth (S)

et al. 1981, Sehgal & Pandey 1984, Reel & Lamb 1985, Wester et al. 1985, Herman & Kincaid 1988, Wester 1991, Dickerson 1997, Johnson et al. 1997, Gimeno et al. in press) and to the effects reported in wild male alligators exposed to dicofol, some DDEs and DDTs (Guillette et al. 1994). Subsequent investigations to this study have also recorded impaired spermatogenesis and fibrosis of the testicular tissue in wild walleyed pike *Stizostedion vitreum* and largemouth bass *Micropterus salmoides* inhabiting ponds receiving sewage discharges in Minnesota, USA (Folmar et al. 1997a, b).

In this study, adverse effects in testes were observed to varying degrees depending on the individual and stage of the reproductive cycle. Testes at the beginning of the annual reproductive cycle, from flounder showing the highest levels of plasma vtg, were the most severely affected and fully mature testes at the end of the annual reproductive cycle the least affected. Recovery of the highly degenerative testes found in May and September is unlikely to have occurred by December. The duration of exposure to oestrogenic sewage will be dependent on the movements of flounder. Although the flounder for most of the year is a relatively stationary species within the estuary, it migrates offshore during the winter months to spawn (Wheeler 1969). It seems likely that the sample examined in December comprised flounders migrating down the estuary to spawn at sea. These flounders would not have been subjected to high levels of sewage effluents during their reproductive development and this may provide an explanation for the presence of relatively healthy testes in December compared to other sampling occasions. Several workers have also demonstrated that the degree on inhibitory effects of testicular growth in rainbow trout *Oncorhynchus mykiss* and cod *Gadhus morhua* exposed to oestrogens (e.g. 17β-oestradiol) and oestrogenic chemicals (e.g. alkylphenols, some PCBs) is both dose-dependent and affected by timing of exposure (Billard et al. 1981, Sangalang et al. 1981, Jobling et al. 1996).

Ovaries collected from the Tyne showing normal gross morphology corresponded to the description of ovaries reported in flounder (Janssen et al. 1995) and other flatfish (Barr 1963b, Htun-Han 1978b). In contrast, ovaries showing abnormal gross morphology revealed the breakdown of developing oocytes and conversion to fully formed corpora atretica (i.e. degenerating oocytes). A range of conditions has been correlated with increased incidence of oocyte atresia, including hypophysectomy (Barr 1963c, Khoo 1975), exposure to sex steroids and synthetic oestrogens (Bretschneider & Duyvené de Wit 1947, Peluso et al. 1979, Iguchi 1985, Piferrer & Donaldson 1992), several oestrogenic compounds (Buckler et al. 1981, Gray et al. 1989, Giesy et al. 1997, Johnson et al. 1997) and some metals (Kirubagaren & Joy 1988). Increased incidence of oocyte atresia has also been reported in a variety of wild fish (e.g. largemouth bass, redbreast sunfish, bluegill- and brown bullet) from sites contaminated with PCBs, metals, bleached kraft mill efflu-

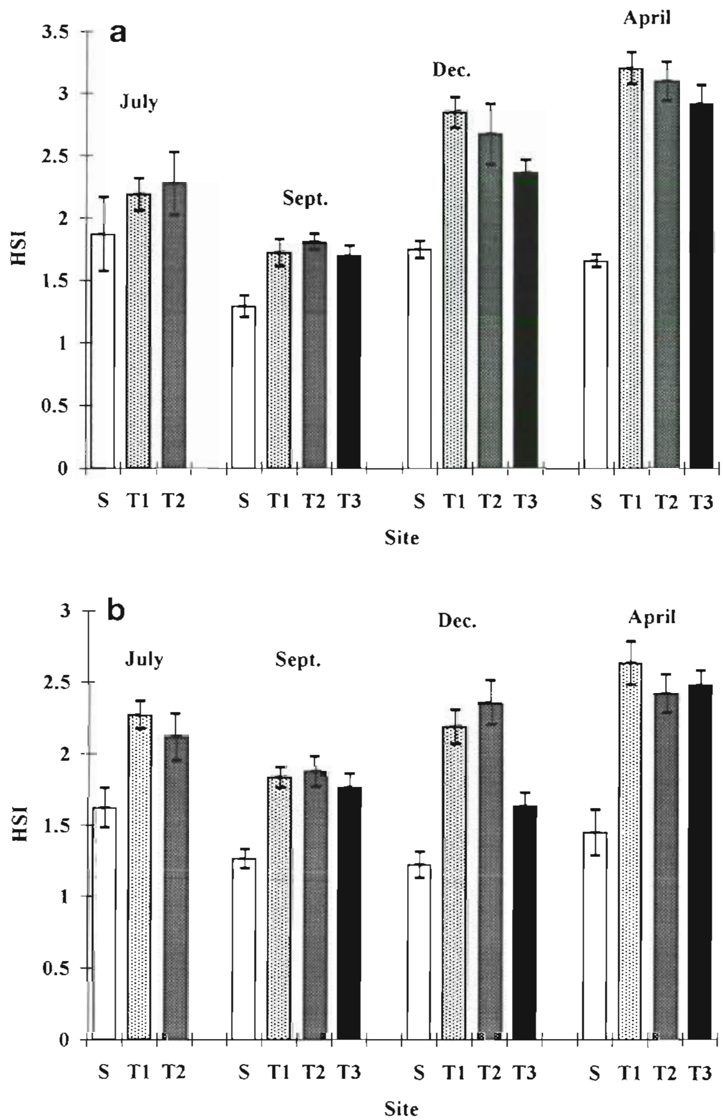

Fig. 12. Mean (± SE) hepatosomatic index (HSI: liver weight [g]/body weight [g]) of (a) female and (b) male flounders *Platichthys flesus* from sites in the Tyne Estuary (T1, T2, T3) and the Solway Firth (S)

ent, fuel components and mixed industrial wastewaters, and has been suggested to be related to the presence of chemical disrupters of the endocrine system (Greeley et al. 1997).

Grossly degenerating oocytes were absent in ovaries found in postspawning or previtellogenic phases (sampled in July) and were most frequent in ovaries in advanced vitellogenic phase (sampled in April), suggesting that the prevalence of degenerated oocytes correspond to the different stages in the reproductive cycle. This observation agrees with reports of other investigators who found atresia mainly restricted to yolked oocytes (Khoo 1975, Babu & Nair 1983, Johnson et al. 1991).

Increases in HSI were observed both in male and female flounders exposed to sewage effluent. Increases in liver weights (HSI) are associated with enhanced detoxification activities in response to the presence of toxic compounds, e.g. PAHs, PCBs (Janssen et al. 1996), cadmium (Pereira et al. 1992), crude oil (Truscott et al. 1992). They are also consistent with increasing levels of endogenous oestrogens in female fish during vtg synthesis (Janssen et al. 1995) and exposure to exogenous oestrogens in male fish, since oestrogen raises the amount of endoplasmatic reticulum in the liver, thus indicating enhanced protein (vtg) synthesis (Emmersen & Emmersen 1976).

In addition to most UK rivers (Harries et al. 1996, 1997), the present study strongly suggests that at least some UK estuaries are also suffering severe contamination by oestrogenic substances. Moreover, the limited data indicate that flounder showing strong vitellogenesis are also suffering adverse reproductive effects that may or may not derive specifically from the action of environmental oestrogens. Although the implications of these observations for fish reproduction and population are not yet known, there must be some concern that flounders showing these effects may have reduced reproductive viability. The present study therefore suggests that further research on oestrogenic contamination of the aquatic environment needs to focus on the crucial relationship between 'early' changes at the biochemical and physiological level (e.g. vtg production), histological changes (e.g. pathological effects on the gonads) and the more serious consequences at the population and community levels (e.g. changes in fecundity and viability).

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