

NOTE

First reported cases of intersex (ovotestis) in the flatfish species dab *Limanda limanda*: Dogger Bank, North Sea

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ABSTRACT: This paper provides the first report of the intersex (ovotestis) condition in the flatfish species dab *Limanda limanda*. Specimens presenting the condition were collected from the North Dogger Bank in the North Sea during the United Kingdom National Marine Monitoring Programme in 2003. Two of the individuals collected (14.3% of the males sampled) presented externally as males but upon histological examination were seen to contain both male and female gonadal tissues. Pre-vitellogenic oocytes were suspended within a matrix of spermatogonia and spermatocytes. One of the specimens also presented dilated testicular follicles filled with masses of mature spermatozoa. The significance of this finding in the United Kingdom's sentinel offshore monitoring species is discussed with relation to potential offshore effects of exposure to endocrine disrupting chemicals (EDCs).

KEY WORDS: Flatfish · Endocrine disruption · *Limanda limanda* · Ovotestis · Ovary · Testis · Reproduction

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INTRODUCTION

In recent years a significant proportion of research into the biological effects of contaminants in the aquatic environment has been devoted to the study of endocrine disrupting chemicals (EDCs) of anthropogenic origin. EDCs have been widely reported to impair fertility, development, growth and metabolism in a range of animal groups (see Colborn et al. 1996). The effects of exposure of fish to such compounds include disturbed maturation and degeneration of the gonads, elevated concentrations of vitellogenin (egg yolk protein) in the plasma of male fish and the presence of intermediate or 'intersex' gonads (Gimeno et al. 1996). Using histological analysis, fish with the intersex condition are seen to possess oocytes within their normal testicular matrix (Sharpe 1997, Bateman et al. 2004).

Until the early 1990s intersex had only rarely been described from fish in the wild (Jafri & Ensor 1979, Slooff & Kloowijk-Vandijk 1982, Blachuta et al. 1991).

However, the condition has now been detected in several wild freshwater and migratory species, including roach *Rutilus rutilus* (Jafri & Ensor 1979, Purdom et al. 1994, Jobling et al. 1998), gudgeon *Gobio gobio* (van Aerle et al. 2001), barbel *Barbus plebejus* (Vigano et al. 2001), chub *Leuciscus cephalus* (Minier et al. 2000), bream *Abramis brama* (Slooff & Kloowijk-Vandijk 1982), white perch *Morone americana* (Kavanagh et al. 2002), stickleback *Gasterosteus aculeatus* (authors' pers. obs.), coregonids (Mikaelian et al. 2002), grayling *Thymallus thymallus* (Blachuta et al. 1991) and Atlantic salmon *Salmo salar* (authors' pers. obs.). Furthermore, detection of elevated prevalences of intersex in some estuarine and marine species such as the European flounder *Platichthys flesus* (Allen et al. 1999), Japanese flounder *Pleuronectes yokohamae* (Hashimoto et al. 2000), bothid flounder *Bothus pantherinus* (Amaoka et al. 1974), common eel *Anguilla anguilla* (Peters et al. 2001) and viviparous blenny *Zoarces viviparus* (Matthiessen et al. 2000, Stentiford et al. 2003) suggest that the effects of anthropogenic

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EDCs extend beyond inland river systems to coastal waters. With the exception of the reports of plasma vitellogenin and ovotestis in male Mediterranean swordfish *Xiphias gladius* (Fossi et al. 2001 and De Metrio et al. 2003, respectively), little evidence has so far been presented on endocrine disruption in the offshore environment.

This study provides the first report of intersex in the United Kingdom National Marine Monitoring Programme's (NMMP) offshore sentinel flatfish species dab *Limanda limanda*. Two fish presenting the condition were present in a sample of 50 dab collected from the Dogger Bank, an NMMP sampling station in the central North Sea, during 2003. This discovery marks the first histologically confirmed case of intersex in a marine fish species collected from an offshore site.

MATERIALS AND METHODS

Dab *Limanda limanda* were captured using 30 min tows of a standard Granton trawl on the Northern Dogger Bank, North Sea (55° 03' 53" N, 02° 04' 26" E), as part of the NMMP, in July 2003. The sex, size (total length) and presence of external signs of disease were recorded for 50 fish using methodology specified by the International Council for the Exploration of the Sea (ICES) (Bucke et al. 1996). Following external disease assessment, fish were sacrificed by a blow to the head, followed immediately by severing of the spinal chord. Samples of liver, kidney, spleen and gonad were fixed in neutral buffered formalin for 24 h followed by transfer to industrial methylated spirit. Fixed samples were processed to wax in a vacuum infiltration processor using standard protocols. Sections were cut at 3 to 5 µm on a rotary microtome and resulting tissue sections were mounted onto glass slides before staining with haematoxylin and eosin (H & E). Stained sections were analysed by light microscopy (Eclipse E800, Nikon) and digital images of histological features were obtained using the Lucia™ Screen Measurement System (Nikon). Histopathological description of the intersex (ovotestis) condition in dab follows that set out for the flounder *Platichthys flesus* by Bateman et al. (2004).

RESULTS

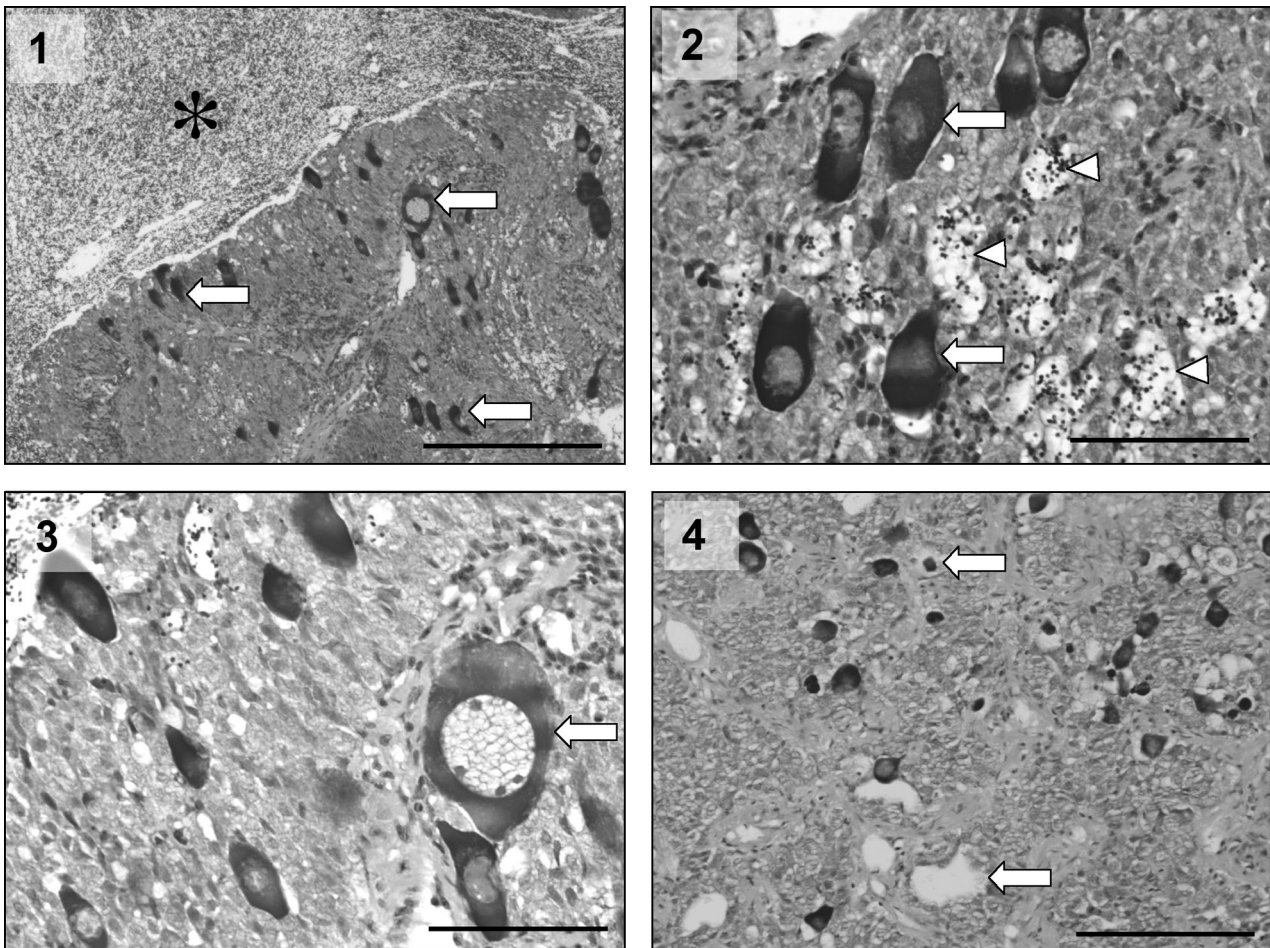
External visual examination revealed 14 male and 36 female dab from the sample collected at the North Dogger Bank site. Following histological preparation of gonadal tissue, 2 of the 14 males (apparent prevalence 14.3%) contained gonads classified as ovotestis by Bateman et al. (2004). The case description for each specimen is given below.

Specimen 1 was a 26 cm (165 g) dab displaying the external diseases of hyperpigmentation (Grade 2) and the fin parasite *Lepeophtheirus pectoralis*. The kidney and spleen appeared histologically normal while the liver exhibited a basophilic focus of cellular alteration (FCA) and melanomacrophage aggregates (MMA). The gonad appeared as a predominantly mature male type consisting of large, dilated follicles filled with masses of mature spermatozoa (Fig. 1). Apparent spermatogonia and spermatocytes lined the connective membranes between follicles and, amongst these, small vacuoles were seen to contain maturing spermatids and mature spermatozoa. Also embedded within the spermatogonia and spermatocytes were oocytes at various stages of development (Fig. 2). The earliest stages contained small but pronounced eosinophilic nuclei with a homogenous basophilic cytoplasm. Larger oocytes also contained prominent nucleoli. The most mature stages observed were late perinuclear oocytes (Fig. 3). Although no oocytes were observed loose within the follicles, several were only partially embedded within the testicular parenchyma. Only 1 testicular lobe was assessed. The specimen was classified as Grade 1 according to the Ovotestis Severity Index (OSI) of Bateman et al. (2004).

Specimen 2 was a 21 cm (93 g) dab displaying the external diseases of hyperpigmentation (Grade 1) and an epidermal ulcer (Grade 1). The kidney and spleen appeared histologically normal while the liver exhibited a basophilic FCA, MMA and inflammation. The gonad appeared as a predominantly immature male type consisting of discrete testicular follicles containing spermatogonia and spermatocytes. Spermatozoa were present in small numbers. Oocytes were present in a focal area within the testis and, as in Specimen 1, were embedded within the matrix of spermatogonia and spermatocytes, occasionally within small vacuoles similar to those containing mature spermatozoa (Fig. 4). All of the oocytes were of immature development, containing a pronounced eosinophilic nucleus and a homogenous basophilic cytoplasm. Several of the oocytes appeared to be undergoing apoptosis, with condensation of chromatin within the nucleus and separation from their supporting matrix. Oocytes were only observed in 1 of the paired testicular lobes. The affected testicular lobe was classified Grade 1 according to OSI of Bateman et al. (2004).

DISCUSSION

The intersex condition is well described from the gonads of several wild gonochoristic teleost fish species inhabiting riverine and estuarine habitats. In several cases, the condition has been linked to the ambient presence of chemicals known to disrupt normal hormonal functioning of the host. On the other hand, re-



Figs. 1 to 4. Intersex (ovotestis) in the dab *Limanda limanda*. **Fig. 1.** Specimen 1. Masses of mature spermatozoa fill the dilated testicular follicles (asterisk) while pre-vitellogenic oocytes (arrows) are suspended in a matrix of spermatogonia and spermatocytes. Scale bar = 100 μ m. **Fig. 2.** Specimen 1. Pre-vitellogenic oocytes (arrows) within spermatogonial matrix. Mature spermatozoa are present within vacuolated regions (arrowheads). Scale bar = 200 μ m. **Fig. 3.** Specimen 1. Perinuclear stage oocytes (arrow) were the most mature stages observed. Scale bar = 50 μ m. **Fig. 4.** Specimen 2. Predominantly testicular matrix consisting of follicles containing spermatogonia and spermatocytes. Pre-vitellogenic oocytes are suspended in follicle matrix and are occasionally present within vacuolated regions (arrows). Scale bar = 50 μ m

ports of the condition in predominantly offshore species are rare. In the present study, we report the first cases of intersex in the marine flatfish species dab *Limanda limanda*. The current study follows an extensive survey of dab reproductive biology from the southern North Sea in the late 1970s. In these surveys, the intersex condition was not recorded (Htun-Han 1978a,b). Dab are utilised as the main offshore sentinel in the United Kingdom NMMP due to their abundance, relative ease of capture and their tendency to express biomarkers of contaminant exposure (including liver cancer) (Anon 2003, Feist et al. 2004). Intersex fish were only found at the North Dogger Bank site in the central North Sea. Fish captured at this site, along with those from nearby sites on this bank, have been shown previously to display relatively elevated levels of disease, including neoplastic lesions of the liver (Anon 2003). Further

work is now required to compare contaminant burdens in sediment and fish tissue at these sites with those where such pathologies are not observed.

Since the intersex condition was not observed in dab captured from other NMMP sites during 2003, it could be proposed that those from the Dogger Bank site are displaying symptoms of exposure to EDCs. However, since hatchlings and juveniles are likely to inhabit different grounds to those where adults are sampled (Dipper 1987) and it is at these early life stages at which sex is determined (and at which disruption may occur) (Gimeno et al. 1997, Devlin & Nagahama 2002), the presence of fish with the intersex condition at the North Dogger Bank site may not necessarily reflect the presence of EDCs at the site but rather their presence at sites where hatching and early growth occurs. Future studies should be directed towards the mea-

surement of plasma vitellogenin in male fish in which intersex is found and to the histological analysis of juveniles. Studies of this kind would allow for assessment of potential endocrine disruption effects in adult fish and would also indicate the apparent prevalence of intersexuality in juveniles. Comparisons of the prevalence of the intersex condition in juvenile and adult fish of the same species may furthermore provide clarification on the population level effects of EDCs in the marine environment and on their long-term ecological effects on sensitive ecosystems. Coupled with studies on the population genetics of these species and the identification of specific spawning grounds for different adult stocks, the potential selective pressures imposed by endocrine disturbances may also be identified.

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