Marine Environmental Research 106 (2015) 42–50

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean

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ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form
13 February 2015
Accepted 23 February 2015
Available online 24 February 2015

Keywords:
Histopathology
Disease
Bay of Biscay
Neoplasia
Ovotestis
Deep-sea fish

ABSTRACT

The deep-sea environment is a sink for a wide variety of contaminants including heavy metals and organic compounds of anthropogenic origin. Life history traits of many deep-water fish species including longevity and high trophic position may predispose them to contaminant exposure and subsequent induction of pathological changes, including tumour formation. The lack of evidence for this hypothesis prompted this investigation in order to provide data on the presence of pathological changes in the liver and gonads of several deep-water fish species. Fish were obtained from the north east region of the Bay of Biscay (north east Atlantic Ocean) by trawling at depths between 700 and 1400 m. Liver and gonad samples were collected on board ship and fixed for histological processing and subsequent examination by light microscopy. Hepatocellular and nuclear pleomorphism and individual cases of ovotestis and foci of cellular alteration (FCA) were detected in black scabbardfish (*Aphanopus carbo*). Six cases of FCA were observed in orange roughy (*Hoplostethus atlanticus*) (n = 50) together with a single case of hepatocellular adenoma. A wide variety of inflammatory and degenerative lesions were found in all species examined. Deep-water fish display a range of pathologies similar to those seen in shelf-sea species used for international monitoring programmes including biological effects of contaminants. This study has confirmed the utility of health screening in deep-water fish for detecting evidence of prior exposure to contaminants and has also gained evidence of pathology potentially associated with exposure to algal toxins.

1. Introduction

Growing interest in fisheries resources and ecosystems of the continental slope is concomitant with depletion of exploitable stocks within continental shelf and inshore zones. Some slope dwelling fish species have significant economic value. Concerns regarding the vulnerability of several species in relation to their life history traits of slow growth, maturation at relatively old age and longevity have prompted stock assessment studies and estimates on the effects of commercial fishing (Lorance et al., 2010; Planque et al., 2012; Trenkel et al., 2012). However, studies on the health status of continental slope fish species, i.e. those occurring mainly from 200 to 2000 m depth, have largely been restricted to investigations of their parasite fauna (Herring, 2007; Klimpel et al., 2001). Such studies have not incorporated histological assessments to detect negative outcomes of such infections but have instead focussed on prevalence and intensity information which may be of use as 'biological tags' for stock discrimination and management (Lester et al., 1988; MacKenzie and Abaunza, 1998). However, Feist and Longshaw (2008) have shown how histopathology can be used to assess the health impacts of parasitism. Such an approach has the benefit of being able to detect other pathologies which may include toxicopathic changes resulting from exposure to anthropogenic contaminants, and other idiopathic lesions of potential detriment to fish health (Stentiford et al., 2009).

It is well known that the deep-sea environment acts as a sink for contaminants including heavy metals (e.g. mercury, cadmium and lead) (Fowler, 1990; Afonso et al., 2007; Costa et al., 2009; Chouvelon et al., 2012) and organic contaminants (e.g. polychlorobiphenyls (PCBs) and persistent pesticides) (Froescheis et al.,

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http://dx.doi.org/10.1016/j.marenvres.2015.02.004

2000; Looser et al., 2000; Mormede and Davies, 2003). In addition, bioaccumulation in the resident fauna is a significant issue for species destined for human consumption (Mormede and Davies, 2003; Afonso et al., 2008).

Assessment of the health status of marine fish species forms an important approach that can be applied internationally under Descriptor 8 of the Marine Strategy Framework Directive (MSFD) to provide assessments of Good Ecological Status (GES) (Lyons et al., 2010). Such approaches use combination assessment of externally visible diseases and internal evaluation of pathology, in particular the presence of toxicopathic-related liver and gonadal pathologies (Bateman et al., 2004; Feist et al., 2004; Lang et al., 2006).

The presence of liver tumours and related lesions are recognised as indicators of previous contaminant exposure (Myers et al., 1991, 1994, 2008; Schiewe et al., 1991; Reichert et al., 1998). The categorisation of lesion types in fish was derived from similar studies investigating hepatic carcinogenesis in rodents (Jones et al., 1997). The progression of initial changes leading to more neoplastic lesions which may culminate in malignant tumours (Bannasch et al., 1997). In fish it is thought that a similar progression occurs and several studies have demonstrated that this process can take several years (Rhodes et al., 1987; Myers et al., 1998; Baumann et al., 1990; Vethaak and Wester, 1996). In a study of 1093 dab from the North Sea, Stentiford et al. (2010) demonstrated that age at onset of different stages of carcinogenesis differed between fish taken from different locations. It is not known whether the occurrence of 'late-stage' liver tumours contribute to mortality in dab or flounder but this has been postulated for other fish species inhabiting contaminated environments and exhibiting liver tumours (Baumann et al., 1990).

Endocrine disrupting chemicals (EDCs) and their biological effects in fish have attracted much attention in recent decades (Allen et al., 1999). Biological effects of exposure to EDCs include overproduction of the egg yolk protein vitellogenin (Purdom et al., 1994) and behavioural disturbances (Sebire et al., 2008). In addition, disturbances in the morphogenesis of the gonads result in the occurrence of 'intersex' condition. In histological sections, the presence of oocytes in testis or testicular tissue in ovaries is denoted ovotestis or testis-ova respectively. In flounder and dab the most common lesion type is ovotestis (Allen et al., 1999; Bateman et al., 2004; Stentiford and Feist, 2005). However, evidence of EDC effects has been detected in several marine fish species other than flatfish, including cod (Scott et al., 2006) and swordfish (De Metrio et al., 2003).

To date, there have been no studies investigating the occurrence of histopathological lesions indicative of anthropogenic contaminant exposure in continental slope fish species. This investigation provides the first such assessment of lesions occurring in the livers and gonads of several fish species from the NEA continental slope, with particular focus on putative toxicologic lesions.

2. Materials and methods

Fish were captured by the *RV Thalassa* in the north east region of the Bay of Biscay (NEA) during the western International Bottom Trawl Survey, November 2009 (Fig. 1). Authorisations to carry out this survey were obtained from Préfecture Martime de l'Atlantique, Brest, France (FAX N° IF 66 CECLANT/OPS/SERPUB of 12 October 2009 and N° IF 68 CECLANT/OPS/SERPUB of 28 October 2009), Ministerio de Asuntos Exteriores y de Cooperacion, Madrid (Nota Verbal N° 1921 of 28th April 2009), Department of Foreign Affairs, Dublin, Ireland (letter No 439/09 of 9th October 2009), Law of the Sea Section, Foreign & Commonwealth Office, London, UK (Note Verbale No 064/2009 of 3d July 2009, including clearance from MOD No 595-10-5 of 2d July 2009) for waters under the jurisdiction of France, Spain, Ireland and UK respectively. The standard programme of this survey covers the upper continental slope down to 700 m. Samples of blackbelly rosefish (*Helicolenus dactylopterus*) (BRF) and greater forkbeard (*Phycis blennoides*) (GFB) were obtained from this region, with juveniles of these two species caught on the shelf and larger adults along the continental slope. In addition, 5 tows were carried out along the mid-slope at 1000–1400 m in the region 47°40′ N and 8°W on a small flat area known as Meriadzek Terrrace. In addition to the species indicated above, all samples of black scabbardfish (*Aphanopus carbo*) (BSF), orange roughy (*Hoplostethus atlanticus*) (ORY) and roundnose grenadier (*Coryphaenoides rupestris*) (RNG) came from this specific area. Sufficient numbers of BSF (n = 32), ORY (n = 50), GFB (n = 36), BRF (n = 32) and a smaller number of RNG (n = 12), were obtained for biological sampling, disease and histological evaluation.

Owing to barotrauma, all slope fish caught during the survey were freshly dead when hauled on the deck of the vessel. Fish were examined individually for signs of disease. However, scale and skin loss during capture and hauling from depth precluded accurate recording of external lesions. Evidence of parasites and length and weight were recorded. Otoliths were removed for age determination of all BSF sampled for organ pathology. Left otoliths were transversely sectioned through the nucleus and 0.5 mm thick sections were mounted on glass slide for assessment (Morales-Nin et al., 2002). Estimates of age for ORY, RNG, GFB and BRF were based on existing age/length keys, growth curves and longevity estimates (Allain and Lorance, 2000; Casas and Pineiro, 2000; Minto and Nolan, 2006; Andrews et al., 2009; Sequeira et al., 2009). The visceral cavity was opened and sex determined by visual examination of the gonads. Depending on the size and species, gonads were removed whole or a 3–5 mm section was dissected and fixed in 10% neutral buffered formalin. In smaller fish, whole liver and gonads were sampled, whilst in larger specimens, a standardised 3–5 mm section was dissected from the central portion of the organ. Fixation was allowed to proceed for a minimum of 24 h before transfer to 70% industrial methylated ethanol until laboratory processing.

Fixed specimens were processed to wax embedded blocks using a vacuum infiltration processor and standard protocols (Feist et al., 2004). Sections were cut at 3–5 μm on a motorised rotary microtome, mounted on glass slides, dried and stained with haematoxylin and eosin (H&E), Periodic Acid Schiff (PAS) and Feulgen stains for selected slides. Stained sections were examined using a Nikon Eclipse E800 microscope and digital images of representative lesions were captured using the Lucia™ Screen Measurement System (Nikon). Liver lesion characterisation followed the method of Feist et al. (2004).

3. Results

The liver of each of the species displayed a trabecular arrangement of hepatocytes and the presence of structures such as bile ducts and blood vessels. However, depending on the amount of intracellular lipid and glycogen, individual hepatocytes displayed a significant variation in appearance within H&E stained sections, with the trabecular arrangement of hepatocytes less apparent. A variety of pathological changes in the liver (Table 1) and gonad were seen and are described in the following sections according to fish species.

3.1. Black scabbardfish

Fish were between 5 and 11 years old (mean 8.6 years). The normal trabecular appearance of the liver was often indistinct although cords of hepatocytes aligning with the bile ductules could