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

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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 to 1400m. 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 scabbard fish (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.

Highlights

►Liver and gonad pathology in continental slope fish from the Bay of Biscay was investigated. ►Continental slope fish demonstrate toxicologic pathology. ►Neoplastic and preneoplastic pathology was detected black scabbardfish and orange roughy. ►Ovotestis was detected in black scabbardfish. ►Algal toxin – like pathology was seen in black scabbardfish livers.

Keywords : Histopathology, Disease, Bay of Biscay, neoplasia, ovotestis, deep-sea fish

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 2000m depth, have largely been restricted to investigations of their parasite fauna (Herring, 2007; Klimpel et al., 2009). 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., 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, et al., 1996). In a study of 1,093 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 over-production of the egg yolk protein vitellogenin (Purdom et al., 2004) 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 to 1400 m in the region 47°40' North and 8°W on a small flat area known as Meriadzek Terrace. 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.

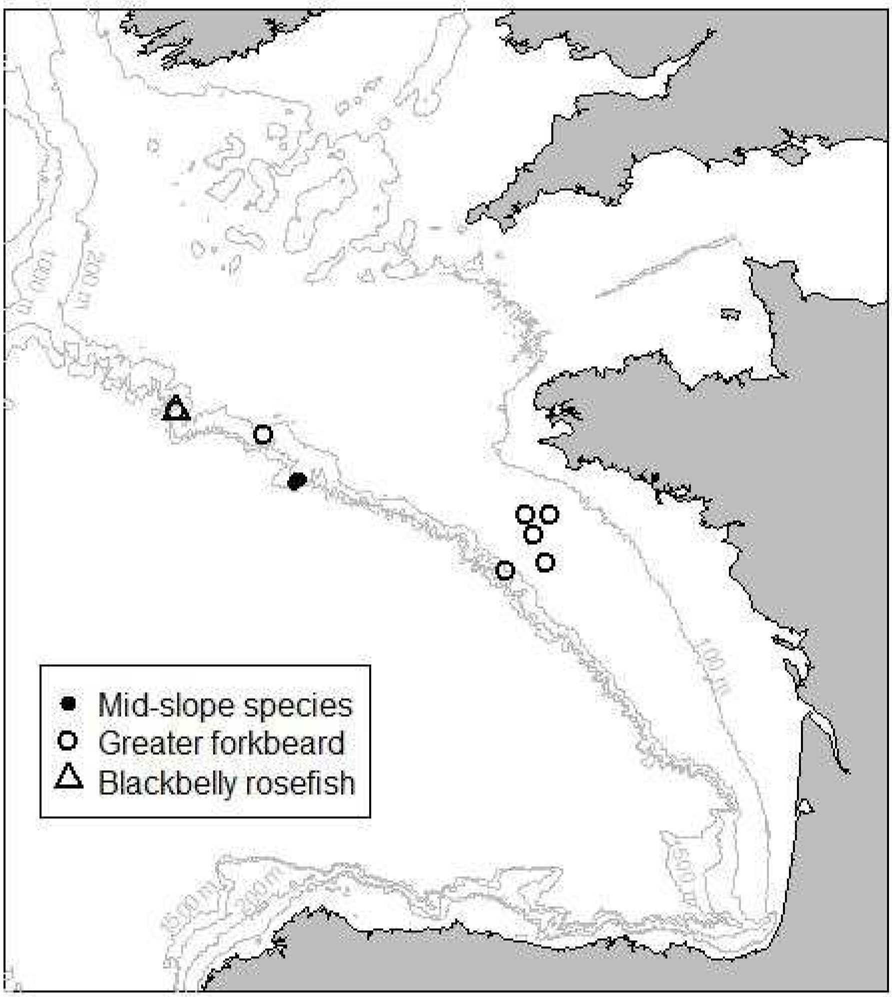

Figure 1. Sample locations in the Bay of Biscay for the five studied species, mid-slope species include black scabbardfish, orange roughy and roundnose grenadier. Depth contours shown are 100, 200, 500, 1000 and 1500 m.

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 0.5mm 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.

Table 1. Prevalence (%) of lesion types detected in the liver of each species examined. Note that individual fish may have more than one lesion type present.

Species	No.	Lesion type (%)											
		NAD	HNP	FCA	Adenoma	Necrosis	MA	Granuloma	Lymphocytic infiltration	Nematodes	Phospho-lipidosis	Steatosis	Spongiosis hepatis
Black scabbardfish	32	0.0	96.9	9.4	0.0	34.4	28.1	62.5	12.5	15.6	3.1	0.0	6.3
Orange roughy	50	8.0	6.0	10.0	2.0	16.0	78.0	0.0	0.0	14.0	2.0	2.0	0.0
Greater forkbeard	36	80.1	0.0	0.0	0.0	0.0	0.0	8.3	11.1	2.8	0.0	0.0	0.0
Bluemouth	32	59.4	0.0	0.0	0.0	3.1	28.1	0.0	18.8	3.1	0.0	0.0	0.0
Roundnose grenadier	12	16.7	0.0	0.0	0.0	0.0	66.7	8.3	0.0	0.0	0.0	0.0	0.0

NAD – No abnormalities detected

HNP – Hepatocellular and nuclear pleomorphism

FCA – Focus of cellular alteration

MA – Macrophage aggregate

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 be easily seen in most cases (Figure 2A). Hepatocytes were generally granular in appearance with varying amounts of vacuolation present and conspicuous regions of relatively basophilic material most likely representing endoplasmic reticulum. Most fish showed hepatocellular and nuclear pleomorphism (HNP) (Fig. 2B). This condition occurred throughout the liver and in several cases was pronounced, with significantly enlarged nuclei containing granular and marginated chromatin. Bi-nucleate hepatocytes were observed but bizarre nuclear morphology was not detected. A number of inflammatory lesions including lymphocytic infiltration and granuloma were seen (see Table 1; Fig. 2C). A single case of pre-neoplastic focus of cellular alteration (FCA) with surrounding low grade HNP was detected in a female fish of 11 years old (Fig 2D). In a single 9 year old fish, macroscopically identified as a female, the gonad displayed a mixture of predominantly primary oocytes interspersed with testicular tissue including foci of spermatocytes. This was identified as ovo-testis (Fig 2E).

Perivascular lymphocytic infiltration was present in four specimens with smaller focal inflammatory lesions also present. The presence of eosinophil granule cells suggests that these lesions may represent the previous location of a parasite infection. Indeed, in some cases these cells were associated with the presence of nematode parasites. In five fish, anisakid nematodes of an undetermined species were observed in section, loosely attached to the surface of the liver. Closer examination discerned a loose ‘capsule’ of connective tissue, apparently derived from the hepatic serosa, attaching the parasites to the liver surface. Associated inflammatory response in the liver tissue was not seen. Granulomas generally had chronic necrotic centres, often with no evidence of pathogen involvement. However, in a few cases, structures reminiscent of collapsed *Ichthyophonus* organisms were observed (Figure

2C). Necrotic foci were present in the livers of 11 fish with no aetiology visible in the sections examined. Affected hepatocytes showed loss of cellular integrity and separation from adjacent cells, cytoplasmic eosinophilia and the presence of strongly basophilic pyknotic nuclei.

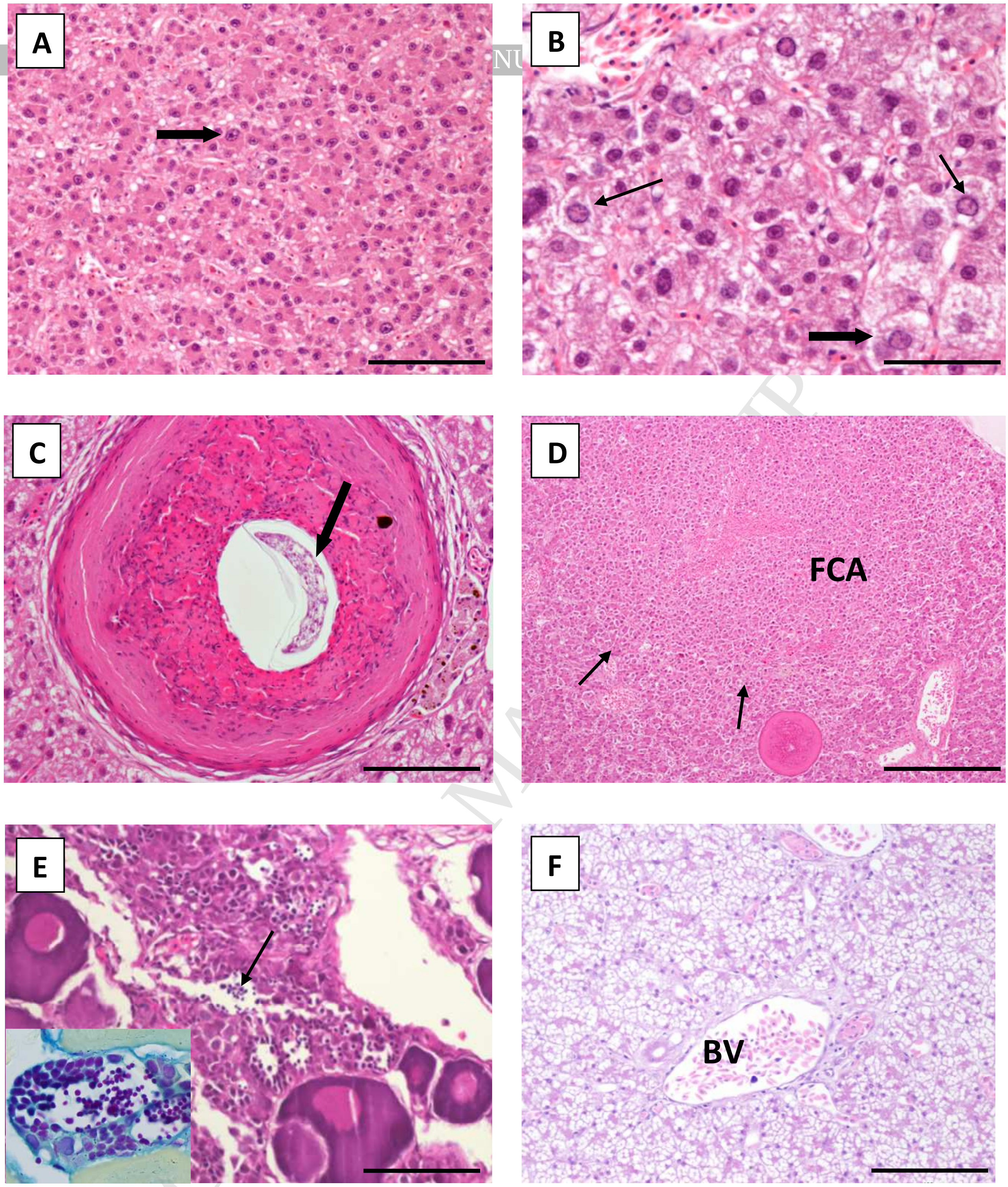

Figure 2 A-F. A-E Histological changes in black scabbardfish liver. All sections stained with H&E unless otherwise stated. (A) Section of normal liver showing indistinct trabecular pattern of hepatocytes and occasional hypertrophied hepatocyte nuclei (arrow). Bar = 100µm. (B) Hepatocellular and nuclear pleomorphism showing enlarged hepatocytes with loose granular cytoplasm (solid arrow) and hypertrophied nuclei with coarse chromatin (arrows). Bar = 50 µm. (C) Granuloma containing at its centre a collapsed *Ichthyophonus* cell and surrounded by a thin layer of flattened epitheloid cells. Bar = 100µm. (D) Focus of cellular alteration (FCA, arrows). Bar = 0.5mm. (E) Ovotestis showing foci of spermatocytes (arrow) amongst ovarian tissue (inset showing detail of spermatocytes stained with Feulgen for DNA). Bar = 50 µm. (F) Normal orange roughy liver showing increased hepatocyte vacuolation due to lipid storage. Blood vessel (BV). Bar = 100 µm.

3.2. Orange roughy

Based upon existing longevity data, and age-length relationships, individuals samples of ORY sampled here were between 30 and 100 years old. Similar to the variation seen in BSF, ORY livers showed a wide range of hepatocyte morphology with the cytoplasm showing dense stain with few vacuoles in some cases with the majority of livers showing moderate to marked cytoplasmic vacuolation based on the degree of storage material (Figure 2F). In several livers examined, the hepatocytes also contained prominent eosinophilic material which was only weakly PAS positive. In addition, three cases of hepatocellular and nuclear pleomorphism were also observed (Figure 3A). Macrophage aggregates (MA) were present in 78% of fish examined and usually occurred with even distribution throughout the liver, sometimes associated with blood vessels. Hepatocellular necrosis affecting individual hepatocytes or focal areas was seen in eight fish, with no evidence of pathogen involvement. Acanthocephalan parasites were observed in liver sections of seven fish, appearing on the surface of the liver or embedded within the liver tissue amongst loose connective tissue with no discernible cellular inflammatory response. Conversely, nematode parasites located within the hepatic parenchyma induced granulomatous lesions. A single case of hyperplasia of the hepatic serosa with small foci of melanin pigmented cells was seen. Six cases of FCA were observed (Fig. 3B) and a single case of hepatocellular adenoma (benign neoplasia) was detected in a female fish measuring 58cm in length. The lesion comprised relatively vacuolated hepatocytes compared to the surrounding cells and showed evidence of characteristic compression of adjacent liver tissue (Fig. 3C). All gonads examined appeared normal.

3.3. Blackbelly rosefish, roundnose grenadier and greater forkbeard

Based upon estimated growth (Sequeira et al., 2009), 29 individuals BRF between 25 and 35 cm total length were estimated to be 12 to 26 years old. Three smaller individuals (20 cm) were estimated as being 8 years old. The liver of 19 of the 32 BRF examined showed no significant pathology. The remaining fish exhibited inflammatory lesions, MAs and a single case of focal necrosis (see Table 1). No lesions known to be directly caused by toxins or contaminant exposure were observed.

Based on existing growth curves, individual RNG were between 5 and 30 years old. The liver of RNG was characterised by extensive vacuolation of hepatocytes, frequently interspersed with small MA (Figure 3D). In 2 fish, hepatocytes were depleted of storage material and were strongly basophilic. In these cases, MA were enlarged and constituent cells were themselves vacuolated (Figure 3E). All gonads from BRF and RNG appeared normal.

Based on estimated growth (Casas and Pineiro, 2000), individual GFB were less than 1 to 7 years old. The smallest individuals were captured on the shelf and were in their first year of life while larger individuals were captured on the continental slope. Lymphocytic infiltration and granulomatous lesions with no discernable pathogen involvement were seen in seven fish. In a single fish, macroscopically identified as female (56cm in length), the gonad displayed a mixture of predominantly primary oocytes interspersed with apparently altered ovarian germ cell lineages (oogonia), which were commonly multinucleate (Fig. 3F). In several regions, small groups of apoptotic cells and structures resembling spermatogenic cytocysts were visible. All other gonads examined appeared normal.

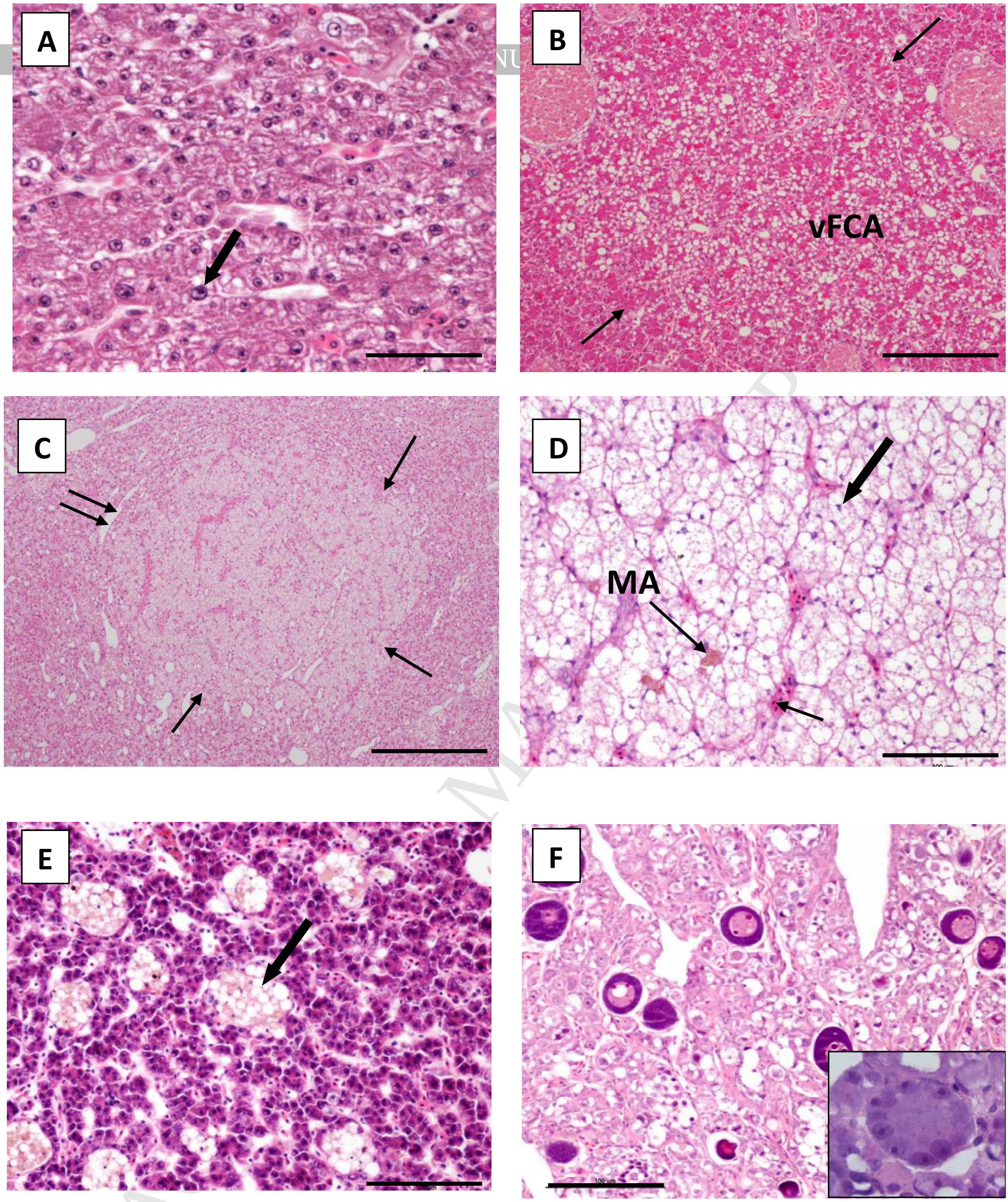

Figure 3 A-F. All sections are stained with H&E. A-C are of orange roughy liver. (A) Hepatocellular nuclear pleomorphism showing several hepatocytes with conspicuously enlarged nuclei (arrow). Bar = 50µm. (B) Vacuolated focus of cellular alteration (vFCA) (arrows show the extent of the lesion). Note the conspicuous macrophage aggregates distributed around the lesion. Bar = 50µm. (C) Well circumscribed adenoma (single arrows show the extent of the lesion) with the double arrow showing a region of hepatocyte compression. Bar = 100µm. (D) Roundnosed grenadier liver showing uniformly vacuolated hepatocytes with condensed nuclei (arrow). Small blood vessels (BV) and pigmented macrophage aggregates (MA) are also visible. Bar = 100µm. (E) Roundnosed grenadier liver hepatocytes with depleted lipid reserves interspersed with vacuolated pigmented macrophage aggregates (arrow). Bar = 100 µm. (F) Greater forkbeard gonad showing ovarian tissue with few previtellogenic oocytes dispersed amongst abnormal tissue containing numerous abnormal oogonia, including multinucleate stages (inset). Bar = 100µm.

4. Discussion

Interest in exploitation of slope fish stocks has led to established fisheries for several species. The species studied here are commercial benthopelagic fish (inhabiting the first few tens of meters above the seafloor, in depths >200m). The deep-sea environment is the ultimate sink for anthropogenic contaminants (Ballschmitter et al., 1997; Froescheis et al., 2000; Looser et al. 2000) and in some regions natural geochemical processes can also contribute to levels of mercury and other trace metals (Afonso et al., 2007). Concern on effects of anthropogenic contaminants on deep-sea fauna has only recently resulted in studies to examine contaminant burden in benthopelagic fish species (Froescheis et al., 2000). However, few authors have commented on the potential contaminant-related health effects in exposed fauna (Storelli et al., 2009), with investigations in the Atlantic Ocean sparse (Barber and Warlen, 1979; Kramer et al., 1984; Froescheis et al., 2000; Afonso et al., 2007; Webster et al., 2009). Bioaccumulation in deep sea fish may be a significant human health issue if those species are destined for human consumption; organic pollutants in benthopelagic fish species may be 10 to 17 times higher than that measured in shelf demersal species (Looser et al., 2000). Studies on polychlorobiphenyls (PCBs) and pesticides have been undertaken in BSF, ORY, RNG and *Bathysaurus ferox* from a number of locations in the NEA (Mormede and Davies, 2003). For several species investigated, contaminant levels were elevated in males compared to females (e.g. mean concentration of PCBs in RNG was 876 vs 664ng g^{-1} lipid weight). This is likely due to elimination of contaminants through egg production in females. Highest levels of contaminants were found in the deepest dwelling species, *B. ferox* with concentrations of PCBs up to 10 times higher than that recorded in other species. More recently Webster et al. (2009) examining chlorobiphenyls in benthopelagic fish from Rockall Trough, off the west of Scotland (NEA) showed that for some fish species, liver burdens were above Oslo and Paris Commission (OSPAR) Background Assessment Concentrations (BAC). Contamination with PCBs (e.g. mean concentration of PCBs in roughsnout grenadier *Trachyrinchus trachyrinchus* was 12,327 ng g^{-1} lipid weight) and organochlorine pesticides (e.g. mean concentration of DDT in *T. trachyrinchus* was 5357 ng g^{-1} lipid weight) in benthopelagic fish from the Mediterranean Sea has also been identified (Storelli et al., 2007, 2009). These authors draw attention to potential adverse health effects on the fish and for the need to undertake further assessments to allow effective management and long-term conservation of the ecosystem in the region. Few investigations into heavy metal contamination have been undertaken (Fowler, 1990). However, studies in the NEA in Portuguese waters showed that levels of mercury, cadmium and lead concentrations in BSF were highest in the liver (mercury and cadmium) and gonad (lead) (Afonso et al., 2007; Costa et al., 2009).

Several species such as ORY and RNG are extremely long-lived, whilst others such as BSF and GFB have longevity similar to continental shelf species (Morales-Nin et al., 2002). This study has afforded the opportunity to observe the normal histological structure of the liver and gonads of such species. The wide variation in appearance of liver tissue observed is apparently dependent on the amount of storage material present within hepatocytes, which in turn is influenced by sex, gonadal maturation, nutritional and disease status. This variation appears to be no greater than that observed in other fish species used for environmental monitoring purposes (Feist et al., 2004). Non-specific and inflammatory lesions including lymphocytic infiltration, granuloma, macrophage aggregates and variable glycogen content; HNP; foci of cellular alteration and benign neoplasms are a similar range of pathologies to that seen in continental shelf and estuarine fish in the NEA (Bucke and Feist, 1983; Stentiford et al., 2003, 2009, 2010; Lang et al., 2006; Fricke et al., 2012). This study has shown that the techniques developed for dab and flounder histopathology are applicable to a range of benthopelagic non-flatfish species and that the risk of barotrauma on the integrity of internal organs prior to sampling appears to be negligible. Whether consistent spatial and temporal patterns in disease presence and prevalence can be observed in benthopelagic fish (as shown for continental shelf species; Vethaak and Jol, 1996; Lang et al., 1999; Wosniok et al., 2000; Stentiford et al., 2009) remains to be shown. If contaminants cause the pathologies observed here, temporal changes might occur over the long-term because of the combination of the species longevity, the time of transfer of contaminants from continental sources and their (unknown) persistence in the deep sea environment. BSF is a highly mobile species probably constituting a single population distributed throughout the NEA (Stefanni and Knutsen, 2007). ORY displays panmixia in the north Atlantic, which has been ascribed to active adult migrations (White et al., 2009). This however does not preclude the possibility that panmixia is maintained by spawning migrations with individuals keeping the same feeding habitats during their life span. RNG however, is a poor swimmer and genetics suggests meta-population structure in the NEA, in particular with a weak difference between individuals from the Bay of Biscay and other regions (White et al., 2010). Population structure is unknown for BRF and GFB. These species live on or close to the seafloor, suggesting limited mobility (Uiblien et al., 2003). Thus, some of the studied species may respond to deep-sea environmental contamination at rather local scale while other may represent the contamination at basin scale. The two extremes might be GFB and BRF versus BSF respectively.

Inflammatory lesions degenerative changes and macrophage aggregates are common findings in many fish species and are generally indicative of host response to pathogens and the process of natural cell turnover (Feist and Longshaw, 2008). The appearances of these and other lesions detected during this study are indistinguishable from those used for the assessment of biological effects of contaminants monitoring programmes in the NEA (Feist et al., 2004). In particular, HNP (an early non-neoplastic toxicopathic lesion) and FCA are considered to be caused by contaminant exposure (Myers et al., 1998) with the latter generally accepted as being a precursor to benign and malignant liver tumours. The presence of HNP within focal lesions is an important feature indicative of malignancy (Feist et al., 2004). However, diffuse HNP (not within lesion) was a prominent feature in livers of nearly all black scabbardfish in this study and has been recorded in several other marine fish species (Myers et al., 1998; Stehr et al., 2003; Stentiford et al., 2003; Lyons et al., 2004; Lang et al., 2006; Fricke et al., 2012). Netpen liver disease in cultured Atlantic salmon presents a very similar histological appearance (Kent et al., 1990) and is caused by exposure to microcystin, an algal toxin (Anderson et al., 1993). This lesion is very common in netpen-reared Atlantic salmon in Washington State, USA and British Columbia, Canada, but is also observed in wild Chinook salmon and other species from marine waters (Stephen et al., 1993; Zimba et al., 2001). However, exposed fish are able to recover (Kent, 1990; Fournie and Courtney, 2002). The association with focal necrosis (in 34.4% of cases affecting BSF) also matches that seen in fish exhibiting microcystin-induced lesions, but histological evidence of recovery was absent. Deceased toxin-producing (demoic acid) algae are known to sink to the deep-sea environment (Potera, 2009) and it is presumed therefore that those producing microcystin would also sink upon death. Exposure to BSF could also be from transport of toxins to the sea floor following the collapse of bloom events. These possibilities require investigation.

In the present study, FCA were detected in the livers of 9.4% of BSF and 10% of ORY but not in the other species examined. In addition, one case of hepatocellular adenoma was seen in ORY which was estimated to be at least 30 years old. It can be concluded that these species are susceptible to hepatocellular neoplasia but further studies with epidemiologically significant numbers of fish and with concomitant contaminant analysis are required to investigate relationships between contaminant burdens and lesion occurrence. The effect of age on the prevalence of liver pathology and other diseases has been shown in a recent study by Stentiford et al. (2010). Although it is accepted that age is an important risk factor for cancer in humans and other animals including fish, that study demonstrated how age of onset of FCA and tumours occurred in dab populations from the Dogger Bank region of the North Sea earlier than in those from the Irish Sea. The authors concluded that other exogenous factors (possibly contaminants) were responsible for this difference. The age range of dab examined by Stentiford et al. (2010) was between 1 and 7 years old with the majority of lesions detected in the older fish. In the current study the single case of FCA in BSF occurred in an 11 year old female, this being one of the oldest individuals sampled. Further samples will be needed to determine whether, as in dab, FCA are more prevalent in older fish.

One example of intersex was detected in BSF. This is the first case identified in deep-water fish species and amongst the very few cases thus far reported in offshore locations (Stentiford and Feist, 2005). The occurrence of intersex in estuarine flatfish species is strongly associated with the presence of EDCs in the environment (Allen et al., 1998) but biomarker measurement for vitellogenin (VTG) levels in the blood provide the most common assessment of oestrogenic endocrine disruption. However, natural EDC's (e.g., phytoestrogens) could also cause intersex (Kiparissis et al., 2003), and intersex fish have been observed in waters considered to be pristine (Schwindt et al., 2009). Whether EDC exposure, either anthropogenic or natural, was a factor in the genesis of the current case is unknown. One case of putative triploidy was detected in the gadoid GFB. Whilst initial appearance of the tissue was suggestive of ovo-testis, the lesion likely represented an idiopathic disturbance in oogenesis. In this respect, the lesion may represent an example of naturally occurring triploidy, similar to that reported associated with triploidy in rainbow trout (Han et al., 2010).

There remains a general lack of information on contaminant burdens in deep-water fish over much of the globe with some areas more extensively studied. Although contaminant burdens in muscle tissue are generally not high enough for human health concern, it has been established that the deep-sea fauna has higher burdens of organochlorine compounds than in continental shelf species (Froescheis et al., 2000). An important factor of specific relevance to many deep-water fish species is their longevity. In benthic flatfish, macroscopic tumour formation occurs from approximately 4 years of age and appears to have a detrimental impact on survival (Myers et al., 2008). This study has shown for the first time that deep-water fish exhibit toxicopathic liver pathologies and are susceptible to tumour formation and intersex condition. Fish health monitoring in continental shelf fish species is well established internationally via OSPAR and includes quality assured assessment of externally visible disease conditions as well as the presence of liver nodules (organic contaminant induced tumours) and microscopic histological lesions (http:/www.bequalm.org/about.htm). Taking into account the contaminant levels detected in deep-water fish and their longevity, the presence of toxicopathic effects, particularly in the liver could be expected in other species not examined in the present study.

Our results suggest that deep-water fish display pathologies that are likely to be caused by their anthropogenic metal and organic contaminant burdens and via exposure to algal toxins. The species studied cover a range of life history traits, including different mobilities, feeding strategies and behaviour; however, all are high trophic levels predators (Lorance et al., 2006). The sampling area is not expected to be one receiving particularly high anthropogenic contamination input and the north Atlantic drift might bring oceanic water on the northern Bay of Biscay slope. Our observations therefore suggest a general impact of anthropogenic contaminants at ocean basin scale and across the whole trophic network.

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Figure legends:

Figure 1. Sample locations in the Bay of Biscay for the five studied species, mid-slope species include black scabbardfish, orange rougy and roundnose grenadier. Depth contours shown are 100, 200, 500, 1000 and 1500 m.

Figure 2 A-F. A-E Histological changes in black scabbardfish liver. All sections stained with H&E unless otherwise stated. (A) Section of normal liver showing indistinct trabecular pattern of hepatocytes and occasional hypertrophied hepatocyte nuclei (arrow). Bar = 100µm. (B) Hepatocellular and nuclear pleomorphism showing enlarged hepatocytes with loose granular cytoplasm (solid arrow) and hypertrophied nuclei with coarse chromatin (arrows). Bar = 50 µm. (C) Granuloma containing at its centre a collapsed *Ichthyophonus* cell and surrounded by a thin layer of flattened epitheloid cells. Bar = 100µm. (D) Focus of cellular alteration (FCA, arrows). Bar = 0.5mm. (E) Ovotestis showing foci of spermatocytes (arrow) amongst ovarian tissue (inset showing detail of spermatocytes stained with Feulgen for DNA). Bar = 50 µm. (F) Normal orange roughy liver showing increased hepatocyte vacuolation due to lipid storage. Blood vessel (BV). Bar = 100 µm.

Figure 3 A-F. All sections are stained with H&E. A-C are of orange roughy liver. (A) Hepatocellular nuclear pleomorphism showing several hepatocytes with conspicuously enlarged nuclei (arrow). Bar = 50µm. (B) Vacuolated focus of cellular alteration (vFCA) (arrows show the extent of the lesion). Note the conspicuous macrophage aggregates distributed around the lesion. Bar = 50µm. (C) Well circumscribed adenoma (single arrows show the extent of the lesion) with the double arrow showing a region of hepatocyte compression. Bar = 100µm. (D) Roundnosed grenadier liver showing uniformly vacuolated hepatocytes with condensed nuclei (arrow). Small blood vessels (BV) and pigmented macrophage aggregates (MA) are also visible. Bar = 100µm. (E) Roundnosed grenadier liver hepatocytes with depleted lipid reserves interspersed with vacuolated pigmented macrophage aggregates (arrow). Bar = 100 µm. (F) Greater forkbeard gonad showing ovarian tissue with few previtellogenic oocytes dispersed amongst abnormal tissue containing numerous abnormal oogonia, including multinucleate stages (inset). Bar = 100µm.