Marine Environmental Research 124 (2017) 54–60

Contents lists available at ScienceDirect

Marine Environmental Research

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

DNA damage in dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) from European seas

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ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form
29 December 2015
Accepted 7 January 2016
Available online 15 January 2016

Keywords:
ICON
Comet
DNA adducts
Genotoxicity
Haddock
Dab
European seas

ABSTRACT

Dab (*Limanda limanda*) and haddock (*Melanogrammus* aeglefinus) were collected from coastal and offshore locations in the Baltic (dab only), North Sea (haddock from one location only) and Iceland. Blood was analysed for DNA strand breaks using the comet assay and liver samples for DNA adduct concentrations. DNA strand breaks were at background levels in dab from the two Iceland locations and from the Dogger Bank. The highest levels were observed in dab from the Firth of Forth, Ekofisk and the German Bight. Hepatic DNA adducts in dab were highest at Ekofisk, in the Baltic and Dogger Bank, below detection limit in dab from Iceland and low in dab from the Firth of Forth and German Bight. There was large variation in DNA strand breaks between locations and individuals for haddock, particularly from Iceland. Adduct concentrations were elevated in haddock from both Iceland and the Firth of Forth. A general linear model (GLM) suggested that, in addition to location, the size of dab and its general condition contributed to explaining the observed variability in DNA strand breaks. A GLM for adducts in dab similarly allocated most of the variability to location, but with a possible contribution from CYP1A activity. There were no apparent differences between male and female dab for any of the methods. There was no obvious relationship between strand breaks and adducts in the same fish although dab from Ekofisk and Iceland had respectively high and low responses using both methods. The results from this large-scale study showed pollution-related genotoxicity for dab, that fish blood samples can be conserved prior to comet analyses and that there are clear species differences in genotoxic responses even when collected at the same location.

1. Introduction

The aquatic environment is the ultimate repository for a major proportion of anthropogenic waste, with a wide range of different chemicals being continuously introduced to coastal and offshore marine areas from rivers, atmospheric inputs as well as coastal and offshore point sources. While most of the chemicals released to the environment have low biological activity and are quickly degraded, some cause toxicity in marine organisms through e.g. interactions with ion regulation (Bjerregaard and Vislie, 1985), hormonal regulation (Kime, 1995), immune functions (Reynaud and Deschaux, 2006), the nervous system (Bocquene et al., 1990), or modifying nucleic acids (Brinkmann et al., 2014). Interaction of chemicals with nucleic acids, potentially resulting in genotoxicity, has been identified as one of the most important mechanism of action since damage to DNA can ultimately cause cell death, carcinogenesis and mutations, hence potentially affecting both individual health and the next generation through germ-line defects (Anderson et al., 1994; Rinner et al., 2011). Nucleic acids, including DNA, may be damaged through many mechanisms, some of which will be promoted through the presence of specific chemicals. Such mechanisms include strand breaks or base modifications, caused by reactive molecules, intercalation of substances between

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http://dx.doi.org/10.1016/j.marenvres.2016.01.001
0141-1136/

double-stranded DNA strands, potentially causing reading and copying errors, and covalent binding of chemicals to bases or sugars. DNA strand breaks have been most widely quantified through single-cell electrophoresis (Singh et al., 1988), a method that has been found its applications in both vertebrate (Collins and Gaivão, 2007; Collins et al., 1997) and invertebrate (Lewis and Galloway, 2008; Mamaca et al., 2005) systems. Covalent binding of chemicals to DNA has generally been quantified through ^{32}P postlabelling, a method similarly used in both human toxicology (Balbo et al., 2014) and ecotoxicology (Cachot et al., 2013; Ericson et al., 1998; Reichert and French, 1994). In addition to direct damage to the DNA thread, chromosomal damage has been widely investigated, most commonly as micronucleus formation (e.g. Lorge et al., 2008).

A potential for different chemicals to cause DNA damage in marine fish species has been amply documented in many laboratory studies. Increased DNA strand breaks, as documented using the comet assay, has been used with a range of fish species, but predominantly freshwater species (reviewed in Frenzilli et al., 2009). There is a scarcity of studies in which comet has been used in marine field studies, but some have been performed, e.g. using dab, Akcha et al. (2004, 2003) or butterfish (Bombail et al., 2001). One reason for the lack of studies is the challenge of keeping cells viable until analysis. Protocols have been developed to maintain fish blood cells for up to 48 h (Ramsdorf et al., 2009), but this is not sufficient for most field surveys where samples may have to be kept for weeks prior to analysis. In contrast to comet, DNA adducts have been used extensively in marine field-based studies (Aas et al., 2003; Ericson et al., 1998; Reichert and French, 1994; Rose et al., 2000) and was as early as in the 1990ies recommended for use in national and international monitoring programmes (OSPAR, 1998).

Any chemical that has a potential to be reactive or increase cellular oxidative stress may cause increased damage to DNA, as will chemicals that can intercalate between strands or bind to the molecule. There has been a particular focus on polycyclic aromatic hydrocarbons (PAHs) in this context, but a swathe of other environmental contaminants may also cause genotoxicity in marine organisms, including dichlorobenzene (Ganesan et al., 2013), natural toxins (Cavas and Konen, 2008) and styrene (Mamaca et al., 2005).

The current study is part of an international project, ICON[1](see Hylland et al., 2017), in which fish, other marine organisms and sediments were investigated at a range of coastal and offshore locations in the Mediterranean. Seine estuary, Wadden Sea, North Sea, Baltic and Iceland. Previous studies have suggested that the entire North Sea may be contaminated by PAHs (Hylland et al., 2006). Concentrations of PCBs and PAHs in areas around Iceland are the lowest reported for any oceanographic region in the North Atlantic (Schulz-Bull et al., 1998) and Iceland was hence selected as a reference area for ICON.

Two fish species were included in the current study: dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*). Dab is a benthic flatfish that will be chronically exposed to environmental contaminants through feeding and direct contact with contaminated sediments. Primary food sources are benthic invertebrates such as crustaceans and polychaetes, but molluscs, small echinoderms and fish are also part of the diet (Braber and de Groot, 1973). Dab is mainly found in shallow waters (less than 100 m depth) from the Bay of Biscay northwards, including the southern part of the Baltic. The species has been used extensively in monitoring programmes in the southern North Sea (Dethlefsen et al., 2000), has been target species in international research workshops (Bayne et al., 1988; Stebbing et al., 1992) and has been recommended by ICES[2] as a monitoring species. Haddock is a gadoid, but has a demersal life-style not that different to that of the two flatfish species above. Haddock mainly feed on sediment-dwelling species and benthic fish eggs. It is common throughout the northern North Sea and northwards, but not found in the southern North Sea, the Baltic or south of the English Channel. Haddock has become a target species in the Norwegian monitoring programme around oil and gas platforms, partly due to its apparent sensitivity to genotoxic contaminants (Balk et al., 2011; Hylland et al., 2006). Surveys off the Norwegian coast as part of the monitoring programme for environmental impacts of offshore oil and gas production have reported increased concentrations of hepatic DNA adducts in haddock collected in areas with high offshore activity, but this was not evident for Atlantic cod (*Gadus morhua*) or saithe (*Pollachius virens*) (Grøsvik et al., 2010).

The aim of the current study was to quantify DNA damage in two fish species from coastal and offshore areas in the Northeast Atlantic with varying inputs of contaminants. The study also aimed to compare the results from two methods to determine genotoxicity, the comet assay and DNA adduct concentration, as well as compare the sensitivity of the two investigated fish species to genotoxic contaminants in their habitat. Finally, the study aimed to identify non-contaminant factors that affect levels of DNA damage.

2. Materials and methods

2.1. Locations

All fish were collected by means of bottom trawling during a research cruise with RV Walther Herwig III in August/September 2008. For the purposes of this study, fish were sampled at five locations in the North Sea: Firth of Forth, Ekofisk, German Bight and Dogger Bank, as well as in the Baltic and at locations southeast and southwest of Iceland (cf Hylland et al., 2017; Tables 1 and 2). The Firth of Forth (FoF) is an area influenced by inputs from industrial activities as well as maritime activities (Elliott and Griffiths, 1987). The German Bight (GB) is another area of extensive maritime activities as well as receiving inputs from the two rivers Elbe and Weser (Ruus et al., 2006). The Dogger Bank (DB) is a feeding and spawning area for commercial fish in the North-East Atlantic, including dab (Heessen and Rijnsdorp, 1989). Ekofisk (EF) is in an area with high density of oil and gas platforms (Brooks et al., 2011). The location in the Baltic is the Mecklenburg bight. Two locations in Iceland were used for reference: Iceland southeast (ISE), located just south of the glacier Vatnajökull, and Iceland southwest (ISW) in the open ocean west of Reykjavik were both designated as reference areas. After trawling, fish were kept alive by rapid transfer to large tanks with pre-cooled, flow-through seawater. Further processing took place within 1 h.

2.2. Sampling

Fish were terminated prior to sampling. Blood was extracted from the caudal vein into heparin pre-coated syringes and immediately diluted 1:400 (by volume) using PBS-EDTA (KH_2PO_4 0.200 g L^{-1}; KCl, 0.200 g L^{-1}; $Na_2HPO_412H_2O$, 2.902 g L^{-1}; NaCl, 8.000 g L^{-1}; EDTA, 3.72 g L^{-1}; pH 7.4) and subsequently kept on ice until further processing. Liver and gonad weights were recorded, and otoliths collected for subsequent age determination. Bile was extracted and liver, gonad and muscle samples were collected from

[1] ICON: international workshop on marine integrated contaminant monitoring.

[2] ICES: International Council for Exploration of the Seas; http://www.ices.dk.