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Liver histopathology in Baltic flounder (*Platichthys flesus*) as indicator of biological effects of contaminants

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Abstract

Results are presented of a study on liver histopathology in Baltic flounder (*Platichthys flesus*) carried out in 2001 and 2002 in four coastal sampling areas of the Baltic Sea: Kvädöfjärden (Swedish east coast, reference area), Klaipeda-Butinge (Lithuanian coast), Gulf of Gdansk (Polish coast), and Wismar Bay (German coast) within the framework of the EU-funded BEEP project. Liver lesions were diagnosed and categorised using standardised methodologies and, for a spatial and temporal assessment of the prevalence and types of lesions detected, a scoring system was applied, involving the calculation of mean histopathology lesion scores. 83.0% of the 436 female flounder examined (size range: 20–43 cm total length, age range: 2–8 years) were affected by liver lesions, out of which 74.3% were assigned to the category of non-specific, 3.4% to the category of early toxicopathic non-neoplastic, 4.6% to the category of pre-neoplastic and 0.7% to the category of neoplastic lesions. Mean lesions scores were highest in the areas at the Lithuanian and Swedish coast and there is indication of an impact of the age structure of the flounder populations studied, the sampling season as well as of contaminant effects.

Keywords: *Platichthys flesus*; Baltic Sea; Liver histopathology; Biological effects of contaminants

1. Introduction

Because of the growing evidence of a cause–effect relationship between environmental contaminants and the occurrence of toxicopathic liver lesions in fish (Malins et al., 1988; Köhler, 1990; Köhler et al., 1992; Stein et al., 1992; Vethaak and Rheinallt, 1992; Moore and Myers, 1994; Myers et al., 1991, 1992, 1994, 1998a,b; Vethaak et al., 1996; Stehr et al., 1998; Stentiford et al., 2003; Feist et al., 2004), studies on liver histopathology in fish have increasingly been incorporated in national marine biological effects monitoring programmes (Lang, 2002a; Feist et al., 2004). On an international level, such studies are in the process of being implemented in integrated environmental monitoring and assessment programmes, e.g., as part of the OSPAR Coordinated Environmental Monitoring Programme (CEMP) for the northeast Atlantic (OSPAR, 2004). Through efforts of the International Council for the Exploration of the Sea (ICES) and the formerly EU-funded project Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM), guidelines for methodologies to be used for monitoring histopathological liver lesions have been developed and partly intercalibrated between laboratories (ICES, 1997; Lang, 2002b; Feist et al., 2004; BEQUALM, 2005). These guidelines have been adopted by OSPAR and have been incorporated

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in the Guidelines/Technical Annexes of the OSPAR Joint Assessment and Monitoring Programme (JAMP) for general and for PAH-specific biological effects monitoring (OSPAR, 1997, 2003, 2004).

The majority of studies on liver histopathology in flatfish species in relation to contaminants has been carried out in coastal waters of the USA (e.g., Malins et al., 1985a,b, 1988; Myers et al., 1991, 1992, 1994, 1998a,b; Stein et al., 1992; Moore et al., 1997; Stehr et al., 1998) and in the North Sea (e.g., Kranz and Dethlefsen, 1990; Bucke and Feist, 1993; Köhler, 1990; Köhler et al., 1992; Vethaak and Wester, 1996; Lang, 2002b; Stentiford et al., 2003). For the Baltic Sea, there is only limited information available, largely resulting from data generated within the 1994 ICES/BMB Workshop on Fish Diseases and Parasites in the Baltic Sea (Lang and Mellergaard, 1999; Lang et al., 1999; Bogovski et al., 1999) and some national studies (Bogovski, 1994; Lang and Dethlefsen, 1994; Wiklund and Bylund, 1994). These studies focused on the European flounder (*Platichthys flesus*) as target species because of its abundance and wide distribution in the Baltic Sea (Aro, 1989) and were largely conducted according to ICES standard methodologies, involving a quantification of macroscopically visible liver nodules >2 mm in diameter and a subsequent histological confirmation of the neoplastic nature of the lesions (ICES, 1989, 1997; Bucke et al., 1996; Feist et al., 2004).

In the present study, which was part of the EU-funded project Biological Effects of Environmental Pollution in Marine Coastal Ecosystems (BEEP) (Lehtonen and Schiedek, this volume; Lehtonen et al., this volume), a different approach was applied. Liver tissue samples were taken randomly from a defined number of flounder from four coastal sampling areas in the Baltic Sea (Swedish east coast, Lithuanian coast, Gulf of Gdansk, Wismar Bay) and were studied histologically for the presence of a wider range of lesions. The categories of liver lesions included in the examination are widely accepted as useful indicators for monitoring biological effects of environmental stressors, in particular contaminants, and ranged from non-specific lesions with a largely unclear linkage to contaminants to early toxicopathic non-neoplastic, pre-neoplastic, benign neoplastic and malignant neoplastic lesions, known to be associated with exposure to carcinogenic or non-carcinogenic contaminants (Malins et al., 1988; Myers et al., 1994; Vethaak et al., 1996; ICES, 1997; Feist et al., 2004). A scoring system was applied, the basic assumption of which is an increasing likelihood for a linkage to contaminant exposure (carcinogenic compounds in particular) from non-specific lesions to malignant tumours.

The aims of the present study were to provide baseline data on the prevalence of histopathological liver lesions in Baltic flounder and to compare the sampling areas in terms of the types and prevalence of lesions present, in the light of potential host-specific (fish size, age) and site-specific (contaminants) spatial and temporal effects, by using a widely applicable and robust categorisation and scoring system designed for monitoring purposes.

2. Materials and methods

Flounder were collected in four coastal sampling areas: Kvädöfjärden, Swedish east coast (reference); Klaipeda-Butinge area, Lithuanian coast; Gulf of Gdansk, coast of Poland and Wismar Bay, German coastal waters, by either bottom trawling or standing gears (the latter only at Kvädöfjärden). Samples were taken within four campaigns in spring 2001 (campaign 1), autumn 2001 (campaign 2), spring 2002 (campaign 3) and autumn 2002 (campaign 4), as described in Lehtonen and Schiedek (this volume). In each of the areas, up to three sampling stations were visited per campaign (Kvädöfjärden: only one station; Lithuanian coast: Butinge, Nemirseta, Palanga; Gulf of Gdansk: Mechelinki, Sobieszewo, Sopot; Wismar Bay: Wismar Harbour, Walfisch, Offentief), characterised by different contaminant exposure conditions (Baršienė et al., this volume; Kopecka et al., this volume; Schiedek et al., this volume; BEEP, unpublished data). Information on size and age spectrum of the flounder sampled is provided in Table 1.

Only live female flounder were processed for histology, either immediately after catching or after being kept alive in tanks with running seawater of ambient water temperature. Fish were sacrificed by a blow on the head, followed by decapitation, and were dissected. Histological sampling and further processing were performed according to standard procedures (Feist et al., 2004). Liver tissue samples (max 2 mm thick) were cut from the central part of the organ using a scalpel, placed into labelled histological cassettes, fixed in 10% neutral buffered formalin and transferred to 70% ethanol after 24–48 h. This was followed by dehydration of tissue samples, embedding in paraffin, sectioning at 3–4 μm using a rotary microtome and staining with haematoxylin and eosin. Four liver sections from each tissue sample were inspected by means of light microscopy. Otoliths were removed for subsequent age determination. From the same fish, various samples were obtained for other biological effects measurements the results of which are reported elsewhere (Baršienė et al., this volume; Broeg and Lehtonen, this volume; Kopecka et al., this volume; Lehtonen et al., this volume; Vuorinen et al., this volume).

Histopathological liver lesions detected were assigned to five main categories recommended for monitoring biological effects of contaminants in flatfish species: non-specific lesions, early toxicopathic non-neoplastic lesions, pre-neoplastic lesions, benign neoplasms and malignant neoplasms (see Table 2) (Feist et al., 2004). Lesions recorded were further staged into mild, medium and severe, depending on the size of the tissue area affected in the sections and the degree of cellular change observed. A scoring systems consisting of 15 scores was applied (see Table 2) and was used for the assessment of spatial and temporal variation in the lesions recorded. After inspection of the liver sections, each fish was assigned a score dependent on the type and severity of the lesion recorded. If more than one lesion category was recorded in one specimen, the highest out of the single