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Effect of age on liver pathology and other diseases in flatfish: implications for assessment of marine ecological health status

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ABSTRACT: Age at disease onset, a familiar concept in human medicine, has not been commonly discussed in relation to diseases of wildlife. Furthermore, while age has been anecdotally linked with disease susceptibility in aquatic animals (e.g. in aquaculture), little attention has been devoted to health of specific cohorts in wild aquatic animal populations. In an attempt to refine current approaches to marine environmental monitoring programmes that utilise fish diseases as a sentinel for ecological status, we have investigated age at onset for 10 disease variables in the flatfish *Limanda limanda*, a commonly utilised species in such programmes in Europe. By comparing the prevalence of these variables (including several associated with liver neoplasia) in different age classes from the same sites, we have demonstrated an increased propensity for 'harm' (a cumulative measure of multiple disease variables) with age. Furthermore, a comparison of age-matched cohorts from geographically distinct offshore marine sites revealed that this tendency for increased harm differs in magnitude between sites. In these scenarios, the age at onset for key disease variables (such as pre-neoplastic and neoplastic pathologies of the liver) also differs between sites, with some locations containing populations with apparently increased susceptibility in younger age classes. We conclude that while age is an important variable when assessing fish population health status, it does not explain all of the differences in disease prevalence observed in natural populations, and further, that age-matched cohorts from certain populations have a higher propensity for disease than fish of the same age from other sites. The basis for these differences in susceptibility is discussed in relation to the prevailing burden of anthropogenic contaminants and to other natural factors (such as population genetics and migration) inherent in sampled populations.

KEY WORDS: Assessment tool · Harm score · Liver cancer · Infectious disease · Marine monitoring · Cohort tracking · Flatfish · Age · Otolith

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INTRODUCTION

The principle of age at onset is used in human medicine to depict the age or period of life at which a disease or the initial symptoms or manifestations of a disease appear in an individual. The principle has been widely used to directly compare type, prevalence or severity of a wide range of diseases in specific age classes or cohorts from distinct geographic, demographic or socio-economic backgrounds. The ability to compare age at onset in this way offers a legitimate route by which disease can be attributed to cause, and whether the cause is uni- or multi-factorial in nature, by assessing which potential causes are associated with specific life stages of the host (Childs & Scriver 1986). While this principle is perhaps more clearly defined for diseases such as cancer that may have multifactorial causes both related to the environment and to the genetics of the host, it can also be applied to those diseases that are associated with infectious agents. In this context, the 'environmental' component is represented by the presence and fitness of the pathogen per se, while the addition of a 'genetic' component to the environmental component is represented by the fitness of the host in dealing with the pathogen (e.g. via competence of the immune system).

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In terms of aquatic animals and their diseases, the outcome of this interaction between host and pathogen can be measured via the susceptibility of given age classes of the host to the same pathogenic agent. Numerous studies have demonstrated differential susceptibility between age cohorts of the same host species, normally in the context of aquaculture, whereby early age classes (such as eggs and larvae) can be afflicted by different diseases (or the same diseases at a different prevalence and severity) to those observed in adult life stages (e.g. Kiran et al. 2002, Bergmann et al. 2003). This concept provides a rationale to consider whether similar phenomena may be apparent in natural (wild) populations of aquatic animals and furthermore, whether differential age of onset for the same disease may occur across geographical regions or temporally. In the latter case, it should then be possible to consider the role of endogenous (host-related) and exogenous (e.g. pathogen-specific, environment-specific) factors in the pattern of disease observed at a given point in time and the stability of this pattern over time — a feature recently identified for populations of marine fish sampled from European waters by Stentiford et al. (2009).

Tumorigenesis is a multi-step, gradual process for which the underpinning molecular steps, and the role of environmental exposure, are rarely known. Most human cancers appear later in life, with one explanation for this pattern proposing that cancer is fundamentally linked to ageing. Studies of lifespan in a taxonomically diverse range of animals (including fish) have however suggested that, although cancer prevalence may be higher in older animals, in laboratory exposure trials using a model carcinogen, animal groups with a lifespan of between 3 and 50 yr developed tumours at a similar rate, with latent periods of ~1 yr between exposure and the presence of tumours. In this context, the time-dependence for tumour development appeared more related to the cumulative dose of carcinogen than to either the lifespan of the host or to the rate of ageing (Lijinsky 1993). In addition, epigenetic factors such as changes in DNA methylation patterns are known to be important in the aetiology of mammalian tumour initiation and progression (Toyota et al. 2009). Studies have shown that gene-specific hyper-methylation and global hypomethylation are induced by environmental contaminants such as PCBs and metals (Desaulniers et al. 2009). The pattern is further complicated by a wide range of studies that have demonstrated how susceptibility to cancer following exposure to a range of model carcinogens is significantly host-type dependent, with large variations in dose-response amongst even closely related taxa (reviewed by Hengstler et al. 1999).

Several studies have assessed the relationship between tumour prevalence and age in fish. Baumann et al. (1987) demonstrated an increased frequency of liver tumours with age in brown bullheads *Ictalurus nebulosus* and also that 4 to 5 yr old fish had a significantly higher prevalence of biliary carcinomas than 2 to 3 yr old ones collected at the same site (Baumann et al. 1990). Furthermore, the latter study demonstrated a relative lack (or even absence) of fish of age ≥ 5 yr at sites with the highest liver tumour prevalence, suggesting an age-selective mortality associated with the disease (Baumann et al. 1990). Similar patterns had previously been observed in the marine flatfish *Parophrys vetulus* whereby fish of age < 4 yr were rarely observed with liver neoplasia (Rhodes et al. 1987, Myers et al. 1998a). Baumann et al. (1990) state that direct comparison of neoplasia frequency between sites or species is problematic since tumour data has not been related to sex or age data from the same fish, a feature also highlighted by Myers et al. (1998a), Rhodes et al. (1987) and Stehr et al. (2004) in their studies on liver tumours in marine flatfish sampled from US waters. Related work on the flatfish dab *Limanda limanda* from European waters has demonstrated how the frequency of DNA lesions (strand breaks and the formation of adducts) increases with age; this likely is an outcome of cumulative biotransformation of pollutants over time in older fish (Akcha et al. 2004). Similar studies on the European flounder *Platichthys flesus* have demonstrated how hepatic concentrations of metallothionein and the heavy metal cadmium increased with age and, interestingly, that maximum induction of metallothionein may occur when fish are still relatively young (Rotchell et al. 2001). Finally, Stentiford et al. (2009) have demonstrated stable patterns over space and time both in the profile and prevalence of diseases (including liver neoplasia) of *L. limanda* sampled from European waters. They stated that at least some of this stable pattern may be explained by life-history features of specific subsets of the metapopulation (e.g. the age of fish sampled at a particular site) and further that marine monitoring programmes that utilise fish disease data must take these life-history parameters into account when assessing site-level impacts, a process termed 'phenotype anchoring' in several recent studies attempting to utilise disease as a marker for assessing marine health status (Stentiford et al. 2005, Ward et al. 2006, Hines et al. 2007, Bignell et al. 2008).

Fish diseases have been recorded in marine and estuarine monitoring programmes for many years (Lang & Dethlefsen 1996, Vethaak et al. 2009). In the UK, the Clean Seas Environmental Monitoring Programme (CSEMP) monitors the disease status of the flatfish dab *Limanda limanda* at offshore sites and flounder *Platichthys flesus* at inshore and estuarine

sites according to procedures of the Oslo and Paris Commission (OSPAR) Coordinated Environmental Monitoring Programme (CEMP) (OSPAR 1998a,b). Grossly visible diseases present in these sentinel species include lymphocystis, epidermal hyperplasia and papilloma, acute and healing ulcerations of the skin and hyperpigmentation of the skin. The presence of grossly visible liver tumours (neoplasms) is also recorded routinely since, in flatfish, liver neoplasia likely represents a biological endpoint of historic exposure to chemicals that initiate and promote carcinogenic pathways (Myers et al. 1990, 1991, 1992, 1994, 2003, Schiewe et al. 1991, Reichert et al. 1998). In addition to the recording of grossly visible neoplasms, histopathological assessment of liver samples from flatfish populations collected under CSEMP allows for the detection and diagnosis of microscopic lesions not visible during gross fish assessments (see Stentiford et al. 2009). The lesions recorded by histopathology include those thought to precede the formation of benign and malignant tumours and include foci of cellular alteration (FCA), non-neoplastic toxicopathic lesions (such as nuclear and cellular polymorphism) and lesions associated with cell death, inflammation and regeneration. Currently, 32 categories of liver lesion in flatfish are classified under the international Biological Effects Quality Assurance in Monitoring Programmes (BEQUALM) initiative (Feist et al. 2004). Similar guidelines exist for diagnosis of liver lesions in medaka *Oryzias latipes* (Boorman et al. 1997) and English sole *Parophrys vetulus* (Myers et al. 1987).

In the present study we report on the prevalence and apparent age at onset for diseases of dab *Limanda limanda* collected at offshore UK CSEMP sites during 2004 and 2007. In addition to the consideration of individual diseases, we also applied a recently developed assessment tool that utilises an overall disease 'harm score' (Stentiford et al. 2009) to specific age classes sampled during 2004 and 2007. This assessment tool can be used to consider the effect of age on the overall harm score measured at specific offshore sites. The development of such assessment tools could be utilised to demonstrate whether maritime waters are achieving Good Environmental Status (GES) under international legislation such as the European Union Marine Strategy Framework Directive (MSFD) (Lyons et al. 2010).

MATERIALS AND METHODS

Scoping survey: 2004. In order to provide age-comparison data for offshore monitoring sites visited under the UK CSEMP, up to 50 dab *Limanda limanda* were sampled from 19 sites during June and July 2004 by use of 30 min tows of a standard Granton trawl fitted with a cod-end liner. This sampling strategy follows the guidelines of Bucke et al. (1996) and Feist et al. (2004). Sites visited (along with coordinates) are given in Table 1 and Fig. 1. The disease profile in dab collected from these sites is summarised in Stentiford et al. (2009). Briefly, these include a range of grossly visible diseases: lymphocystis (LY), acute/healing skin ulceration (U), epidermal papilloma (EP), skin hyperpigmentation (HYP) and so-called 'liver nodules' (LN); the latter required confirmation of specific type via histology. In addition, a series of liver pathologies are recorded and grouped under the headings of non-neoplastic toxicopathic lesions (such as hepatocellular and nuclear pleomorphism, HNP), non-specific inflammatory lesions (such as melanomacrophage aggregates, MMA), pre-neoplastic foci of cellular alteration (FCA), benign neoplasms (such as hepatocellular adenoma, HCA) and malignant neoplasms (such as hepatocellular carcinoma, HCC).

Upon landing, live dab were immediately removed from the catch and placed into flow-through tanks containing aerated seawater. All dab sampled were in the size range of 19 to 24 cm in length (according to Feist et al. 2004). The sex, total length and presence of grossly visible signs of disease (LY, U, EP, HYP) were recorded in each fish by use of methods specified by the ICES (Bucke et al. 1996). Following assessment of grossly visible diseases, fish were euthanised and upon opening of the body cavity, the liver was assessed for the presence of visible LN according to the guidelines set out by Feist et al. (2004). Samples of liver from all fish (includ-

Table 1. UK Clean Seas Environmental Monitoring Programme (CSEMP). (*) Geographic coordinates of the sites surveyed during the scoping survey in 2004 and the targeted survey in 2007

UK CSEMP site	Mid-tow co-ordinates
Amble	55° 16.01′ N, 01° 15.26′ W
NE Dogger Bank	55° 18.05′ N, 02° 53.82′ E
N Dogger Bank*	55° 04.08′ N, 02° 05.40′ E
W Dogger Bank	54° 46.76′ N, 01° 17.69′ E
Central Dogger Bank	54° 30.00′ N, 02° 42.53′ E
Tees Bay	54° 45.25′ N, 01° 08.31′ W
Flamborough	54° 14.72′ N, 00° 29.91′ E
Off Humber	54° 03.92′ N, 01° 47.46′ E
Indefatigable Bank	53° 33.40′ N, 02° 04.92′ E
Rye Bay*	50° 46.74′ N, 00° 46.83′ E
Camarthen Bay	51° 32.82′ N, 04° 35.13′ W
N Cardigan Bay	52° 42.44′ N, 04° 32.29′ W
Inner Cardigan Bay	52° 18.00′ N, 04° 16.35′ W
Burbo Bight	53° 28.24′ N, 03° 20.47′ W
Liverpool Bay*	53° 28.32′ N, 03° 41.91′ W
St. Bees	54° 30.71′ N, 03° 47.63′ W
Red Wharf Bay	53° 22.46′ N, 04° 12.84′ W
Morecambe Bay	53° 55.31′ N, 03° 23.23′ W
SE Isle of Man	54° 03.36′ N, 03° 52.47′ W

Fig. 1. UK Clean Seas Environmental Monitoring Programme (CSEMP) sites surveyed during the scoping survey in 2004 and the targeted survey in 2007 (★). For geographic coordinates of the sites, see Table 1

ing those containing LN) were removed and fixed for 24 h in 10% neutral buffered formalin (NBF) before transfer to 70% industrial methylated spirit (IMS) for subsequent histological assessment. In order to prevent the appearance of post-mortem artefacts, only live fish were sampled. Fixed liver samples were processed to paraffin wax in a vacuum infiltration processor by using standard protocols (Feist et al. 2004). Blocks were cut at 3 to 5 µm on a rotary microtome and resulting tissue sections mounted onto glass slides before staining with haematoxylin and eosin (H&E). Stained sections were analysed by light microscopy (Eclipse E800, Nikon) and diagnosis of liver lesion type followed the guidelines set out by Feist et al. (2004) for the flatfish liver. Digital images of histological features were obtained by use of the Lucia™ Screen Measurement System (Nikon). Representative images of grossly visible pre-neoplastic, benign neoplastic and malignant neoplastic liver lesions are given in Fig. 2.

For ageing, otoliths were removed from individual fish and stored in vials for subsequent processing in the laboratory. Preparation, mounting, cutting, staining and reading of otoliths followed the methods of Easey & Millner (2008) and age data (in years) was generated for each fish sampled for disease assessment.

Targeted survey: 2007. In order to provide enhanced resolution on the effect of age on the prevalence and profile of diseases monitored under the UK CSEMP, up to 250 dab were sampled from each of 3 UK CSEMP sites (identified in Table 1, Fig.1), during June and July 2007, by use of 30 min tows of a standard Granton trawl. The sites were chosen to represent geographically separated sites to the west, south and east of the UK mainland respectively: Liverpool Bay (Irish Sea), Rye Bay (English Channel) and North Dogger Bank (North Sea). Fish were treated in the same way as above but were separated into the following size classes: 10–14, 16–19, 20–24 and ≥25 cm. The size classes were chosen to represent the range of size classes retained by the trawl and, accordingly, the likely spread of age classes present at the target site. Grossly visible diseases (LY, U, EP, HYP, LN) and the presence of histological liver pathologies were assessed as in the 2004 scoping survey. Similarly, for ageing, otoliths were removed from individual fish and stored in vials for subsequent processing in the laboratory.

Data analysis. The 2004 scoping survey was designed to identify broad effects of age on the prevalence and profile of diseases across sites sampled in the

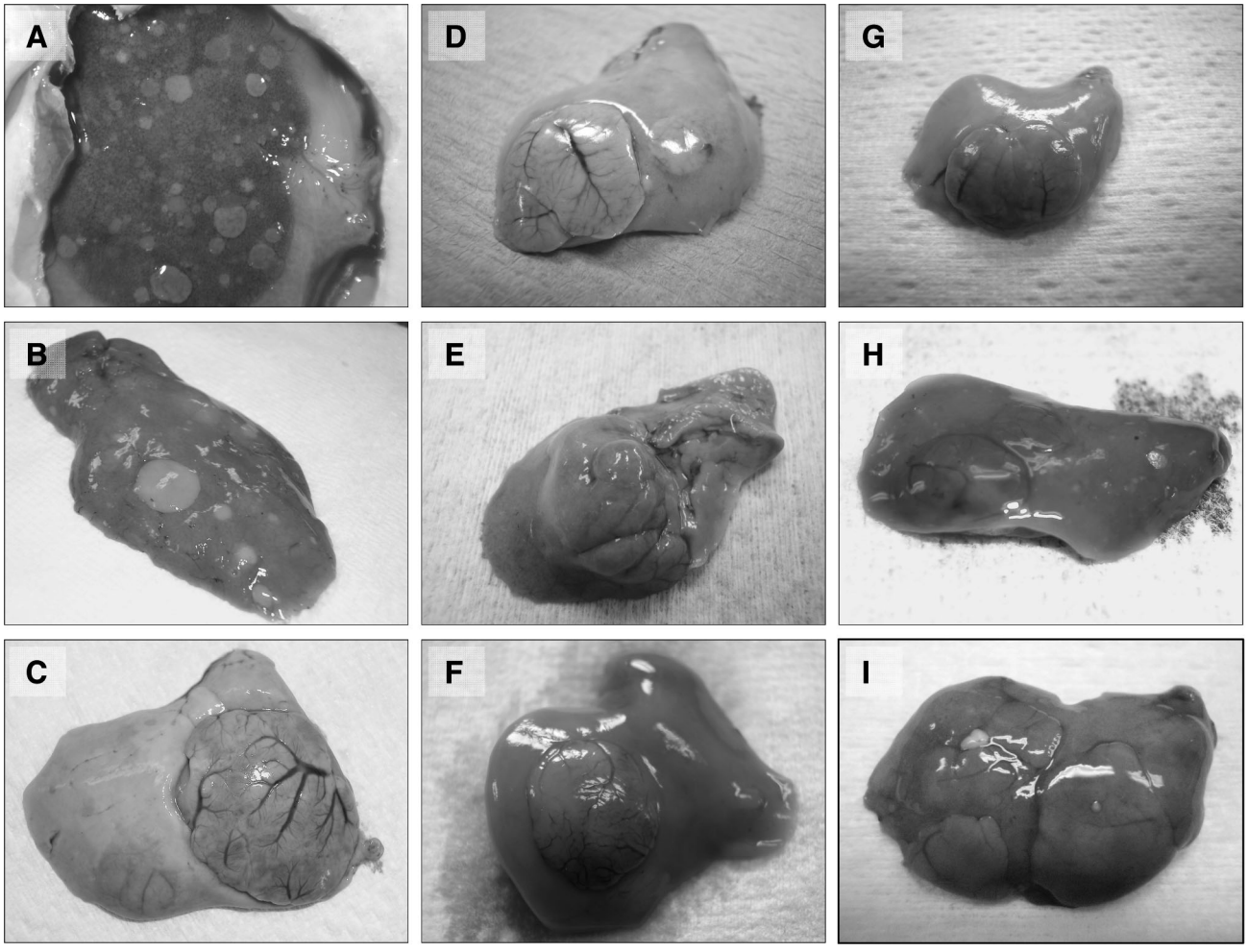

Fig. 2. *Limanda limanda*. Representative images of the grossly visible liver nodules and other lesions corresponding to (A,B) preneoplasia (foci of cellular alteration, FCA); (C–E) benign neoplasm (hepatocellular adenoma, HCA); and (F–I) malignant neoplasm (hepatocellular carcinoma, HCC) in the liver of dab collected from UK CSEMP sites. Field diagnosis of 'liver nodules' was later confirmed for lesion type according to the diagnostic principles detailed by Feist et al. (2004)

UK CSEMP, particularly to provide an understanding of potential age range in the currently adopted sampling strategy as defined by Bucke et al. (1996) and Feist et al. (2004). As such, data from the 2004 survey was analysed to provide basic age profiles for dab sampled from UK offshore sites under the UK CSEMP. These included: variation in age by fish length, variation in age by sampling site, the age at first observed onset of particular diseases (albeit in this limited size range data set) and the effect of sex (and age) on disease prevalence. The more detailed survey of 3 sites carried out during 2007 was designed to assess the effect of age on disease within a population sampled from a particular site. The data was analysed to assess age at length at these sites and, further, the age at first observed onset of each disease variable. Previous work by Stentiford et al. (2009) has developed a 'harm score' index for dab populations sampled, using quality assurance procedures for disease diagnosis as detailed by Bucke et al. (1996) and Feist et al. (2004). The harm score is based on a cumulative assessment of the prevalence of specific diseases measured in dab populations from marine sites. It assigns a score (0 to 3) to the 10 disease variables (5 grossly visible diseases and 5 liver pathology categories); the cumulative score (maximum 30) leads to an overall 'harm score' that depicts the relative departure of those populations from an empirical baseline. The harm score is further divided into 3 'site types' (A, B, C) that have been proposed as an assessment tool for classifying UK marine sites based upon fish disease profiles at those sites. In the present study, we applied the harm score principle to the target site data collected during 2007. The application of the harm score to the different age classes sampled at these sites allows for an assessment of the effect of age on disease profile (harm) and, further-

more, a numerical comparison of the disease profile between fish of the same age (and sex) sampled from geographically distinct sites. The ability to measure harm (from disease) by age and sex provides an essential tool for deciphering the effects of these life-history parameters from potential anthropogenic causes.

RESULTS

Scoping survey: 2004

All dab (n = 843) sampled during the 2004 scoping survey were within the size range 19 to 24 cm total length. Otolith data relating to these fish revealed an apparent range of between 1 and 12 yr of age (mean 4.98 yr). Dominant year groups were observed at 5 and 6 yr of age. Sites with the youngest and oldest sampled populations were Rye Bay (mean 2.94 yr, range 1 to 6 yr) and West Dogger Bank (mean 6.44 yr, range 4 to 11 yr), respectively. Comparison between mean age and mean length for dab from the 19 UK CSEMP sites sampled during 2004 demonstrates that populations at some sites are relatively large for their age (e.g. Rye Bay, Carmarthan Bay, Red Wharf Bay) while other populations are relatively small for their age (e.g. Inner Cardigan Bay, North Dogger Bank, St. Bees) (Fig. 3). Since current survey techniques (according to Bucke et al. 1996, Feist et al. 2004) do not stipulate the use of specific sexes or ratios for fish disease assessments, the data shown in Fig. 3 is not intended to represent an accurate age-length assessment for fish captured at a given site (which is expected to vary by sex) but rather, the actual age-length relationship of all fish sampled from a given site within 2004. This analysis shows that selection of fish based only on total length (19 to 24 cm) leads to a wide variation in mean age of the sample. Taken together, these data reveal that unless accurate age-length keys (separated by sex) are available for given sites, the selection of fish for surveys based only upon total length is not an accurate proxy for age, particularly when fish of both sexes selected randomly from multiple geographic sites are to be compared.

In terms of those diseases regularly monitored in dab sampled during the UK CSEMP, it is pertinent to consider the age (and sites) at which these diseases are first

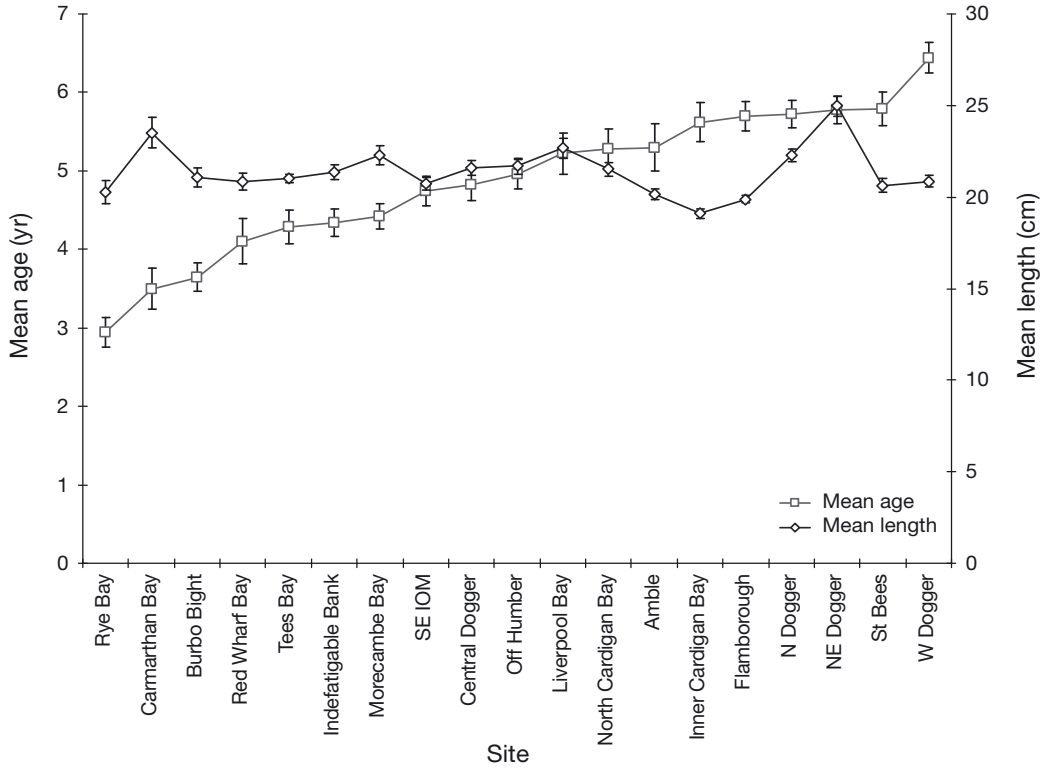

Fig. 3. *Limanda limanda*. Mean age and length (±SE) for dab captured from all sites sampled during the 2004 scoping survey. The 2004 survey was based upon targeting of dab between 19 and 24 cm total length according to sampling protocols provided by Feist et al. (2004). Fish populations captured from sites shown on the left of the chart are generally larger by age than those populations captured from sites shown on the right. Note: Current survey techniques (according to Bucke et al. 1996, Feist et al. 2004) do not stipulate the use of specific sexes or ratios for fish disease assessments. As such, the data shown are not intended to represent an accurate age–length assessment for fish captured at a given site (which is expected to vary by sex) but rather the actual age–length relationship of all fish sampled from a given site within 2004. This analysis shows that selection of fish based only on total length (19 to 24 cm) leads to a wide variation in mean age of the sample. SE IOM: SE Isle of Man

observed. A summary of age at first observation of specific grossly visible diseases and liver pathologies is given in Table 2. In terms of grossly visible diseases, LY and U appear in the youngest fish (2 yr) while HYP and LN appeared at age 3 yr. LN were first observed in populations from Tees Bay (North Sea) and Liverpool Bay (Irish Sea), while HYP was first observed in fish from Rye Bay (English Channel), Tees Bay, Off Humber and Central Dogger Bank (North Sea) and Inner Cardigan Bay (Irish Sea). Non-specific inflammatory lesions were the first pathologies to appear in the liver of dab sampled from UK CSEMP sites; inflammatory (IF) and regenerative (RG) lesions appeared in fish of age 1 yr from Carmarthan Bay (Irish Sea) and Rye Bay (English Channel). Non-neoplastic toxicopathic liver pathologies were first observed in fish of age 2 yr sampled from several sites in the Irish Sea (Burbo Bight, Liverpool Bay, North Cardigan Bay and SE Isle of Man). For pre-neoplastic lesions, initial onset was observed at age 2 yr in dab from several Irish Sea sites (Carmarthan Bay, Red Wharf Bay and SE Isle of Man), with sites in the Irish Sea also first to register benign neoplastic lesions such as HCA (age 3 yr) and malignant neoplastic lesions such as HCC (age 4 yr).

Table 3 provides a summary on the effect of sex on individual disease prevalence for all fish sampled during the 2004 scoping survey. Overall, despite some significant differences between the mean age of fish sampled from specific sites, the mean age of all male (5.3 yr) and all female (4.8 yr) fish sampled during the programme was similar. In addition Table 3 demonstrates a very similar prevalence of specific diseases in male and female dab. Grossly visible diseases showing the highest prevalence in male and female fish respectively were: HYP (16.2 and 13.6%) and U (11.2 and 10.7%). Liver pathologies with the highest prevalence were: MMA (66.1 and 64.8%), IF (37.4 and 35.1%) and RG (21.2 and 19.8%). It is interesting to note that lesions associated with pre-neoplasia: basophilic FCA (bFCA; 15.6 and 18%) and eosinophilic FCA (eFCA; 2.8 and 2.7%), and with benign (HCA: 4.7 and 7.7%) and malignant (HCC: 1.6 and 1.1%) neoplasia, show a high degree of similarity between male and female subsets of the UK dab population.

Table 4 further dissects the effect of age and sex on the prevalence of specific diseases. In this case, it is apparent that certain grossly visible diseases become more prevalent with age in both sexes (HYP, LN) while

Table 2. *Limanda limanda*. Age at first-observed onset of grossly visible diseases and liver pathologies based upon data collected from 19 UK CSEMP sites surveyed in 2004. Grossly visible diseases: LY (lymphocystis), U (ulceration), EP (epidermal papilloma), HYP (hyperpigmentation), LN (liver nodule). Liver pathologies: PL (phospholipidosis), HNP (hepatocellular and nuclear pleomorphism), LI (lipidosis), IF (inflammation), MMA (melanomacrophage aggregates), RG (regeneration), ccFCA (clear-cell focus of cellular alteration), vFCA (vacuolated focus of cellular alteration), bFCA (basophilic focus of cellular alteration), eFCA (eosinophilic focus of cellular alteration), HCA (hepatocellular adenoma), HCC (hepatocellular carcinoma), CH (cholangioma). Sites: BB (Burbo Bight), CB (Carmarthan Bay), NC (N Cardigan Bay), SE IOM (SE Isle of Man), IC (Inner Cardigan Bay), RW (Red Wharf Bay), LB (Liverpool Bay), RB (Rye Bay), OH (Off Humber), TB (Tees Bay), IB (Indefatigable Bank), CD (Central Dogger Bank), WD (W Dogger Bank). Sites in **bold** are located in the Irish Sea. All other sites are located in the English Channel or North Sea

Disease	Age (yr) at first observation	Sites
LY	2	TB
U	2	**BB**, **CB**, **NC**
EP	4	**BB**, CD, IB, **IC**, **SE IOM**
HYP	3	CD, **IC**, OH, RB, TB
LN	3	**LB**, TB
PL	8	**LB**
HNP	2	**BB**, **LB**, **NC**, **SE IOM**
LI	1	RB, **CB**
IF	1	RB, **CB**
MMA	1	RB, **CB**
RG	1	RB
ccFCA	2	**CB**
vFCA	2	**CB**
bFCA	2	**RW**
eFCA	3	**SE IOM**
HCA	3	**BB**, **LB**
HCC	4	**NC**
CH	6	WD

Table 3. *Limanda limanda*. Effect of sex on prevalence of grossly visible diseases and liver pathologies in dab sampled during the 2004 scoping survey. M: males (mean age: 5.3 yr), F: females (mean age: 4.8 yr). See Table 2 legend for disease abbreviations

Disease	Prevalence (%)	
	M	F
LY	1.8	0.96
U	11.2	10.7
EP	1.6	1.7
HYP	16.2	13.6
LN	3.4	4.2
PL	0.3	0
HNP	4.7	3.1
LI	5.6	13.2
IF	37.4	35.1
MMA	66.1	64.8
RG	21.2	19.8
ccFCA	1.6	0.4
vFCA	1.9	2.9
bFCA	15.6	18
eFCA	2.8	2.7
HCA	4.7	7.7
HCC	1.6	1.1
CH	0	0.2

others do not show a clear pattern with either age or sex (LY, EP, U). Similarly, while the prevalence of certain liver pathologies (such as pre-neoplastic and neoplastic lesions) are clearly related to age in both sexes of dab (e.g. bFCA, HCA, HCC), others (such as IF, RG and lipidosis [LI]) show no clear pattern with either age or sex. Interestingly, some liver pathologies, such as HNP, appear to be more prevalent in younger fish of both sexes.

Targeted survey: 2007

Three sites, Rye Bay (English Channel), Liverpool Bay (Irish Sea) and North Dogger Bank (North Sea), were selected for further targeted sampling during the 2007 UK CSEMP. In addition to their geographical separation, the 2004 scoping survey revealed a different relationship between age and length in fish captured from these sites. Furthermore, data presented recently by Stentiford et al. (2009) for disease profiles of dab from UK CSEMP sites revealed an apparent difference between these sites, with dab from Dogger Bank sites displaying disease profiles that led to higher 'harm scores' than those observed in dab populations from Liverpool Bay and from Rye Bay, respectively. Detailed sampling of the population captured at each of these sites during 2007 has allowed for an assessment of the effect of age on disease onset, prevalence and overall 'harm' within these sites and importantly, for a comparison between these factors in age-matched fish from the 3 sites. The prevalence of key disease variables measured in dab of different age classes sampled from the 3 sites during the targeted survey in 2007 is shown in Table 5.

Table 4. *Limanda limanda*. Effect of sex on prevalence of grossly visible diseases and liver pathologies in example year classes (age 2, 4 and 6 yr) using data from all sites sampled during the 2004 scoping survey. M: males, F: females. See Table 2 legend for disease abbreviations

Disease	Prevalence (%)					
	Age 2 yr		Age 4 yr		Age 6 yr	
	M	F	M	F	M	F
LY	0	3.7	2.6	0.96	1.23	0.9
U	23.1	0	7.9	12.5	7.4	13.5
EP	0	0	7.9	2.9	1.2	1.8
HYP	0	0	0	4.8	29.6	26.1
LN	0	0	0	3.9	3.7	3.6
PL	0	0	0	0	0	0
HNP	15.4	7.4	8.1	1.9	2.5	4.5
LI	15.4	18.5	2.7	10.6	2.5	11.7
IF	53.9	22.2	51.4	41.4	39.5	38.7
MMA	46.2	29.6	51.4	52.9	80.3	87.4
RG	38.5	3.7	13.5	13.5	22.2	27.9
ccFCA	0	3.7	0	0	3.7	0
vFCA	7.7	0	0	0.96	1.23	7.2
bFCA	0	3.7	16.2	16.3	23.5	29.7
eFCA	0	0	0	2.9	3.7	3.6
HCA	0	0	2.7	6.7	6.2	10.8
HCC	0	0	0	0.96	3.7	2.7
CH	0	0	0	0	0	0.9

Fig. 4 shows the age-length profiles for male and female subsets of the population of dab sampled from the 3 sites. No dab (of either sex) of age >4 yr were captured from the Rye Bay site, while for the remaining 2 sites, due to relatively low numbers, all fish of age >7 yr were grouped for analysis. Female fish from all sites attain a higher mean length than that observed in males by the age of 3 yr. Furthermore, after age 3 yr, female fish remain larger than males in any given age class. The growth rate in terms of length (depicted by the flattened portions of the male and female curves for all sites) appears to slow considerably after age 3 yr. While fish of age >4 yr were not observed from Rye Bay, the mean length of male and female dab from this site appears to be larger at age 3 yr than that observed in the same age fish of both sexes from the other 2 sites, suggesting a more rapid initial growth rate of dab at Rye Bay compared to those captured at these sites.

Data collected during the 2004 scoping study demonstrated an ability to investigate earliest onset of specific diseases in dab within given age classes, and further, provided an insight into which sites these diseases were first observed to occur. A more detailed assessment of this issue can be achieved by analysis of data from the targeted survey of 2007 by investigating earliest onset in age-matched subsets of the population sampled from distinct geographic sites. In this context, the prevalence of key disease variables measured in dab of different age classes sampled from the 3 sites during the targeted survey in 2007 is shown in Table 5. In terms of grossly visible diseases, HYP and LN displayed an increase in prevalence with age, particularly in dab sampled from North Dogger Bank. Over half of all fish sampled above age 5 yr displayed HYP, while prevalence of LN reached ~18% in age 5 and 6 yr fish, and 50% in age 7 yr fish from the same site. In comparison, the maximum prevalence of HYP in fish from Liverpool Bay was observed at age 5 yr, while LN prevalence peaked at 10% in age 7 yr fish. For liver pathologies associated with carcinogenesis, FCA types showed a general increase in prevalence with age, reaching 70% (for bFCA) in fish of age 7 yr at North Dogger Bank and 40% (for bFCA) in fish of the same age from Liverpool Bay. Since fish of age >4 yr were not obtained from the Rye Bay site, the maximum prevalence of bFCA (~18%) was comparable to the prevalence of this condition observed in fish from Liverpool Bay. However, by age 4 yr, the prevalence of

Table 5. *Limanda limanda*. Summary of prevalence data for age classes of dab captured from target fishing stations sampled in 2007. L: mean total length, W: mean weight, M: percentage of male fish in age class, F: percentage of female fish in age class. See Table 2 legend for disease abbreviations

Site	Age (yr)	n	L (cm)	W (g)	M (%)	F (%)	Prevalence (%) of disease																
							LY	U	EP	HYP	LN	PL	HNP	ccFCA	vFCA	eFCA	bFCA	HCA	CH HCC	LI	MMC	IF	RG
N Dogger Bank	1	73	14.15	27.75	54.79	45.21	0	0	0	2.7	0	0	1.37	0	0	1.37	4.11	0	0	41.1	15.07	54.79	19.18
	2	29	18.22	57.22	41.38	58.62	0	6.9	0	20.7	0	0	10.34	0	6.9	0	17.24	0	0	17.24	75.86	48.28	20.69
	3	14	23.12	125.71	14.29	85.71	0	0	0	28.6	0	0	7.14	0	0	0	42.86	7.14	0	42.86	85.71	71.43	7.14
	4	27	23.79	131.60	25.93	74.07	0	14.9	0	48.1	3.7	0	3.85	0	3.85	3.85	46.15	7.69	0	15.38	100	53.85	7.69
	5	34	25.78	176.89	14.71	85.29	0	26.5	0	58.9	17.6	5.9	2.94	2.94	8.82	5.88	61.76	17.65	0 8.82	29.41	100	55.88	41.18
	6	11	24.33	153.18	36.36	63.64	0	0	0	72.7	18.2	0	0	9.09	9.09	9.09	81.82	54.55	0 0	9.09	100	54.55	9.09
	7+	10	26.09	195.31	40	60	0	30	0	60	50	0	0	10	30	20	70	80	0 0	40	100	70	30
Liverpool Bay	1	37	13.25	21.55	48.6	51.4	0	0	0	0	0	0	0	0	0	0	0	0	0 0	0	0	28.57	7.14
	2	37	17.76	56.94	41.7	58.3	0	5.4	0	0	0	0	2.7	0	0	0	5.41	0	0 0	37.84	27.03	43.24	24.32
	3	25	20.50	86.32	40	60	0	4	0	0	0	0	4	0	4	4	8	4	0 0	56	52	36	4
	4	30	23.95	139.38	20	80	0	13.3	3.3	6.7	3.3	0	3.33	0	3.33	0	10	0	0 0	26.67	90	53.33	16.67
	5	23	23.57	124.78	17.4	82.6	0	13	0	13	4.3	0	4.35	0	8.7	4.35	26.09	4.35	0 0	34.78	95.65	56.52	17.39
	6	26	25.35	163.11	19.2	80.8	0	19.2	7.7	7.7	0	0	3.85	0	7.69	19.23	23.08	7.69	0 0	26.92	96.15	42.31	15.38
	7+	10	26.62	188.03	0	100	0	10	0	10	10	0	10	0	0	20	40	40	0 0	30	100	40	0
Rye Bay	1	55	13.53	27.17	33.3	66.7	0	0	0	0	0	1.82	0	0	0	0	1.82	0	0 0	25.45	1.82	43.64	9.09
	2	146	20.10	92.92	36	64	0	0	0	0	0	0	1.37	0	1.37	0	6.85	0	0 0	30.14	6.85	43.84	6.85
	3	32	23.55	148.49	33.3	66.7	0	6.1	0	6.1	0	0	0	3.03	3.03	3.03	15.15	0	0 0	24.24	18.18	60.61	21.21
	4	11	22.39	125.28	58.33	41.67	0	0	9.1	18.2	0	0	0	0	0	0	18.18	0	0 0	27.27	54.55	45.45	9.09

this condition in fish sampled from North Dogger Bank was >46%. Similar age-related increases in prevalence were observed for the benign neoplasm HCA. In fish from North Dogger Bank, prevalence of this condition rose steadily from age 3 yr onwards, reaching >17% by age 5 yr and 80% by age 7 yr. Similar increases in prevalence were observed in fish from Liverpool Bay, though prevalence at age 7 yr was less (40%) than that observed in fish from North Dogger Bank. HCA was not observed in fish collected from Rye Bay. Malignant neoplasms (HCC) were only observed in fish of age 5 yr captured from North Dogger Bank (8.82%). No older fish, at any site, were observed with this condition.

The age at first-observed onset for specific grossly visible diseases and liver pathologies recorded during the 2007 survey is shown in Table 6. In terms of grossly visible diseases, U showed earlier onset at North Dogger Bank and Liverpool Bay sites (age 2 yr) compared to Rye Bay (age 3 yr), while HYP occurred earlier at North Dogger Bank (age 1 yr) compared to Rye Bay (age 3 yr) and Liverpool Bay (age 4 yr). Liver nodules were not observed in fish from Rye Bay, while they were first observed in fish of age 4 yr from both North Dogger Bank and Liverpool Bay. In terms of liver pathology, non-specific inflammatory lesions (such as MMA, IF, RG) generally occurred at age 1 yr at all sites, while non-neoplastic toxicopathic lesions such as HNP occurred at age 1 yr in populations from both Rye Bay and North Dogger Bank (Liverpool Bay at age 2 yr). While pre-neoplastic lesions (FCA) were observed at all sites, the earliest onset of both eFCA and bFCA occurred at North Dogger Bank at age 1 yr. Similar lesions were not observed in fish from Liverpool Bay until age 2 yr and in Rye Bay until age 3 yr (for eFCA). Benign (HCA) neoplasms were only observed in dab from North Dogger Bank and Liverpool Bay (both at age 3 yr) while malignant lesions (HCC) were only observed at North Dogger Bank (age 5 yr).

The 'harm score' assessment tool of Stentiford et al. (2009) allows generation of a cumulative numerical value based upon the site prevalence of 10 key disease variables measured in dab populations. Application of the tool to age and sex subsets from the 3 targeted sites from 2007 provides an indication of cumulative harm in fish captured from these sites and further, allows for direct comparison of harm in fish of the same age (and sex) from different sites. A summary of individual and cumulative harm scores for each age class and sex of dab captured at Rye Bay, Liverpool Bay and North Dogger Bank is given in the form of a 'heatmap' in Fig. 5. Pale yellow boxes depict baseline prevalence of a particular disease variable (lower empirical quartile), while light orange, dark orange and red boxes depict mid-low, mid-high and high empirical quartile preva-

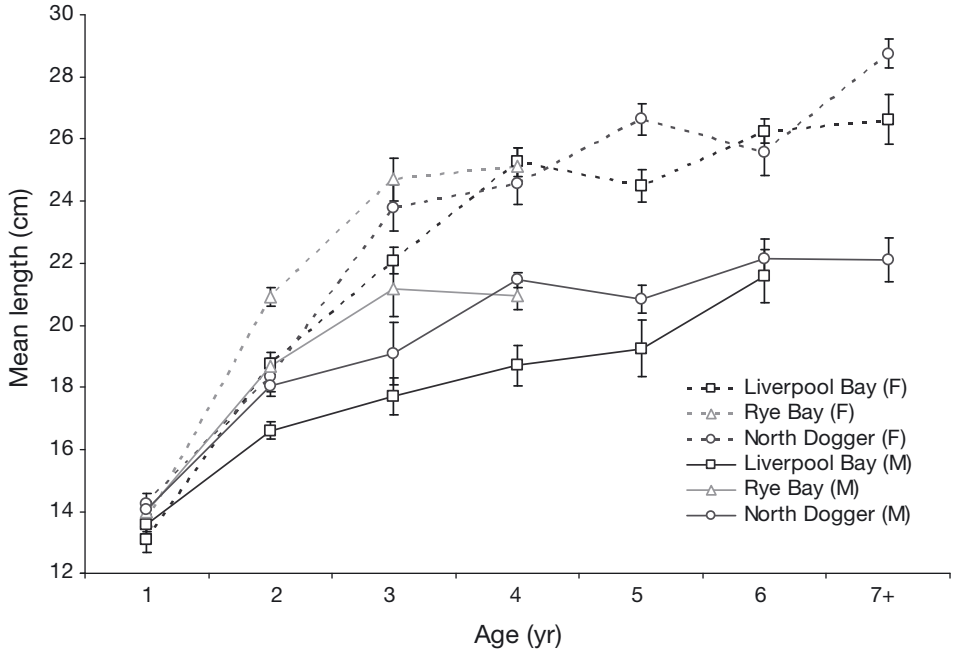

Fig. 4. *Limanda limanda*. Mean length (±SE) at age by sex and site for dab captured during targeted survey sites during 2007. No fish of age >4 yr were captured from the Rye Bay site. Due to low sample numbers, fish of age ≥7 yr were grouped for analyses. M: males, F: females

Table 6. *Limanda limanda*. Age at first observed onset of grossly visible diseases and liver pathologies at targeted sites sampled during the 2007 survey. nd: not detected. See Table 2 legend for disease abbreviations

Disease	Age (yr) at first observed onset		
	Rye Bay	Liverpool Bay	N Dogger Bank
LY	nd	nd	nd
U	3	2	2
EP	4	4	nd
HYP	3	4	1
LN	nd	4	4
PL	nd	nd	5
HNP	1	2	1
LI	1	2	1
IF	1	1	1
MMA	1	2	1
RG	1	1	1
ccFCA	3	nd	5
vFCA	2	3	2
bFCA	2	2	1
eFCA	3	3	1
HCA	nd	3	3
HCC	nd	nd	5
CH	nd	nd	nd

lence, respectively. The increasing proportion of red boxes in older age classes of both sexes from Liverpool Bay and particularly North Dogger Bank suggest an overall increase in cumulative harm with age at these sites. Since fish aged >4 yr were not found in catches from Rye Bay, it is not possible to directly compare age classes of >5 yr from Rye Bay to the same age classes from Liverpool Bay and North Dogger Bank.

Fig. 6 provides a summary of data presented in Fig. 5 as mean harm scores (from all fish within particular age classes) measured in populations from the 3 target sites. From this, it is seen that, while harm scores are similar at age 1 yr and increase with age at all sites, the harm score measured in fish from North Dogger Bank rises more rapidly with age than in populations from either Liverpool Bay or Rye Bay. As such, the harm score reached by age 2 yr in fish (particularly males) from North Dogger Bank is not reached until approximately age 4 yr in populations from Liverpool Bay and Rye Bay. Similarly, this ~2 yr phase-shift in the harm score curve is maintained in older age classes of fish from North Dogger Bank and Liverpool Bay, with populations from the former maintaining consistently higher harm scores by age than those from the latter. Direct comparison of mean harm scores measured at age 4 yr at each site (dashed vertical line in Fig. 5) shows that, by this age, assessment of populations of dab from Rye Bay and Liverpool Bay would classify these sites as Type A, while those from North Dogger Bank would classify this site as Type B (according to the assessment criteria of Stentiford et al. 2009). It is interesting to note that for Liverpool Bay, Type B classification is not reached until ~2 yr after North Dogger Bank (Figs. 5 & 6). The increased mean harm score achieved in the population of dab from North Dogger Bank is primarily due to elevated prevalence of HYP and pathologies associated with the formation of liver cancer (FCA, benign neoplasm, malignant neoplasm) at this site. Interestingly, however, while fish with benign liver neoplasms were found in older age classes sampled from North Dogger Bank, no cases of malignant neoplasms were observed in fish of age >5 yr at this site.

DISCUSSION

Detailed assessment of fish disease data collected from the flatfish *Limanda limanda* sampled as part of the UK CSEMP has revealed a tendency for increasing prevalence of diseases such as liver neoplasms with age but also, differences in the age of onset of specific diseases at geographically distinct sites and variation in the degree of change in disease prevalence with progressive age in fish sampled from these sites. The present study confirmed that length is not a suitable proxy for age when comparing mixed-sex fish of the same species sampled from different offshore sites, and furthermore, that age data, either from direct measurement or from accurate age-length keys for given sexes and sites, should be included in marine monitoring studies that attempt to utilise fish diseases as a biomarker of anthropogenic impact. The present study has also revealed that overall 'harm' (following the concept of Stentiford et al. 2009) is cumulative with age — older fish being more diseased than younger fish sampled from the same sites. The different approaches for the monitoring of fish diseases at specific sites between the 2004 (scoping survey) and 2007 (targeted survey) demonstrated that age is a more important factor than sex in disease onset and prevalence in dab. In addition, the future discrimination of sex at the time of sampling will allow for a more accurate assessment of likely age of individual fish using sex- and site-specific age-length keys, thereby providing increased capability to accurately compare disease type and profile between sites and years. This is particularly important where individual ageing of fish by otolith assessment is cost-prohibitive.

Age-cohort analysis of dab collected from the North Dogger Bank site in the North Sea revealed an almost linear increase in the apparent prevalence of liver neoplasms after age 3 yr (7.14 to 80 % of fish of age ≥7 yr). A similar pattern of increasing prevalence (albeit at lower magnitude) was also observed in dab collected from the Liverpool Bay site on the west coast of the UK (up to 40 % liver neoplasms in fish of age ≥7 yr). The trend for increasing prevalence of liver neoplasms with age (predominated by HCA) was also supported by similar increases in the prevalence of the pre-neoplastic condition FCA (predominated by bFCA) in fish from North Dogger Bank, Liverpool Bay and Rye Bay. Since FCA was observed in fish of approximately 2 yr younger than the age of onset of neoplasms at the same sites, it is presumed that FCA can be utilised as an indicator of initiation of the carcinogenic pathway in dab sampled from these sites and, further, provides an indicator for the presence of more advanced lesions in the histogenesis of liver neoplasia in older age classes of fish collected from the same sites. It also provides a predictor of likely presence of neoplasms in the same cohort of fish sampled at future time points. Similar patterns have previously been described for flatfish collected from contaminated estuaries (Myers et al. 1991, Baumann & Harshbarger 1995) and in mammalian studies of carcinogenesis (Bannasch et al. 1989). Although the absence of neoplastic changes in fish collected from the Rye Bay site appears partly due to an absence of fish of age > 4 yr at this site, it is also noteworthy that by age 4 yr, neoplasms had been observed in up to 4 and 7 % of the dab from Liverpool Bay and North Dogger Bank, respectively. The apparent omnipresence of fish of up to age 4 yr at the 3 sites targeted for detailed study in 2007 and the differences in the prevalence of FCA and neoplasms in fish of up to this age appears to indicate a differential carcinogenic process (or rate) at these sites. As a result, it is feasible to compare the prevalence and profile of grossly visible diseases and liver pathologies in age-matched cohorts collected from distinct sites. Comparison of the age 4 yr cohort, for instance, by which time pre-neoplastic and neoplastic liver lesions have the possibility of being detected (in at least a proportion of the population), offers a feasible basis to compare sites for these disease types. In this way, the effect of age as a significant confounding factor on the overall prevalence of neoplasms (and other diseases) is removed. Furthermore, it allows for inclusion of certain sites in monitoring programmes that for some reason (e.g. migration) display an absence of older age classes of fish within the sampled population. It is recommended that age-cohort matching be applied to monitoring programmes that utilise fish disease data from multiple sites for the assessment of the health status of marine ecosystems but also that collection of data from other age classes will also provide valuable data for age at onset and for the detection of differential disease profiles in specific age classes. Alternative approaches to normalisation of disease data, utlising a model based upon fish length relative to age have been developed under the auspices of the ICES Working Group for Parasites and Diseases of Marine Organisms (WGPDMO) in their Fish Disease Index (FDI). The FDI is an assessment tool that will be utilised for defining disease trends in fish captured from open-ocean monitoring sites (ICES 2007). In this context, the FDI also recognises the importance of age as a confounding factor in multi-site or multi-year comparisons.

In light of the high prevalence of liver neoplasia in various age classes of dab collected during UK CSEMP surveys, it is pertinent to compare susceptibility to cancer in this species with other published evidence from wildlife. Martineau et al. (2002) observed cancer (of intestinal tract and mammary glands) in 27 % of all beluga found dead in the St. Lawrence estuary, Canada, and, from this, calculated an estimated annual rate of

Site	Disease	Age 1		Age 2		Age 3		Age 4		Age 5		Age 6		Age 7	
		F	M	F	M	F	M	F	M	F	M	F	M	F	M
Rye Bay	LY	0	0	0	0	0	0	0	0	na	na	na	na	na	na
	U	0	0	0	0	0	1	0	0	na	na	na	na	na	na
	EP	0	0	0	0	0	0	0	3	na	na	na	na	na	na
	HYP	0	0	0	0	0	0	2	1	na	na	na	na	na	na
	LN	0	0	0	0	0	0	0	0	na	na	na	na	na	na
	NNT	0	0	0	0	0	0	0	0	na	na	na	na	na	na
	FCA	0	0	0	0	1	1	1	0	na	na	na	na	na	na
	BN	0	0	0	0	0	0	0	0	na	na	na	na	na	na
	MN	0	0	0	0	0	0	0	0	na	na	na	na	na	na
	NSI	1	3	1	1	1	3	1	2	na	na	na	na	na	na
Harm score		1	3	1	1	2	5	4	6	na	na	na	na	na	na
Liverpool Bay	LY	0	0	0	0	0	0	0	0	0	0	0	0	0	na
	U	0	0	0	1	0	0	1	1	1	0	1	2	1	na
	EP	0	0	0	0	0	0	1	0	0	0	2	0	0	na
	HYP	0	0	0	0	0	0	0	0	1	0	0	1	0	na
	LN	0	0	0	0	0	0	0	3	1	0	0	0	2	na
	NNT	0	0	0	1	0	1	0	0	0	0	0	0	1	na
	FCA	0	0	0	0	1	0	0	1	2	3	2	2	3	na
	BN	0	0	0	0	1	0	0	2	0	0	0	3	3	na
	MN	0	0	0	0	0	0	0	0	0	0	0	0	0	na
	NSI	0	0	1	3	1	1	3	3	3	3	3	3	3	na
Harm score		0	0	1	5	3	2	5	10	8	6	8	11	13	na
North Dogger	LY	0	0	0	0	0	na	0	0	0	0	0	0	0	0
	U	0	0	0	0	0	na	1	1	3	0	0	0	3	0
	EP	0	0	0	0	0	na	0	0	0	0	0	0	0	0
	HYP	0	0	0	2	1	na	3	3	3	3	3	3	3	3
	LN	0	0	0	0	0	na	0	3	3	3	3	0	3	3
	NNT	0	0	0	2	1	na	0	0	1	3	0	0	0	0
	FCA	0	0	0	2	2	na	3	3	3	3	3	3	3	3
	BN	0	0	0	0	1	na	0	2	2	3	3	3	3	3
	MN	0	0	0	0	0	na	0	0	3	3	0	0	0	0
	NSI	2	2	3	2	3	na	3	3	3	3	3	3	3	3
Harm score		2	2	3	8	8	na	10	15	21	21	15	12	18	15

Fig. 5. *Limanda limanda*. Heatmap generated by application of 'harm score' tool of Stentiford et al. (2009) to male and female dab of specific age classes captured during the targeted survey in 2007. The overall harm score is based on cumulative scoring of prevalence of 10 disease variables: pale yellow boxes (score 0) depict baseline prevalence of a particular disease variable (lower empirical quartile), while light orange (score 1), dark orange (score 2) and red (score 3) boxes depict mid-low, mid-high and high empirical quartile prevalence respectively. According to the protocol of Stentiford et al. (2009), cumulative scores <10 classify sites as Type A, between 10 and 20 as Type B, and scores 20 and 30 as Type C. na: not available, F: females, M: males, LY: lymphocystis, U: ulceration, EP: epidermal papilloma, HYP: hyperpigmentation, LN: liver nodule, NNT: non-neoplastic toxicopathic lesion, FCA: foci of cellular alteration, BN: benign neoplasm, MN: malignant neoplasm, NSI: non-specific inflammatory lesion

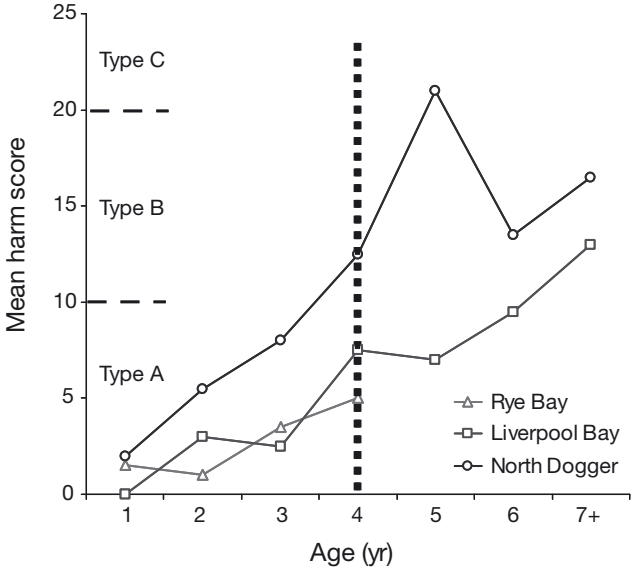

Fig. 6. *Limanda limanda*. Application of 'harm score' tool of Stentiford et al. (2009) to targeted survey sites sampled in 2007. Mean scores by age and site. Harm scores for Rye Bay and Liverpool Bay are similar for fish up to age 4 yr while scores for N Dogger Bank are higher than for the other 2 sites from age 2 yr onwards. Vertical dashed line allows comparison of harm score in age 4 yr fish from the 3 sites. Horizontal dashed lines depict cut-off points for classification of 'Site Types' A, B and C as proposed by Stentiford et al. (2009). Using an age 4 yr control point, dab from Rye Bay and Liverpool Bay classify the site as Type A, while fish from N Dogger Bank classify the site as Type B. After age 4 yr, fish from N Dogger Bank classify the site as at least Type B (Type C for 5 yr old fish) while those from Liverpool Bay classify the site as Type B after approximately age 6 yr

cancer of 163/100 000, a figure similar to that found in humans and certain hospitalised domesticated mammals. Since estimation of population size (in terms of numbers of individuals) is problematic for dispersed marine fish populations such as dab, calculation of the estimated annual rate is not feasible. However, data from the current survey and from previous work on this species by Stentiford et al. (2009) has indicated that grossly visible liver neoplasms can be present in up to 18% of dab populations sampled at some UK marine sites, with histopathological prevalence of liver neoplasia even higher than this (up to 24%). Martineau et al. (2002) state that cancer causes 23% of all deaths in humans, a percentage comparable to data presented in their study of beluga from the St. Lawrence estuary. Furthermore, they state that, with the exception of virus-induced cancers in woodchuck and in rodents and due to difficulties in estimating population size in most wild animals, cancer has not been reported as a major cause of mortality in wild animal populations. In this context, since reports of cancer prevalence in captive or domesticated animals may be artificially inflated due to increased lifespan or veterinary intervention to extend life in the animals (MacVean et al. 1978, Priester & McKay 1980, Fowler 1987), the 27% of beluga exhibiting cancer in the St. Lawrence estuary population was considered to represent a higher propensity for cancer in animals from this site compared to that observed in other open-water populations of the same cetacean species, or in humans or domesticated animals. In this instance, the highest risk factor was considered to be exposure to carcinogenic contaminants present in the sediments and infauna of the estuary (Martineau et al. 2002), a similar conclusion to that for the cause of liver neoplasms in estuarine flatfish (Myers et al. 2003).

Since calculation of an estimated annual rate of cancer is difficult for dab populations sampled as part of the UK CSEMP, it is only possible to compare apparent prevalence in those sub-populations sampled with the prevalence of cancer in other wildlife species. In this respect, the prevalence of neoplasia in dab captured from offshore sites in the UK is similar to that observed in beluga from the St. Lawrence estuary (Martineau et al. 2002) and to several fish species sampled from estuarine environments (see Myers et al. 1987, 1994, 1998a,b, 2003, 2008, Rhodes et al. 1987, Stehr et al. 1997, Vogelbein et al. 1990, Stentiford et al. 2003). Furthermore, the prevalence of pre-neoplastic liver lesions in dab sampled from several UK sites is similar to that observed in the flatfish *Parophrys vetulus* captured from PAH-contaminated sites in Puget Sound in the 1980s, prior to the remediation of harbour sediments via capping (Myers et al. 2008). While the capacity to link elevated levels of carcinogenic contaminants is potentially feasible for those animals sampled from relatively constrained water bodies (e.g. estuaries, rivers, lakes) (Köhler 1990, Stein et al. 1990, Myers et al. 1991, 1998a, 2003, 2008, Vethaak et al. 1996), it is more problematic when attempting to investigate similar relationships in the offshore environment, where contaminant burdens are invariably lower and population migrations are more pronounced (Cefas 2006, 2007, Lyons et al. 2006, Vethaak et al. 2009). Nevertheless, repeatable patterns of disease (including cancer) are reported from geographically distinct sites sampled over multiple years using standardised approaches for disease assessment, suggesting that a basis for this pattern exists (Stentiford et al. 2009). The present study has demonstrated that age does play a role in this pattern (with certain sites containing an older sampled population than other sites, with older fish having higher disease burdens) but also that following analysis of disease prevalence (and overall 'harm') in specific age classes, age at onset and overall disease burden by age does clearly differ between sites; the latter is suggestive of a causal factor or factors that manifest as effects at the individual and population level.

Some studies have proposed a single metapopulation of dab around the coast of the UK and in the North Sea (Rijnsdorp et al. 1992). However, more recent information, utilising a microsatellite marker-based approach to analyse populations of dab in the Irish Sea, English Channel and North Sea, has suggested that distinct sub-structuring of these populations actually exists (Tysklind 2009, Tysklind et al. 2009). In light of data presented in the present paper and in Stentiford et al. (2009), it is important to investigate whether such genetic distinction can describe the differential susceptibility to diseases such as liver cancer observed in European flatfish populations. By utilising a microsatellite approach to analyse fish of different age classes collected from the same site, it should be possible to define whether cohorts of fish collected at the same site (as in the present study) reside at that site for their whole lifetime, or just portions of it. Furthermore, by analysis of young and old age classes collected from different sites, it may be feasible to construct a 'migrational map' that describes broad movements of fish from sites where they reside as juveniles, to those occupied during later life stages. In this way, linking any early life stage exposure of juveniles to contaminated waters or sediments may be utilised to predict disease profile (particularly for those diseases thought to be linked to contaminant exposure) in older age classes captured at distant sites (a concept discussed by Vethaak et al. 2009). As stated by Stentiford et al. (2009), recognising the contributing role of such factors will significantly refine our approaches to marine monitoring and lead to a greater understanding of cause-effect pathways, particularly with respect to the biological effects of specific contaminant exposure.

In previous studies, we have suggested that for the purposes of monitoring diseases to assess the biological effects of exposure of fish populations to contaminants, diseases not thought to be directly linked to contaminant exposure (e.g. pathogens, parasites) provide a different level of information concerning health status to those diseases with at least some direct natural or experimental link with exposure (e.g. liver cancer, intersex) (Stentiford et al. 2009). In the context of the present analysis, several of the grossly visible diseases (LY, U, EP) are caused by pathogens that suggest some indirect effect of exposure to the wider environment on the immuno-competence of the host. As such, while they provide a very useful indicator of the overall health status of individuals (or populations), it is unlikely that they can be directly linked with exposure of individual fish to anthropogenic contaminants. Recently published work has suggested that these types of disease may be better utilised as so-called 'phenotypic anchors' (with sex, species, etc.) against which contaminant-related biomarker (or disease) expression can be assessed (Bignell et al. 2008). It should be noted that we do not advocate that the recording of grossly visible diseases (including pathogens and parasites) has no value in monitoring the health of fish within the ocean. On the contrary, the recording of infectious agents that may lead to elevated mortality in fish populations has a key role in assessing the health and sustainability of finfish and shellfish fisheries. In terms of development of objective assessment tools for the measurement of the biological effects of exposure to anthropogenic contaminants, however, we believe that it is important to concentrate efforts on the measurement of those diseases for which the link between presence and exposure is at least demonstrable from laboratory and field surveys (Myers et al. 2003). In light of data presented in the present study, it will now be important to consider the relationship between elevated liver cancer prevalence and potential causal factors at offshore sites. Studies demonstrating potential offshore endocrine disruption effects in populations of dab from the Dogger Bank region of the North Sea (Stentiford & Feist 2005, Scott et al. 2007) and the recent finding of over-expression of vitellogenin mRNA in liver tumours of dab (Small et al. 2010) are provocative directors for further research investigating the relationship between liver-cancer formation and the endocrine system of marine fish and their elevated association with particular geographic locations (Stentiford et al. 2009).

The concept of age at onset, which is discussed extensively in the medical literature, has not been applied extensively to studies of wild animals (including fish). In such a way, it is perhaps not surprising that clear patterns exist between disease expression and age in these organisms. The future utilisation of age at onset of key diseases (such as liver cancer) in marine monitoring programmes will provide important insights into initiation processes for these diseases based upon host genetics, migration or exposure to environmental perturbation. Controlling for fish age during marine monitoring programmes removes the bias inherent in the sampling of populations using total length and further, may be utilised in appropriate scenarios to follow specific cohorts of fish through successive years, particularly where the underlying migratory pattern of sentinel species are either limited (e.g. in some estuarine fish species) or otherwise well described. This technique has previously been used to assess the effect of intervention strategies on the health status of estuarine fish populations (Baumann & Harshbarger 1998, Myers et al. 2008). Additionally, assessment of age at onset for infectious diseases may be utilised in fish stock assessment to provide clarity on specific pressures driving mortality in juvenile cohorts prior to their recruitment to the fished stock.

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