Vol. 103: 9–24, 2013
doi: 10.3354/dao02554

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published March 13

OPEN ACCESS

Hyperpigmentation in North Sea dab *Limanda limanda*. I. Spatial and temporal patterns and host effects

F. Grütjen[1], T. Lang[2,*], S. Feist[3], D. Bruno[4], P. Noguera[4], W. Wosniok[5]

[1]Department of Biological Sciences, Zoology, University of Rostock, 18055 Rostock, Germany
[2]Thünen Institute of Fisheries Ecology, 27472 Cuxhaven, Germany
[3]Centre for the Environment, Fisheries and Aquaculture Science, Weymouth, Dorset DT4 8UB, UK
[4]Marine Scotland Science, Aberdeen AB11 9DB, UK
[5]Institute of Statistics, University of Bremen, 28334 Bremen, Germany

ABSTRACT: Hyperpigmentation is a term describing a specific pigment anomaly affecting common dab *Limanda limanda* in the North Sea and, less frequently, in adjacent areas, e.g. the English Channel, Irish and Celtic Seas, western Baltic Sea and Icelandic waters. Other North Sea flatfish species are also affected, but at a markedly lower prevalence. The condition is characterised by the occurrence of varying degrees of green to black patchy pigment spots in the skin of the upper (ocular) body side and pearly-white pigment spots in the skin of the lower (abocular) body side. In the course of fish disease monitoring programmes carried out by Germany and the UK (England and Scotland), a pronounced spatial pattern of hyperpigmentation has been detected in the North Sea. An increase in prevalence has been recorded in almost all North Sea areas studied in the past 2 decades. The prevalence recorded in hot spot areas of the condition increased from 5 to >40% between 1988 and 2009. Analysis of the German data indicates that the prevalence and intensity (degree of discolouration) of hyperpigmentation increase with size and age, indicating a temporal progression of the condition with size and age. Intense hyperpigmentation is associated with increased growth (length) and decreased condition factor. Potential causes of the condition (UV-B radiation nutrition, water temperature increase, demographic changes) and, in particular, of the spatial/temporal patterns recorded as well as the relationship to host-specific factors (sex, age, length, growth, condition factor) are discussed.

KEY WORDS: Pigment anomaly · Flatfish · Growth · Condition factor

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Hyperpigmentation is a specific pigment anomaly of the skin known since the early 1980s to occur in North Sea common dab *Limanda limanda* and, less frequently, in other flatfish species (T. Lang unpublished data). Pigment anomalies of some other flatfish species are well documented and have been described to affect wild populations (De Veen 1969, Gartner 1986, Macieira et al. 2006) as well as farmed fish (Ottesen & Strand 1996, Venizelos & Benetti 1999, Bolker & Hill 2000, Copeman & Parrish 2002, Bolker et al. 2005, Yamanome et al. 2005, 2007, Noguera et al. 2013, this issue). However, hyperpigmentation observed in dab may be a distinct phenomenon and differs from other types recorded because of its high prevalence and cellular and tissue characteristics (Noguera et al. 2013).

Hyperpigmentation is one of the externally visible diseases of dab recommended as a target disease for fish disease surveys in the North Sea and adjacent areas and is included in the methodological guidelines

*Corresponding author. Email: thomas.lang@vti.bund.de

© Inter-Research 2013 · www.int-res.com

for general biological effects monitoring under the OSPAR Joint Assessment and Monitoring Programme (JAMP; Bucke et al. 1996, OSPAR 1997). Consequently, it has been incorporated in the range of diseases recorded in the German and UK fish disease monitoring programmes, and long-term data are available for the North Sea and adjacent as well as reference areas, including the English Channel, Irish Sea, Celtic Sea, western Baltic Sea and Icelandic waters. German and UK fish disease data have been submitted to the Data Centre of the International Council for the Exploration of the Sea (ICES) and have been analysed and assessed repeatedly, e.g. for the OSPAR Quality Status Report 2010 (OSPAR 2010).

The common dab is characterised by a wide distribution in the North Sea and adjacent areas in connection with high abundance (Bohl 1957, Daan et al. 1990, Rijnsdorp et al. 1992, Knijn et al. 1993). It is a relatively sedentary species (Bohl 1957, Lozan 1988, Damm et al. 1991) and is considered to react sensitively to environmental stressors (Dethlefsen et al. 1987, Lang et al. 2003). Therefore, the dab is widely used as a bioindicator in national and international programmes for monitoring and assessing effects of anthropogenic stressors (e.g. contaminants) on fish health in the North Sea and adjacent areas, and methodological guidelines for sampling and the measurement of various parameters are available (Dethlefsen et al. 1986, McVicar et al. 1988, ICES 1989, Bucke et al. 1996, OSPAR 1997, 2003, Lang 2002, Feist et al. 2004).

The present study was aimed at providing information on spatial and temporal patterns in the prevalence of hyperpigmentation in dab, on the impact of host-specific factors (e.g. size, age, sex), and on effects of hyperpigmentation on the host. For this purpose, we used data obtained in fish disease monitoring programmes carried out by Germany and the UK (England and Scotland). In an additional contribution on this topic, Noguera et al. (2013) provide information on macroscopic, histological and ultrastructural characteristics of the phenomenon as well as on the involvement of pathogens.

MATERIALS AND METHODS

Sampling and disease examination

The German long-term fish disease monitoring programme of the Thünen Institute of Fisheries Ecology has been carried out since the early 1980s during annual winter (December) and summer (August/September) cruises with RVs 'Walther Herwig' II and III (Table 1). Currently, diseases in dab have been recorded in up to 12 main sampling areas in the North Sea and at least 3 areas in the Baltic Sea (for locations, see Table 2 and Fig. 1). More limited studies in terms of spatial and temporal coverage have also been carried out in adjacent waters, such as the English Channel, Irish Sea, Celtic Sea and in reference areas at the south coast of Iceland.

Fishing was carried out by means of bottom trawling with standard gears (GOV or 140 ft [ca. 42.7 m] bottom trawl), with a towing time of 30 to 60 min and a speed of 3 to 4 knots. Per sampling area, usually 3 to 5 hauls were performed.

Marine fish disease monitoring in England and Scotland has been carried out by the Centre for Environmental and Aquaculture Science (Cefas) and Marine Scotland Science, respectively. Data are available for the North Sea and adjacent waters such as the English Channel, Irish Sea, Celtic Sea and North Scottish waters (Cefas 2009, Noguera et al. 2013).

All methodologies applied followed ICES and BEQUALM guidelines (ICES 1989, Bucke et al. 1996, Feist et al. 2004, www.bequalm.org) and were intercalibrated repeatedly; thus, data generated are comparable. Fish for disease examination were sorted from the catches immediately after hauling and kept alive in tanks with running seawater. The total length (in cm, rounded down) and wet weight were measured in specimens taken for disease examination, facilitating the calculation of a condition factor (CF = wet weight [g] / total length^3 [cm] × 100). Sex was determined visually by the shape of the gonads, and doubtful cases were confirmed by dissection and inspection of the gonads.

Dab from representative subsamples (encompassing all size groups in the catch) were inspected externally for signs of hyperpigmentation and a wide range of other diseases and parasites, and a size-stratified sample (specimens ≥20 cm total length) was inspected internally for the presence of liver anomalies (including macroscopic liver neoplasms) as well as externally for other diseases, both using standardised ICES methodologies (ICES 1989, Bucke et al. 1996, Feist et al. 2004) as well as guidelines provided through the fish disease component of the BEQUALM quality assurance programme (www.bequalm.org). From the specimens inspected for liver anomalies, otoliths for subsequent age determination were taken and stored individually. The presence of hyperpigmentation was recorded according to 3 BEQUALM severity grades (grade 1: ≤10% of the skin surface covered by hyperpigmentation; grade 2:

Table 1. Research cruises of RVs 'Walther Herwig' II and III in 12 North Sea areas and 3 Baltic Sea areas. Each sampling campaign lasted 17 to 22 d. S: summer cruise, W: winter cruise; blank: no cruise

Date (mm/yyyy)	Cruise ID	Sampling area														
		North Sea												Baltic Sea		
		N01	N02	N03	N04	N05	N06	N07	N10	N11	N22	P01	P02	B01	B12	B11
06/1988	WH090	S	S	S	S	S	S	S	S	S						
12/1988	WH094	W	W	W	W	W	W	W		W						
06/1989	WH098	S	S	S	S	S	S	S	S	S						
12/1989	WH103	W	W	W	W	W	W	W		W						
06/1990	WH107	S	S	S	S	S	S	S	S	S						
12/1990	WH112	W	W	W	W		W	W	W	W	W			W		
06/1991	WH116	S			S	S	S	S	S		S					
12/1991	WH120	W	W		W	W	W	W	W	W	W			W	W	W
06/1992	WH125	S	S		S	S	S	S	S	S						
12/1992	WH130	W	W		W	W	W	W		W	W					
06/1993	WH135	S			S	S	S	S	S	S						
12/1993	WH142	W	W		W		W	W	W	W	W			W	W	W
06/1994	WH148	S		S	S		S	S		S						
12/1994	WH155	W			W	W	W	W		W	W					
06/1995	WH161	S		S	S	S	S	S		S	S					
12/1995	WH167	W	W	W	W	W	W	W	W	W	W			W	W	W
06/1996	WH172	S		S	S	S	S	S		S	S					
12/1996	WH178	W												W	W	
06/1997	WH185	S		S	S	S	S	S	S	S	S					
12/1997	WH191	W			W		W				W			W	W	W
06/1998	WH195	S		S	S	S	S	S	S	S	S					
12/1998	WH200	W		W	W	W	W	W	W	W	W			W	W	
08/1999																
12/1999	WH212	W		W	W	W	W	W	W	W	W			W	W	
08/2000																
12/2000	WH223	W		W	W	W	W		W	W	W	W		W		
08/2001																
12/2001	WH234	W			W	W	W				W	W		W	W	
08/2002	WH242	S			S		S					S	S	S	S	
12/2002	WH245	W			W	W	W				W	W		W		
08/2003	WH255	S			S		S			S	S	S	S	S	S	S
12/2003	WH258	W					W					W		W	W	W
08/2004	WH267	S	S	S	S	S	S	S		S	S	S	S	S	S	S
12/2004	WH269	W		W	W	W	W			W	W	W	W	W	W	
08/2005	WH278	S			S		S			S		S	S	S	S	
12/2005	WH281	W								W				W	W	
08/2006	WH291	S			S		S			S	S	S	S	S	S	
12/2006	WH294	W			W	W	W			W	W	W		W	W	
08/2007	WH303	S			S		S		S	S		S	S	S	S	
12/2007	WH306	W		W	W	W	W		W	W	W	W		W	W	
08/2008	WH315	S			S		S			S		S	S			
12/2008	WH317						W			W		W	W	W	W	
08/2009	WH325	S												S	S	
12/2009	WH328													W	W	

between 10% and up to twice the area of the spread-out caudal fin covered by hyperpigmentation; grade 3: more than twice the area of the spread-out caudal fin covered by hyperpigmentation).

Fish disease database analysed

All single fish data generated in the German programme on board RVs 'Walther Herwig' II and III were entered into a PC interface and incorporated into the fish disease database of the Thünen Institute of Fisheries Ecology. The database is structured in a descriptive part with variables providing temporal and spatial information as well as host-specific individual information (sex, length, wet weight, age) and a binary part with variables providing information on the presence/absence of diseases and their severity grades assigned. The database comprises data on >400 000 specimens of dab ≥10 cm from a total of

Table 2. *Limanda limanda*. Location of the sampling areas (12 North Sea and 3 Baltic Sea areas) of the German monitoring programme in the North Sea and the Baltic Sea from summer 1988 to winter 2009. N: total number of dab examined

Area code	ICES rectangle code	Geographical coordinates	N
North Sea			
N01	37F7	54° 15′ – 54° 26′ N, 07° 25′ – 07° 39′ E	24342
N02	37F4	54° 06′ – 54° 29′ N, 03° 21′ – 04° 08′ E	10052
N03	35F3	52° 46′ – 53° 10′ N, 03° 28′ – 04° 10′ E	9270
N04	37F2	54° 25′ – 54° 50′ N, 02° 00′ – 02° 32′ E	24351
N05	39E9	55° 15′ – 55° 29′ N, 00° 00′ – 00° 25′ W	14547
N06	41E8	56° 15′ – 56° 26′ N, 01° 44′ – 02° 14′ W	21691
N07	44E9	57° 45′ – 57° 59′ N, 00° 46′ – 01° 20′ W	9021
N10	42F5	56° 43′ – 56° 56′ N, 03° 30′ – 03° 55′ E	12023
N11	40F7	55° 30′ – 55° 41′ N, 06° 49′ – 07° 24′ E	20032
N22	36F1	53° 30′ – 53° 44′ N, 01° 18′ – 01° 47′ E	13943
P01	39F4	55° 22′ – 55° 47′ N, 04° 40′ – 05° 14′ E	9809
P02	41F2	56° 16′ – 56° 41′ N, 02° 40′ – 03° 24′ E	6136
Baltic Sea			
B01	38G0	54° 28′ – 54° 45′ N, 10° 12′ – 10° 59′ E	11578
B12	37G1	54° 12′ – 54° 27′ N, 11° 22′ – 11° 51′ E	10537
B11	38G3	54° 40′ – 54° 55′ N, 13° 00′ – 13° 55′ E	934
		Total	**198266**

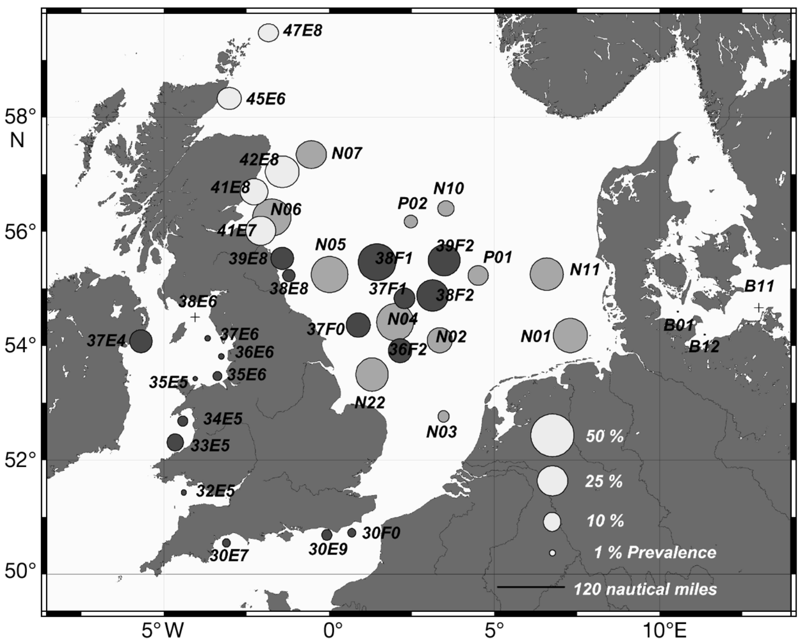

Fig. 1. *Limanda limanda.* Mean prevalence of hyperpigmentation in dab from the North Sea and adjacent waters in the time period 2002 to 2008, with prevalence of hyperpigmentation (%) as arithmetic means; males and females and all size classes (>10 cm) combined; +: 0% prevalence. Scottish data: light grey; English data: dark grey; German data: medium grey (Software: Ocean Data View) (see Table 3 for the number of sampling campaigns at each station; for spatial patterns over the whole sampling period 1988 to 2008, see Fig. 2)

70 research cruises conducted in the period 1981 to 2009. For the present study, data on 198 266 specimens fulfilling the criteria defined in terms of spatial and temporal coverage were used for the analysis, covering the period from summer 1988 to winter 2009 (see Tables 1 & 2) and standardised sampling areas. Since 2009, data from the North Sea are limited to German Bight areas (Table 1). For comparative purposes, UK data submitted by Cefas and Marine Scotland Science (see above) were extracted from the ICES Data Centre, covering the periods 2003 to 2007 (23 646 specimens) and 2002 to 2008 (32 536 specimens), respectively (Table 3). Despite a successful intercalibration of diagnostic methodologies (see above), the English and Scottish data were treated separately from the German data since they covered different time spans and sampling seasons.

The number of fish examined is shown in Table 2 (German data, entire period 1988 to 2009) and Table 3 (according to the prevalence of hyperpigmentation in the North Sea and adjacent waters: German and Scottish data, 2002 to 2008; English data, 2003 to 2007), together with information on geographical coordinates and coding of the sampling areas as well as on mean prevalences of hyperpigmentation recorded in the respective periods (Table 3). For the German areas, internal codes were used, whereas for the English and Scottish data, we used the codes for the ICES statistical rectangles.

Data treatment

The prevalence of hyperpigmented fish was calculated by sampling area (see Table 3) and research cruise, using the fish disease database of the Thünen Institute of Fisheries Ecology. Temporal trends in hyperpigmentation prevalence were identified by fitting a generalized additive model (GAM; Hastie & Tibshirani 1990) with logistic link and binomial error to the prevalence data recorded during external inspection of dab. The model involved a spline function for the temporal

Table 3. Location of sampling areas in the North Sea and adjacent waters from 2002 to 2008 (Scottish data, German data) and 2003 to 2007 (English data); geographical coordinates are mean locations of each area; prevalence of hyperpigmentation shown as arithmetic means (see also Fig. 1)

Area code	ICES rectangle code	Geographical coordinates		$N_{examined}$	$N_{affected}$	Prevalence (%)	No. of sampling campaigns	Data origin
North Sea								
N01	37F7	54° 15′ N,	07° 25′ E	7690	2519	32.76	13	German
N02	37F4	54° 06′ N,	03° 21′ E	938	165	17.59	1	German
N03	35F3	52° 46′ N,	03° 28′ E	1985	74	3.73	3	German
N04	37F2	54° 25′ N,	02° 00′ E	7510	2891	38.20	11	German
N05	39E9	55° 15′ N,	00° 01′ E	2890	1065	36.07	6	German
N06	41E8	56° 15′ N,	01° 44′ W	7873	2938	37.61	13	German
N07	44E9	57° 45′ N,	00° 46′ W	409	87	21.27	1	German
N10	42F5	56° 43′ N,	03° 30′ E	1380	91	6.55	2	German
N11	40F7	55° 30′ N,	06° 49′ E	7550	2173	29.10	11	German
N22	36F1	53° 30′ N,	01° 18′ E	4397	1287	30.63	8	German
P01	39F4	55° 22′ N,	04° 40′ E	8922	939	10.69	13	German
P02	41F2	56° 16′ N,	02° 40′ E	6136	263	4.50	9	German
	47E8	59° 29′ N,	01° 50′ W	2188	190	8.68	2	Scottish
	45E6	58° 20′ N,	03° 01′ W	11273	1486	13.18	2	Scottish
	42E8	56° 63′ N,	01° 25′ W	7394	2056	27.81	2	Scottish
	41E8	56° 42′ N,	01° 76′ W	6097	1159	19.01	2	Scottish
	41E7	56° 01′ N,	02° 04′ W	5584	1302	23.32	2	Scottish
	39E8	55° 32′ N,	01° 25′ W	693	94	13.56	3	English
	39F2	55° 30′ N,	02° 89′ E	3071	824	26.83	5	English
	38F1	54° 88′ N,	01° 27′ E	1305	490	37.55	5	English
	38F2	54° 53′ N,	02° 68′ E	1215	332	27.33	4	English
	38E8	54° 74′ N,	01° 13′ W	1245	52	4.18	5	English
	37F0	54° 22′ N,	00° 53′ E	1318	216	16.39	5	English
	37F1	54° 50′ N,	01° 77′ E	1210	143	11.82	4	English
	36F2	53° 55′ N,	02° 09′ E	1239	197	15.90	3	English
English Channel								
	30F0	50° 85′ N,	00° 78′ E	1575	32	2.03	5	English
	30E9	50° 76′ N,	00° 04′ W	338	11	3.25	1	English
	30E7	50° 61′ N,	03° 04′ W	308	6	1.95	3	English
Celtic Sea								
	32E5	51° 54′ N,	04° 58′ W	823	7	0.85	4	English
Irish Sea								
	33E5	52° 28′ N,	04° 30′ W	1423	116	8.15	5	English
	34E5	52° 69′ N,	04° 53′ W	238	7	2.94	2	English
	35E5	53° 35′ N,	04° 12′ W	1632	9	0.55	5	English
	35E6	53° 47′ N,	03° 34′ W	2776	62	2.23	5	English
	36E6	53° 92′ N,	03° 38′ W	1785	15	0.84	5	English
	37E6	54° 05′ N,	03° 89′ W	1355	12	0.89	5	English
	37E4	54° 05′ N,	05° 62′ W	97	14	14.43	1	English
	38E6	54° 54′ N,	03° 84′ W	1165	0	0.00	4	English
Baltic Sea								
B01	38G0	54° 36′ N,	10° 33′ E	7133	3	0.04	13	German
B11	37G1	54° 40′ N,	13° 00′ E	511	0	0.00	4	German
B12	38G3	54° 12′ N,	11° 22′ E	6119	3	0.04	12	German

change of prevalence and linear terms for the effects of sex, length and season. Model fits were calculated by geographic regions. To remove effects due to sampling variation in sex and length distributions and due to sampling season and to facilitate a comparison of trend estimates from different regions, predicted trends were calculated for each area for a common fictitious standard population (direct standardisation) and by adjusting for sex, body length and season effects. The standard population was derived as the mean composition of all samples with regard to sex and length. The mean sample size per region served as the population size for prediction. When adjusting for season, winter conditions were assumed.

Areas N01, N04, N06 and N22 showed a constantly high prevalence over the time series and were considered as 'high prevalence areas' (HPAs). Data from these areas were combined for analyses of age and length classes with severity grades. The CFs of hyperpigmented fish and non-affected fish were compared by 1-factorial analysis of variance (ANOVA). If the *F*-test revealed significance, a Tukey HSD post hoc test was used to identify differences between groups. The condition factor was tested for normal distribution (the ANOVA precondition) by the Kolmogorov-Smirnov test. Age and body length (growth) of affected and non-affected fish were compared in the same way.

The body length of dab in different North Sea areas in an early (1990–1998) and a later (2000–2008) time window was compared by a *t*-test. Changes in body length over a time period were tested for monotone trends using Spearman's rank correlation.

Statistical analyses on trends and relationship to host-specific factors were only done for the German data. All calculations were carried out using the computer software Statistica (SoftwareSystem for Data Analysis, StatSoft) and R software version 2.13.1 (R Development Core Team 2011)

RESULTS

Spatial and temporal patterns in the prevalence of hyperpigmentation

The prevalence of hyperpigmentation in North Sea dab differed markedly between regions and developed persistent spatial patterns (Fig. 1). In the period 2002 to 2008, the mean observed prevalence over all size groups ranged from <10% in areas in the northern, central and southernmost North Sea (N10, P01, P02, N03) to >35% at the Dogger Bank and in the Firth of Forth area (N04, N06). Data from the northern North Sea revealed a high mean prevalence of 28% in area 42E8 (Marr Bank) and a decrease in prevalence in a northerly direction, with 13% in area 45E6 (Moray Firth) and 9% in area 47E8 (Fair Isle). Data from the period 2003 to 2007 showed a high mean prevalence of 38% in area 38F1, similar to the high prevalence recorded in areas N04 and N05. There were considerable small-scale variations in prevalence between neighbouring areas, e.g. between areas P01 and 39F2 or areas N04/N22 and 36F2/37F0/37F1. In dab from areas outside the North Sea, the prevalence of hyperpigmentation was lower in the period 2003 to 2007, with values of 2 to 3% in the English Channel (areas 30F0, 30E9, 30E7), 1% in the Celtic Sea (area 32E5) and 1 to 14% in the Irish Sea (areas 33E5, 34E5, 35E5, 35E6, 36E6, 37E4, 37E6, 38E6; Fig. 1, Table 3). In the western Baltic Sea, the condition was either absent (area B11) or occurred at a markedly low prevalence of <1% (areas B01, B12).

As can be seen from Fig. 2 (based on German data only), illustrating both the observed prevalence and the predicted prevalence for the standard population (adjusted for effects of length, sex and season; see 'Materials and methods'), in the period 1988 to 2008, there were marked differences between sampling areas in the general prevalence level of hyperpigmentation, with e.g. high levels in areas N01, N04, N06 and N22 and low levels in areas N03, N10 and P02. These findings correspond to the data for the years 2002 to 2008 shown in Fig. 1.

From the observed and predicted prevalence, there is evidence that the prevalence of hyperpigmentation in the North Sea has increased since 1988 (Fig. 2). A significant upward trend was apparent in all 12 North Sea sampling areas (GAM, $p < 0.001$). Areas N01, N04, N05 and N06 showed a particularly strong increase, and area N22 showed a generally high prevalence over the whole time series. The observed prevalence of hyperpigmentation at the beginning of the observation period (1988) was <5% in areas N01, N05 and N06, while it was >10% in areas N04 and N22. The maximum observed prevalence of >50% was reached in the period after 2005 and was recorded in areas N01, N04, N05, N06 and N11. However, in some areas, a decrease in prevalence was recorded in recent years, e.g. since 2006 in areas N01 (especially in summer) and area N11.

Comparing the adjusted trend over the areas suggests a spatio-temporal process of increasing prevalence over time, which did not occur synchronously over all areas. The peaks of the trend curves showed a certain delay, with N01 and N11 exhibiting the earliest maxima, followed by N06, while in other areas a clear peak was not identifiable.

Impact of host-specific factors on the prevalence of hyperpigmentation

In the following subsections effects of sex, body length, age, season, severity grade on the prevalence of hyperpigmentation are described. For the analysis of the combined factors length and severity grade or age, only German data from the 4 HPAs N01, N04, N06 and N22 were used.

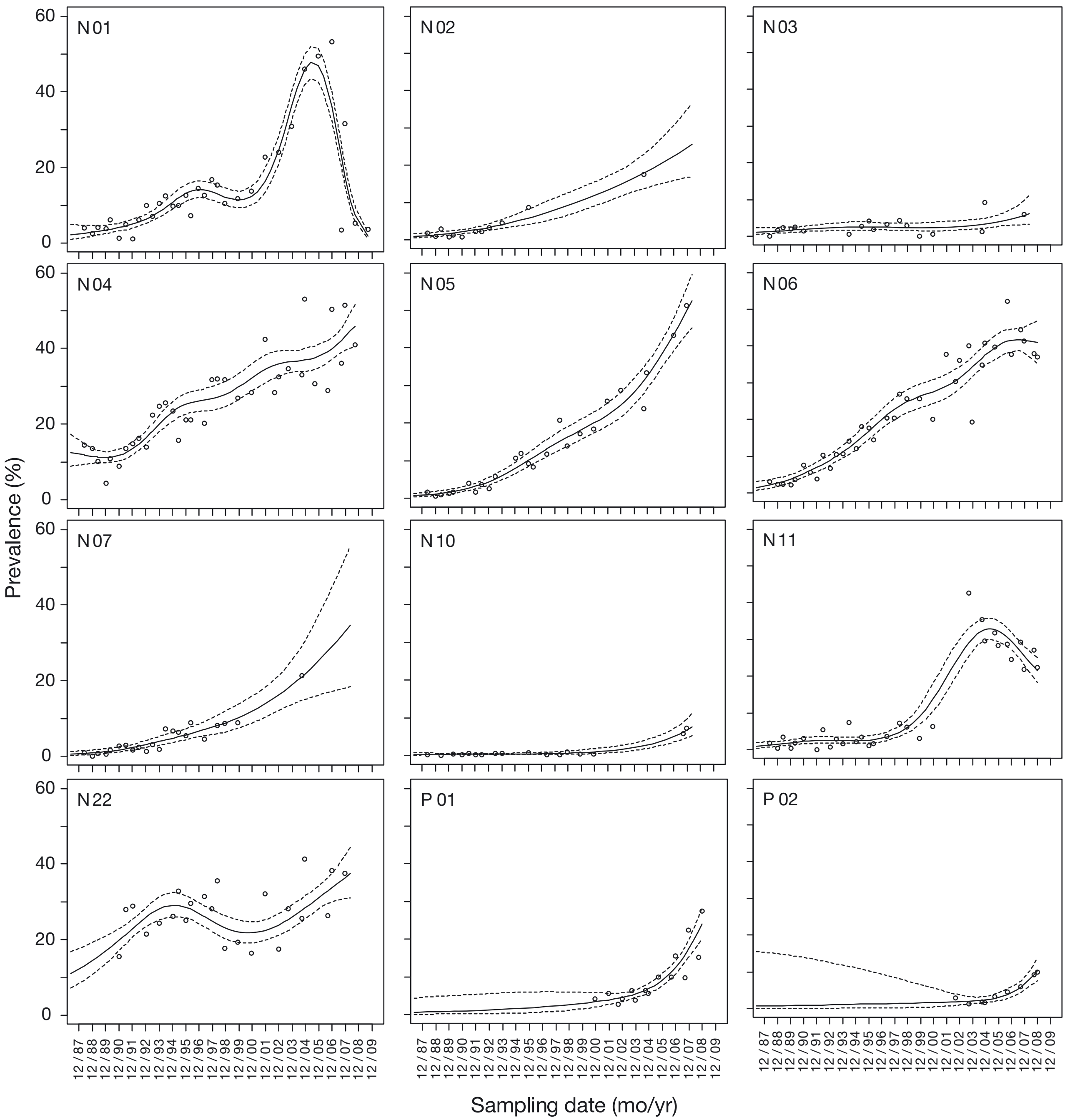

Fig. 2. *Limanda limanda.* Temporal trends in hyperpigmentation prevalence in dab from sampling areas in the North Sea (German sampling campaigns 1988 to 2009), obtained by fitting a generalized additive model with logistic link and binominal error to the prevalence data. Solid line: predicted trend curve for the standard population (see 'Materials and methods'); dotted lines: confidence intervals for the mean trend; circles: observed prevalence. Males and females and all size classes (>10 cm) combined

Sex

Significant differences in the prevalence of hyperpigmentation between males and females were recorded in all areas (GAM, $p > 0.05$), with the prevalence in males always being higher. When looking globally (without distinguishing between areas) at different size groups of dab, there were some differences in prevalence between males and females (data not shown); in small fish (size group 16–20 cm), the prevalence was higher in males. In medium-sized fish (21–22 cm), there were no differences. In large fish (23–28 cm), females were more frequently affected than males.

Body length

To test the relationship between total body length and the prevalence of hyperpigmentation, data from female and male dab were used separately, because sex-specific differences in growth of dab are well documented in the literature (Bohl 1957, Knust 1990, Lozan 1992, Rijnsdorp et al. 1992) and may have affected the prevalence of hyperpigmentation. Fig. 3 shows that the prevalence of hyperpigmentation (all severity grades combined) in female dab increased with length, especially in the size range 17–25 cm. Thereafter, it levelled off and remained at a more or less constant prevalence level of 40 to 45% up to the largest size class of 32 cm. Prevalence tended to decrease in the largest size classes (>28 cm, with the exception of 31 cm). However, due to the relatively low number of large specimens in the samples, some uncertainties remain. The length class 31 cm showed the highest overall prevalence of >50%. The total length of the smallest specimens recorded displaying hyperpigmentation was 12 cm, and the smallest dab with hyperpigmentation severity grade 3 were 13 cm (male) and 14 cm (female). The prevalence of hyperpigmentation grade 1 was generally higher than that of grades 2 and 3. However, with increasing length, there was a shift towards a higher prevalence of severity grades 2 and 3. In the largest specimens (31–32 cm), grade 1 was less prevalent than grade 2. Similar patterns were recorded in male dab (data not shown). The overall prevalence of all length classes of males was similar to females (see above). As in females, there was a shift in prevalence towards higher severity grades with increasing length.

The joint effect of sex and length was also seen in the GAM fit, which showed significant effects of sex and length in all areas, but with a variable extent in the areas. Therefore, to allow an assessment of temporal trends unaffected by size and body length effects, predicted trends adjusted for size and length effects were calculated from the GAM (Fig. 2; see above).

Since 2006, a strong decline in prevalence of hyperpigmentation was recorded in the German Bight (N01), especially in summer, but also in winter (Fig. 2). This corresponded to a markedly increased proportion of small dab of the size group 10–14 cm in the catches, 45 to 70% in summer and 25% in winter, while the average proportion of small dab in the period 1988 to 2006 was 15%. The data from the other HPAs did not reveal a change in the proportion of small fish. Besides area N01, a decline in prevalence was only recorded in area N11 (starting in 2003).

Considering the length data over the entire observation period, Table 4 shows that the mean body length of dab decreased in 7 out of 11 sampling areas when comparing the periods 1990–1998 and 2000–2008. A significant decrease in length was recorded in the 4 HPAs N01, N04, N06 and N22 as well as in area N03 (Spearman's rank correlation; *t*-test, $p < 0.05$). In contrast, data from areas N05, N10 and N11 indicated an increase in length which was significant in areas N05 and N11 (Spearman's rank correlation; *t*-test, $p < 0.05$). These changes could be observed both in hyperpigmented and unaffected fish, albeit with differing significance levels. In the 3 Baltic Sea areas, no significant changes in body length were recorded. The results indicate changes in population structure of dab over the past 20 yr.

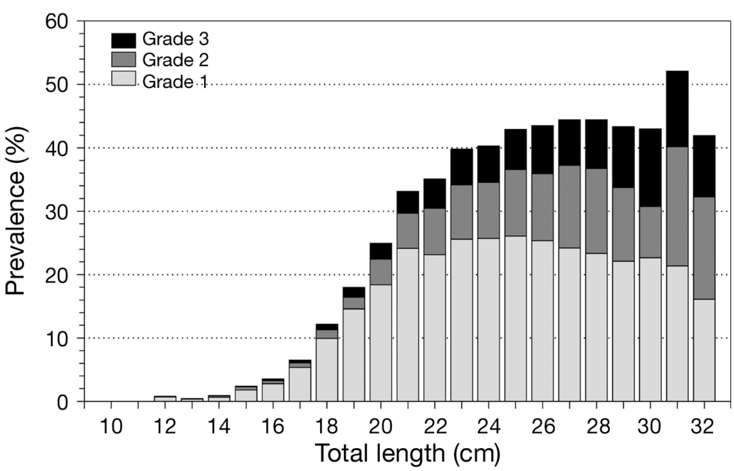

Fig. 3. *Limanda limanda*. Prevalence of hyperpigmentation according to length class (10–32 cm) in female dab from the high prevalence areas (HPA) examined for externally visible diseases (accumulation of hyperpigmentation severity grades 1, 2, 3; sampling campaigns 1988 to 2009 combined)

Age

The analysis of age data was based on female dab because male dab were less abundant, and age data from males did not cover all age groups sufficiently. Furthermore,

Table 4. *Limanda limanda*. Arithmetic mean body lengths of dab (affected and unaffected fish combined) in the sampling periods 1990 to 1998 and 2002 to 2008 and Spearman's rank correlation as an indicator of monotone temporal trends in body length in different areas of the North Sea and Baltic Sea (**bold**: significant at $p < 0.05$; *t*-test). Males and females and all size classes (>10 cm) combined. N: number of dab examined; HPA: high prevalence areas (N01, N04, N06, N22 combined); nd: no data

Area	Spearman's rank correlation	1990–1998 Length (cm) Mean	SE	N	2000–2008 Length (cm) Mean	SE	N	Difference (cm)
HPA	**−0.8301**	18.94	0.07	44053	17.90	0.08	28735	**−1.04**
N01	**−0.6519**	18.02	0.10	11822	16.77	0.13	10566	**−1.25**
N04	**−0.4947**	19.27	0.09	13125	18.60	0.12	8607	**−0.67**
N06	**−0.8456**	19.67	0.18	10841	17.82	0.21	8809	**−1.85**
N22	**−0.6265**	19.20	0.13	8265	18.12	0.16	5163	**−1.08**
N02	0.2167	17.43	0.09	6536	17.43	0.08	938	0.00
N03	**−0.7418**	17.11	0.15	5531	15.72	0.18	2164	**−1.39**
N05	**0.7882**	16.96	0.09	8544	18,11	0.12	3839	**+1.15**
N07	−0.0490	19.03	0.13	7479	18.89	0.17	409	−0.14
N10	0.3571	18.15	0.11	8094	18.26	0.12	1723	+0.11
N11	**0.5980**	16.34	0.15	8793	18.48	0.15	7867	**+2.14**
P01	0.0500	nd	–	–	18.89	0.11	9809	–
P02	0.2857	nd	–	–	18.28	0.08	6136	–
B01	0.4324	22.20	0.27	3179	20.50	0.21	8399	−1.71
B11	0.1429	20.69	0.37	464	21.75	0.25	470	+1.07
B12	0.1059	21.95	0.42	2507	20.96	0.26	8029	−0.99

it has to be noted that age data were only available for fish ≥20 cm total length which were examined for liver anomalies (see 'Materials and methods'); therefore, the age/prevalence data shown in Fig. 4 cannot directly be compared to length/ prevalence data shown in Fig. 3. Fig. 4 shows that the prevalence of hyperpigmentation in age classes 2 to 4 yr was similar (20–22 %), but that there was a decreasing trend with increasing age in the age groups 5 to 7 yr, corresponding to the tendency observed in the length data (see above). With respect to the proportion of the 3 severity grades, the data were also in line with the length data and revealed an increasing proportion of severity grades 2 and 3 with higher age.

Season

Only areas N01, N11 and P01 in the south-eastern North Sea showed significant differences in prevalence of hyperpigmentation between the sampling seasons summer and winter (GAM, $p < 0.01$). In the other North Sea areas, no significant differences between season and the prevalence of hyperpigmentation occurred. Season therefore does not affect the prevalence of hyperpigmentation in general. However, a more complex GAM with interaction terms between season and sampling area showed overfitting (data not shown), with differences between sampling campaigns of summer and winter in areas N01 (since 2003), N11 (since 2003) and P01 (since 2006).

Severity grade

Considering the whole data set used for the analyses, it is obvious that severity grade 1 showed the highest prevalence in general, while severity grades

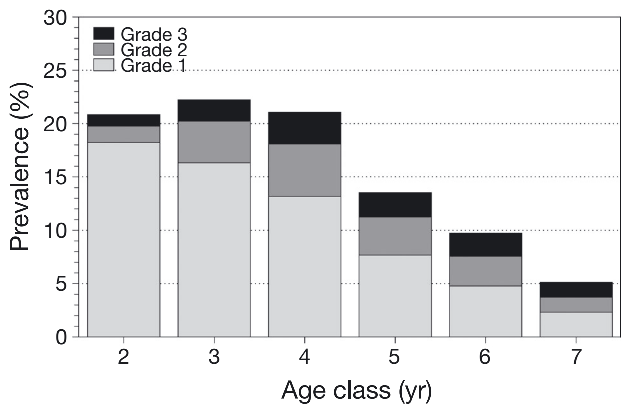

Fig. 4. *Limanda limanda.* Prevalence of hyperpigmentation according to age class (2–7 yr) in female dab ≥20 cm total length from the high prevalence areas (HPA) examined for liver anomalies and externally visible diseases (cumulation of hyperpigmentation severity grades 1, 2, 3; sampling campaigns 1994 to 2009 combined)

2 and 3 did not differ markedly. However, the ratios of the grades were influenced by the length and age classes (see Figs. 3 & 4). Especially the prevalence of grade 1 increased in all 4 HPAs in the period 1993 to 1995, a development that continued especially after the years 1999 to 2000. Areas N22 and N04 showed signals indicating a temporal relationship between lower and higher severity grades since there was a time lag in the increase in prevalence of grade 3 in relation to the increase recorded for grade 1. From these data (not shown) and the data on the relationship between hyperpigmentation and body length and age, there is evidence of a progression of hyperpigmentation with age in terms of its severity.

Effects of hyperpigmentation on the host

In order to assess the effects of hyperpigmentation on affected hosts, effects on growth and on condition factors were analysed.

Growth

The growth parameters age and length indicated differences between the growth of hyperpigmented and non-affected fish (12 North Sea areas; female dab; age 2–7 yr; sampling campaigns 1994–2009). Fish with hyperpigmentation (severity grades combined) were significantly larger (difference: arithmetic mean ± SE 0.39 ± 0.074 cm) than fish without hyperpigmentation (ANOVA, $p < 0.05$). Especially fish with hyperpigmentation grade 3 were significantly larger (difference: arithmetic mean 1.18 ± 0.20 cm) compared to control and grade 1 fish (Tukey HSD test, $p < 0.05$). There was no significant difference in total length between unaffected and grade 1 fish (Tukey HSD test, $p > 0.05$).

Condition factor

The data (CFs of dab according to the severity grade of hyperpigmentation; sampling areas N01, N04, N06, N22 and all 12 North Sea areas combined; all size classes combined; winter 2002 to winter 2008) indicate differences in mean CF between fish with and without hyperpigmentation (only females were taken into account for consistency reasons). In all North Sea areas, including their combinations, there was a tendency for lower CF in hyperpigmented fish, particularly in those with hyperpigmentation grade 3 (12 North Sea areas combined: severity grade 3, arithmetic mean CF = 1.0041 ± 0.0056; no hyperpigmentation, arithmetic mean CF = 1.0468 ± 0.0020; HPAs: severity grade 3, arithmetic mean CF = 1.0038 ± 0.0177; no hyperpigmentation, arithmetic mean = 1.0677 ± 0.0089). Taking data from all 12 areas together, this difference was significant (ANOVA, $p < 0.05$). The CF in fish with lower severity grades (1, 2) were not significantly different (Tukey HSD test, $p > 0.05$). However, there was an indication of lower CF in grade 2 and 1 fish compared to non-hyperpigmented fish. Grade 3 dab showed significant differences (Tukey HSD test, $p < 0.05$) to the controls and to grade 1 fish.

DISCUSSION

The aims of this study were to analyse spatial and temporal patterns in the prevalence of hyperpigmentation in dab from the North Sea and adjacent areas, to assess the impact of host-specific factors on its prevalence, and to provide information on the effects of hyperpigmentation on the host. Noguera et al. (2013) addresses macroscopic and microscopic characteristics of the condition as well as results from virological and bacteriological testing of the involvement of pathogens as aetiological factors.

The results indicate that hyperpigmentation is a specific and prevalent condition in dab that is different from other types of pigment anomalies reported from wild and farmed flatfish in its macroscopic and microscopic characteristics (Noguera et al. 2013), its prevalence and spatial patterns in the North Sea and adjacent areas as well as its marked temporal changes recorded over the past 2 decades. Besides dab, the condition has been also observed in other flatfish species from the same habitats, e.g. in long rough dab *Hippoglossoides platessoides*, solenette *Buglossidium luteum*, lemon sole *Microstomus kitt* and flounder *Platichthys flesus*; T. Lang et al. unpubl. data). Although quantitative data are lacking, observations revealed a generally lower prevalence in these other species compared to dab. No information is available on spatial and temporal patterns of the condition in these species.

Noguera et al. (2013) discuss a number of potential causes of hyperpigmentation, such as a localized expression of intrinsic melanisation stimulatory or inhibitory factors in the skin, affecting the differentiation of specific pigment cell types, nutritional factors at early life stages, with vitamin A, fatty acids and thyroid hormones all shown to affect pigmentation, and UV-B radiation associated with a protective 'sunscreen' role of pigments as a reaction against radiation. Here, we focus on environmental and host-specific factors that may have an impact on the observed spatial and temporal patterns of hyperpigmentation.

The fact that the prevalence of hyperpigmentation increased in all 12 North Sea study areas of the German fish disease monitoring programme and that similar changes were recorded in the UK studies indicates that there has been a general North Sea-wide shift in environmental or host-specific conditions favouring the development of hyperpigmentation, the degree of which, however, apparently varied depending on the area. In some areas (N04, N05, N06), the prevalence increased almost linearly over a more or less identical time period and reached similar levels, although the geographical distances between the areas are large. Other areas showed no clear trends over the first observation period, which was, however, followed by an extraordinarily steep increase (areas N11, P01, P02). Only in areas N03 and N10 was the increase low and did not lead to high values. In area N22, the prevalence has always been comparatively high, possibly indicating that environmental conditions favouring hyperpigmentation have had an impact here for a longer period (Fig. 2).

It is interesting to note that the strongest increase in prevalence occurred in hyperpigmentation severity grade 1, whereas the increase was less pronounced in the higher severity grades. There was some indication of a delay effect in the increase of the 3 severity grades (the increase in prevalence of severity grades 2 and 3 started later in time than the increase in severity grade 1), revealing a temporal progression of hyperpigmentation in the population from severity grade 1 towards severity grades 2 and 3 (data not shown). Because of the positive relationship between fish size (length), which is also connected with age, and the prevalence of hyperpigmentation, length and age are considered as risk factors for hyperpigmentation.

The spatial pattern of hyperpigmentation observed in dab, revealing marked differences between areas in the North Sea and a generally low prevalence in areas outside the North Sea (especially in the dab population of the western Baltic Sea, but to a lesser degree also in the English Channel and the Celtic and Irish Seas), indicates a strong impact of region-specific factors on the aetiology and development of the condition. General spatial patterns in the North Sea are mainly a result of physical factors like hydrodynamics, substrate type as well as the structure and topography of the seabed (Becker 1990, Otto et al. 1990). Within the North Sea, there are 4 main water masses (fronts), which differ in their density characteristics (temperature, salinity) and turbidity (Joseph 1953, Kalle 1953, Becker 1990). The 4 main fronts are North Atlantic water (high salinity, nutrient-poor, low turbidity), Atlantic Channel water (high salinity, warm, nutrient-poor, low turbidity), UK coastal water (medium salinity, nutrient-rich, high turbidity) and continental coastal water (low salinity, nutrient-rich, high turbidity). As a result of the differences in density of these waterbodies, their mixing is limited. The waterbodies are characterised by differences in current direction and speed, depending on water inflow, wind direction and wind speed. These may be factors that have an influence on the spatial distribution of hyperpigmentation. Considering the spatial patterns in prevalence (Figs. 1 & 2), it is obvious that central North Sea areas (N03, N02, P01, P02, N10) were characterised by a lower mean prevalence, similar to that recorded at sites in the Celtic and Irish Seas. However, these low values were still high compared to the prevalence in dab from the western Baltic Sea population (Table 3). The latter population differs from the others in many respects, some of which may be causally involved in the spatial pattern of hyperpigmentation. For example, the western Baltic Sea is characterised by different bottom topography and sediment composition, lower salinity, partly higher contaminants levels and different biodiversity in terms of the number of species and their abundance (Kolp 1966, Voipio 1981, Rheinheimer et al. 1989, Bergström & Carlsson 1993, Kammann 2007, HELCOM 2009). Furthermore, dab from the western Baltic Sea are characterised by a different food spectrum (Arntz 1970, Temming 1989), faster growth (Lozan 1988, Temming 1989) and higher CFs compared to their conspecifics from the North Sea (Baumgart 2007), suggesting that nutrition may play a role in the onset of hyperpigmentation (Grütjen 2012). On the other hand, dab eggs and embryos are pelagic and, in the less saline Baltic Sea, due to their specific weight, they drift in deeper water layers than in the more saline North Sea where they are located closer to the water surface (Nissling et al. 2002). Therefore, the early life stages of North Sea dab may be less protected from an increasing impact of UV-B radiation (due to increasing global ozone depletion), which is known to affect pigmentation in various taxonomic groups, including fish (Blazer et al. 1997, Jokinen et al. 2000, Alemanni et al. 2003). It can therefore not be excluded that increased UV-B radiation is responsible for the increase in prevalence of hyperpigmentation (Noguera et al. 2013). However, considering the spatial distribution of hyperpigmentation in the North Sea with its almost uniform salinity range, there does not seem to be an apparent direct link between salinity, vertical distribution of early life stages of dab, UV-B radiation and hyperpigmentation. If UV-B is effective, it is more likely that it acts in concert with other factors, possibly driven by climate change.

Change in nutrition is a factor possibly linked to climate change and the associated rise in water temperature (Beaugrand 2004) that has also been reported for the southern North Sea (Wiltshire & Manly 2004, Loewe et al. 2005). There is some indication that there have been changes in food composition of North Sea dab over the past 2 decades (Knust 1990, Mintenbeck 2007), and it is known that food composition and nutritional value are critical factors with respect to the development of pigmentation in young flatfish (Kanazawa 1993, Naess & Lie 1998, Estevez et al. 1999), especially the quality of lipid nutrition (McEvoy et al. 1998, Hamre et al. 2007). In fish, as in terrestrial mammals, polyunsaturated fatty acids are involved in maintaining cell membrane structure and function (Sargent et al. 1999), play a critical role in the development and function of neural tissues like brain and eyes and are accumulated by these tissues (Kanazawa 1993, Bell et al. 1995, Koven et al. 2001, Christie 2003, Marszalek & Lodish 2005), and can influence the endrocrine system, both controlling pigmentation (Estevez & Kanazawa 1996, Campos et al. 2010). In addition to the importance of a balanced lipid nutrition, the total amount of lipids influences the growth characteristics in flatfish (Lee et al. 2000, Regost et al. 2001, Borges et al. 2009, Campos et al. 2010), possibly explaining the faster growth and higher condition factors in Baltic dab in combination with the very low prevalence of hyperpigmentation. The finding of significantly lower CFs in severely hyperpigmented dab from the North Sea reveals that food composition (or quantity) may be causally involved. The low CF of hyperpigmented fish possibly reflects a worse nutritional status compared to unaffected fish. However, for more conclusive statements on the role of nutrition, studies on food composition of different life stages of dab with and without hyperpigmentation and an analysis of the fatty acid patterns of dab are needed.

There is evidence of a significant increase in water temperature in the North Sea (Becker & Pauly 1996, MacKenzie & Schiedek 2007), especially in winter temperature (Wiltshire & Manly 2004) and in its southern part (Loewe et al. 2005). The increase in water temperature correlates with the long-term increase in the prevalence of hyperpigmentation and thus the possible existence of a causal link cannot be excluded. Dab belong to the boreal species (Tulp et al. 2008), and the temperature increase in some areas may exceed their adaptation capacities and result in unexpected biological reactions. However, possible mechanisms are at present unknown and more research is required to investigate the relationship. It is interesting to note that in Scottish waters, prevalence decreases in a northerly direction (Fig. 1, Table 3), which could be related to a lower water temperature in the northern North Sea.

Another potential causative factor with an impact on spatial and temporal patterns in hyperpigmentation, and related to climate change, is changes in demography or migratory patterns of dab populations in the North Sea. Since the data presented provide evidence that the prevalence of hyperpigmentation is linked to length and age of the host and is different in size groups of males and females, any change in these demographic entities may have an impact on the spatial and temporal patterns of hyperpigmentation. In fact, the decrease in prevalence recorded in some areas over the last years of the observation period was linked to an increased ratio of small fish in the samples examined, which are in general characterised by a low prevalence of hyperpigmentation. However, when looking at long-term trends since 1988, the data reveal that the mean length of dab in the samples from many of the study areas decreased more or less steadily while the prevalence of hyperpigmentation increased in the same period, indicating that causality with respect to body length is more complex (Baumgart 2007). From the south-eastern North Sea (German Bight and adjacent areas, including area N01) there is indication from stock assessment work for changes in the catch per unit effort data in recent years, revealing a strong increase in the number of fish in the standard catches but, at the same time, only a moderate increase in the weight (A. Sell pers. comm.). This indicates a change in population structure towards a dominance of smaller and younger dab and corresponds to the results of the present study, indicating both a short-term strong increase in the proportion of small dab since 2006 and a long-term continuous decrease in mean length of dab. Since these results were also obtained from other North Sea areas, a general shift in population structure cannot be excluded. The findings are further confirmed by results of a study in Dutch coastal waters, where a decline in fish biomass but a constant total fish density were observed, also indicating an increase in the proportion of small fish in the catches (Tulp et al. 2008). As a result, the prevalence of hyperpigmentation has recently decreased.

A cause of the observed demographic change, possibly affecting the prevalence of hyperpigmentation, could be variation in migration patterns associated with changing climatic conditions (i.e. increasing water temperature) in the North Sea, first affecting shallow and more coastal areas. For adult dab, seasonal migration connected to reproduction in the German Bight was described by Bohl (1957) and Saborowski & Buchholz (1997) and was supported by tagging experiments (Damm et al. 1991).

The strong prevalence increase in area N11 in the period 2000 to 2003 (Fig. 2) could be a result of adult dab migrating from warmer waters in area N01 (German Bight) to colder water in area N11 (Horns Reef, Denmark) as an avoidance reaction. The significant decline in body length in area N01 and the significant increase in body length in area N11 (Table 4) underline the migration hypothesis. Juvenile dab, on the other hand, may prefer warmer waters in area N01 to support faster growth and to avoid food competition. The increased proportion of small fish may also have been due to avoidance migration from even warmer areas closer to the coast, e.g. the Wadden Sea, to the deeper areas in the German Bight (area N01).

As to the effects of hyperpigmentation on the host, there is evidence that it is a chronic and irreversible condition (Grütjen 2012, Noguera et al. 2013). Its prevalence as well as intensity (severity) increase with length and age (the latter at least in young fish; Figs. 3 & 4), indicating a progression of the condition with age. Since the prevalence decreases in the largest and oldest fish, it cannot be excluded that severe hyperpigmentation causes mortality. The finding of decreased CFs in severely affected specimens and the observation that mortality in severely affected dab during maintenance in tanks is higher than in non-affected or less affected specimens (T. Lang unpubl. data) supports this hypothesis. However, other mechanisms, e.g. a higher predation risk, may also lead to a removal of hyperpigmented fish from the population. Length-at-age data indicate that hyperpigmentation is linked to faster growth. This finding is hard to explain, but may be linked to differences in the food spectrum between hyperpigmented and non-affected fish (see above) or a hormesis effect. Nevertheless, more research addressing the causes, pathogenesis and effects of hyperpigmentation is required.

In conclusion, hyperpigmentation in dab is a conspicuous external condition characterised by a marked spatial pattern in the North Sea and adjacent areas and a significant overall increase in prevalence over the past 2 decades. Its underlying causes are yet unknown, but there is indication that the condition may be linked to nutritional factors and/or effects of UV-B radiation on early life stages. Parts of the temporal changes recorded (particularly in recent years) can be explained by changes in population structure, possibly linked to climate change induced migration. Because of its adverse effects on the host and the distinct histological, ultrastructural and partly pathological findings described in detail by Noguera et al. (2013), it seems justified to use the term 'disease' in the context of hyperpigmentation. Further research is required to identify causes and to assess possible consequences of hyperpigmentation in affected dab populations.

Acknowledgements. We thank the officers and crews of the RVs 'Walther Herwig' II and III, 'Scotia', 'Endeavour' and 'Cirolana' for collecting the samples. We also thank the scientific staff onboard the RVs and in the German and UK institutes involved in the national fish disease monitoring programmes for generating the data used for the analysis. Input and information provided by stock assessment experts (including A. Sell, Thünen Institute of Sea Fisheries) is gratefully acknowledged. This study was supported by a grant from the Landesgraduiertenförderung Mecklenburg-Vorpommern.

LITERATURE CITED

Alemanni ME, Lozada M, Zagarese HE (2003) Assessing sublethal effects of ultraviolet radiation in juvenile rainbow trout (*Oncorhynchus mykiss*). Photochem Photobiol Sci 2:867–870

Arntz WE (1970) Das Makrobenthos der Kieler Bucht im Jahre 1968 und seine Ausnutzung durch die Kliesche (*Limanda limanda* L.) [The macrobenthos of Kiel Bight in the year 1968 and its utilisation by the dab (*Limanda limanda L.*)]. PhD thesis, University of Kiel

Baumgart F (2007) Hyperpigmentierung bei Klieschen (*Limanda limanda*) in Nord- und Ostsee: regionale und zeitliche Muster sowie mögliche Ursachen [Hyperpigmentation in dab (*Limanda limanda*) from the North Sea and Baltic Sea: regional and temporal patterns and possible causes]. MS thesis, University of Rostock

Beaugrand G (2004) The North Sea regime shift: evidence, causes, mechanisms and consequences. Prog Oceanogr 60:245–262

Becker GA (1990) Die Nordsee als physikalisches System. In: Lozan JL, Lenz W, Rachor E, Watermann B, Westernhagen H (eds) Warnsignale aus der Nordsee: wissenschaftliche Fakten. Paul Parey, Hamburg, p 11–27

Becker GA, Pauly M (1996) Sea surface temperature changes in the North Sea and their causes. ICES J Mar Sci 53:887–898

Bell MV, Batty RS, Dick JR, Fretwell K, Navarro JC, Sargent JR (1995) Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). Lipids 30:443–449

Bergström S, Carlsson B (1993) Hydrology of the Baltic Basin. Inflow of fresh water from rivers and land for the period 1950–1990. SMHI Rep Hydrol 7:1–21

Blazer VS, Fabacher DL, Little EE, Ewing MS, Kocan KM (1997) Effects of ultraviolet-B radiation on fish: histologic comparison of a UVB-sensitive and a UVB tolerant species. J Aquat Anim Health 9:132–143

Bohl H (1957) Die Biologie der Kliesche (*Limanda limanda*) in der Nordsee [The biology of the dab (*Limanda limanda*) in the North Sea]. Dtsch Wiss Kommis Meeresforsch 15:1–57

Bolker JA, Hill CR (2000) Pigmentation development in hatchery-reared flatfishes. J Fish Biol 56:1029–1052

Bolker JA, Hakala TF, Quist JE (2005) Pigmentation development, defects, and patterning in summer flounder (*Paralichthys dentatus*). Zoology 108:183–193

Borges P, Oliveira B, Casal S, Dias J, Conceição L, Valente LMP (2009) Dietary lipid level affects growth performance and nutrient utilization of Senegalese sole (*Solea senegalensis*) juveniles. Br J Nutr 3055:17–53

Bucke D, Vethaak D, Lang T, Mellergaard S (1996) Common diseases and parasites of fish in the North Atlantic: training guide for identification. ICES techniques in marine environmental sciences no. 19. International Council for the Exploration of the Sea, Copenhagen

Campos C, Valente LMP, Borges P, Bizuayehu T, Fernandes JMO (2010) Dietary lipid levels have a remarkable impact on the expression of growth-related genes in Senegalese sole (*Solea senegalensis*). J Exp Biol 213: 200–209

Cefas (Centre for the Environment, Fisheries and Aquaculture Science) (2009) Monitoring the quality of the marine environment, 2006-2007. Sci Ser Aquat Environ Monit Rep 62. Cefas, Lowestoft. Available at www.cefas.defra.gov.uk/publications/aquatic/aemr62.pdf

Christie WW (ed) (2003) Lipid analysis — isolation, separation, identification and lipidomic analysis. The Oily Press, Bridgwater

Copeman LA, Parrish CC (2002) Lipid composition of malpigmented and normally pigmented newly settled yellowtail flounder, *Limanda ferruginea* (Storer). Aquacult Res 33:1209–1219

Daan N, Bromley PJ, Hislop JRG, Nielsen NA (1990) Ecology of North Sea fish. Neth J Sea Res 26:343–386

Damm U, Lang T, Rijnsdorp AD (1991) Movements of dab (*Limanda limanda*) in the German Bight and Southern Bight: results of German and Dutch tagging experiments in 1988, 1989. ICES CM Pap /E 22, International Council for the Exploration of the Sea, Copenhagen

De Veen JF (1969) Abnormal pigmentation as a possible tool in the study of the populations of the plaice (*Pleuronectes platessus* L.). J Cons Int Explor Mer 32: 344–383

Dethlefsen V, Egidius E, McVicar AH (1986) Methodology of fish disease surveys. Report of an ICES sea-going workshop held on RV 'Anton Dohrn' 3–12 January 1984. ICES Coop Res Rep 140. International Council for the Exploration of the Sea, Copenhagen

Dethlefsen V, Watermann B, Hoppenheit M (1987) Disease of North Sea dab (*Limanda limanda*) in relation to biological and chemical parameters. Arch Fischwiss 37: 107–237

Estevez A, Kanazawa A (1996) Fatty acid composition of neural tissues of normally pigmented and unpigmented juveniles of Japanese flounder using rotifer and *Artemia* enriched in n-3 HUFA. Fish Sci 62:88–93

Estevez A, McEvoy LA, Bell JG, Sargent JR (1999) Growth, survival, lipid composition and pigmentation of turbot (*Scophthalmus maximus*) larvae fed live prey enriched in arachidonic and eicosapentaenoic acids. Aquaculture 180:321–343

Feist SW, Lang T, Stentiford GD, Köhler A (2004) Biological effects of contaminants: the use of liver pathology of the European flatfish, dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring biological effects of contaminants. ICES techniques in marine environmental sciences no. 28. International Council for the Exploration of the Sea, Copenhagen

Gartner JV (1986) Observations on anomalous conditions in some flatfish (Pisces: Pleuronectiformes), with a new record of partial albinism. Environ Biol Fishes 17: 141–152

Grütjen F (2012) Untersuchungen zu Ursachen der Hyperpigmentierung bei Klieschen (*Limanda limanda*) in Nord- und Ostsee [Research into the causes of hyperpigmentation in dab (*Limanda limanda*) in the North Sea and Baltic Sea]. PhD thesis, University of Rostock

Hamre K, Holen E, Moren M (2007) Pigmentation and eye migration in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae: new findings and hypotheses. Aquacult Nutr 13:65–80

Hastie T, Tibshirani R (1990) Generalized additive models. Chapman & Hall, London

HELCOM (Helsinki Commission) (2009) Integrated thematic assessment on biodiversity and nature conservation in the Baltic Sea. Baltic Sea Environment Proceedings 116B. Helsinki Commission, Helsinki

ICES (International Council for the Exploration of the Sea) (1989) Methodology of fish disease surveys. Report of an ICES Sea-going Workshop held on RV U/F 'Argos' 16-23 April 1988. ICES Coop Res Rep 166:1–33

Jokinen EI, Salo HM, Markkula SE, Aaltonen TM, Immonen AK (2000) Effects of ultraviolet light on immune parameters of the roach. Toxicol Lett 112-113:303–310

Joseph J (1953) Die Trübungsverhältnisse in der südwestlichen Nordsee während der Gauss-Fahrt im Februar/März 1952 [Attenuation in the southwestern North Sea during a survey with RV Gauss in February/March 1952]. Dtsch Wiss Kommis Meeresforsch 13:93–103

Kalle K (1953) Der Einfluss des englischen Küstenwassers auf den Chemismus der Wasserkörper in der südlichen Nordsee [The influence of English coastal water on the chemistry of the water bodies in the southern North Sea]. Dtsch Wiss Kommis Meeresforsch 13:130–135

Kammann U (2007) PAH metabolites in bile fluids of dab (*Limanda limanda*) and flounder (*Platichthys flesus*) — spatial distribution and seasonal changes. Environ Sci Pollut Res Int 14:102–108

Kanazawa A (1993) Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. J World Aquacult Soc 24:162–166

Knijn RJ, Boon TW, Heessen HJL, Hislop JRG (1993) Atlas of North Sea fishes based on bottomtrawl survey data for the years 1985–1987. ICES Coop Res Rep 194: 12–68

Knust R (1990) Ernährung der Kliesche (*Limanda limanda* (L.)) in der zentralen und südlichen Nordsee und die Bedeutung des Ernährungszustandes für Erkrankungen dieses Fisches [Nutrition of the dab *Limanda limanda* (*L.*) in the central southern North Sea and implications of the nutritional status on disease of this species]. PhD thesis, University of Bremen

Kolp O (1966) Die Sedimente der westlichen und südlichen Ostsee und ihre Darstellung [The sediments of the western and southern Baltic Sea and their illustration]. Beitr Meereskd 17-18:9–60

Koven W, Barr Y, Lutzky S, Ben-Atia I and others (2001) The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. Aquaculture 193: 107–122

Lang T (2002) Fish disease surveys in environmental monitoring: the role of ICES. ICES Mar Sci Symp 215: 202–212

Lang T, Dethlefsen V, von Westernhagen H (2003) Fischkrankheiten und embryonale Missbildungen. In: Lozan JL, Rachor E, Reise K, Sündermann J, Westernhagen H (eds) Warnsignale aus Nordsee und Wattenmeer: eine aktuelle Umweltbilanz. Wissenschaftliche Auswertungen, Hamburg, p 219–228

Lee SM, Cho SH, Kim KD (2000) Effects of dietary protein and energy levels on growth and body composition of juvenile flounder *Paralichthys olivaceus.* J World Aquacult Soc 31:306–315

Loewe P, Klein H, Schmolke S, Müller-Navarra S and others (2005) Nordseezustand 2003 [North Sea status 2003]. Berichte des Bundesamtes für Seeschifffahrt und Hydrographie (German Federal Maritime and Hydrographic Agency) Nr. 38. Available at www.bsh.de/de/Produkte/Buecher/Berichte_/Bericht38/index.jsp

Lozan JL (1988) Verbreitung, Dichte und Struktur der Population der Kliesche (*Limanda limanda*) in der Nordsee mit Vergleichen zu den Populationen um Island und in der Ostsee anhand meristischer Merkmale [Distribution, density and structure of the populations of dab (*Limanda limanda*) in the North Sea and a comparison with populations around Iceland and in the Baltic Sea by means of meristic characteristics]. Arch Fischwiss 38:165–189

Lozan JL (1992) Sexual differences in food intake, digestive tract size, and growth performance of dab, *Limanda limanda.* Neth J Sea Res 29:223–227

Macieira RM, Joyeux JC, Chagas LP (2006) Ambicoloration and morphological aberration in the sole *Achirus declivis* (Pleuronectiformes: Achiridae) and two other cases of color abnormalities in achirid soles from southeastern Brazil. Neotrop Ichthyol 4:287–290

MacKenzie BR, Schiedek D (2007) Long-term sea surface temperature baselines — time series, spatial covariation and implications for biological processes. J Mar Syst 68: 405–420

Marszalek JR, Lodish HF (2005) Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function. Annu Rev Cell Dev Biol 21:633–657

McEvoy LA, Naess T, Bell JG, Lie O (1998) Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture 163:237–250

McVicar AH, Bruno DW, Fraser CO (1988) Fish disease in the North Sea in relation to sewage sludge dumping. Mar Pollut Bull 19:169–173

Mintenbeck K (2007) Durchführung von Mageninhaltsanalysen von Fischen zur Umsetzung der Ergebnisse des Forschungsprojektes: Identifizierung von organischen Schadstoffen in Nord- und Ostsee [Stomach content analysis in fishes for the application of results of the research project 'Identification of organic contaminants in the North Sea and Baltic Sea']. Project Report to the German Federal Environmental Agency, FKZ 363 01 141; internal report

Naess T, Lie O (1998) A sensitive period during first feeding for the determination of pigmentation pattern in Atlantic halibut, *Hippoglossus hippoglossus* L., juveniles: the role of diet. Aquacult Res 29:925–934

Nissling A, Westin L, Hjerne O (2002) Reproductive success in relation to salinity for three flatfish species, dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and flounder (*Pleuronectes flesus*), in the brackish water Baltic Sea. ICES J Mar Sci 59:93–108

Noguera PA, Feist SW, Bateman KS, Lang T, Grütjen F, Bruno DW (2013) Hyperpigmentation in North Sea dab *Limanda limanda*. II. Macroscopic and microscopic characteristics and pathogen screening. Dis Aquat Org 103: 25–34

OSPAR (Oslo-Paris Commission) (1997) JAMP guidelines for general biological effects monitoring. OSPAR Commission Ref No 1997-7. OSPAR Commission, London

OSPAR (2003) JAMP guidelines for contaminant-specific biological effects monitoring. OSPAR Commission Ref No 2003-10. OSPAR Commission, London

OSPAR (2010) Quality status report 2010. OSPAR Commission, London

Ottesen OH, Strand HK (1996) Growth, development, and skin abnormalities of halibut (*Hippoglossus hippoglossus*) juveniles kept on different bottom substrates. Aquaculture 146:17–25

Otto L, Zimmermann JTF, Furnes GK, Mork M, Saetre R, Becker G (1990) Review of the physical oceanography of the North Sea. Neth J Sea Res 26:161–238

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at www.R-project.org/

Regost C, Arzel J, Cardinal M, Robin J, Laroche M, Kaushik SJ (2001) Dietary lipid level, hepatic lipogenesis and flesh quality in turbot (*Psetta maxima*). Aquaculture 193: 291–309

Rheinheimer G, Gocke K, Hoppe HG (1989) Vertical distribution of microbiological and hydrographic-chemical parameters in different areas of the Baltic Sea. Mar Ecol Prog Ser 52:55–70

Rijnsdorp AD, Vethaak AD, Van Leeuwen PI (1992) Population biology of dab *Limanda limanda* in the southeastern North Sea. Mar Ecol Prog Ser 91:19–35

Saborowski R, Buchholz F (1997) Some observations on the seasonal distributions of dab, *Limanda limanda*, in the southern North Sea. Helgol Meersunters 51:41–51

Sargent JR, McEvoy LA, Estevez A, Bell JG, Bell MV, Henderson RJ, Tocher DR (1999) Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217–229

Temming A (1989) Biologie und Populationsdynamik der Kliesche (*Limanda limanda*) in der Ostsee [Biology and population dynamics of the dab (*Limanda limanda*) in the Baltic Sea]. PhD thesis, University of Kiel

Tulp I, Bolle LJ, Rijnsdorp AD (2008) Signals from the shallows: in search of common patterns in long-term trends in Dutch estuarine and coastal fish. J Sea Res 60:54–73

Venizelos A, Benetti DD (1999) Pigment abnormalities in flatfish. Aquaculture 176:181–188

Voipio A (ed) (1981) The Baltic Sea. Elsevier Oceanographic Series. Elsevier, Amsterdam

Wiltshire KH, Manly BFJ (2004) The warming trend at Helgoland Roads, North Sea: phytoplankton response. Helgol Mar Res 58:269–273

Yamanome T, Amano M, Takahashi A (2005) White background reduces the occurrence of staining, activates melanin-concentrating hormone and promotes somatic growth in barfin flounder. Aquaculture 244:323–329

Yamanome T, Chiba H, Takahashi A (2007) Melanocyte-stimulating hormone facilitates hypermelanosis on the non-eyed side of the barfin flounder, a pleuronectiform fish. Aquaculture 270:505–511

Editorial responsibility: Bernd Sures, Essen, Germany

Submitted: May 3, 2012; Accepted: October 31, 2012
Proofs received from author(s): March 4, 2013