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Report

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The Dutch Contribution to PLACES (the plaice and cod egg survey of the North Sea in 2004)

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Summary

The Netherlands participates in the ICES Planning Group on North Sea Cod and Plaice Egg Surveys (PGEGGS) and has contributed substantially to the Plaice and Cod Egg Survey (PLACES) carried out in 2004. The main objectives of this survey were to map the spawning grounds of plaice and cod. Genetic techniques were used to distinguish between cod, haddock, whiting and saithe eggs, which have overlapping distributions in egg diameter. Plaice were found to spawn in similar locations as described in the past, although with relatively more spawning in the German Bight. Cod were also found to spawn in previously documented locations, although with relative less spawning in the Southern Bight. No relative increase in spawning in the northern North Sea was found, which is in contrast to the expectations based upon the distribution of mature fish from research vessel surveys.

Samenvatting

Nederland neemt deel aan de ICES Planning Group on North Sea Cod and Plaice Egg Surveys (PGEGGS) en heeft een belangrijke bijdrage geleverd aan de internationale schol en kabeljauw eisurvey van 2004 (PLACES). De belangrijkste doelstellingen van deze survey waren het in kaart brengen van de paaigronden van schol en kabeljauw. Genetische technieken zijn gebruikt om onderscheid te maken tussen de eieren van kabeljauw, schelvis, wijting en koolvis, die overlappende eidiameter-verdelingen hebben. Schol paait in dezelfde gebieden als in het verleden gerapporteerd, maar de paaiactiviteit in de Duitse Bocht lijkt relatief te zijn toegenomen. Kabeljauw paait ook in de gebieden die voorheen bekend stonden als paaigronden, maar met een relatieve afname van de paaiactiviteit in de Zuidelijke Bocht. Er is geen toename in paaiactiviteit waargenomen in de noordelijke Noordzee, hetgeen in strijd is met de verwachting op grond van de verspreiding van volwassen vis zoals waargenomen in onderzoeksreizen.

1. Introduction

There is currently considerable concern about the health of some North Sea fish stocks, in particular cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*). Under the Common Fisheries Policy, stocks assessed as being below precautionary reference points must be managed under recovery plans designed to allow the population to rebuild to sustainable levels. A Scientific Expert Conference related to the Fifth North Sea Conference (Bergen, 20-22 February, 2002) recommended as one of their short-term high priority areas for research that spawning grounds of commercial fish be mapped and monitored and this forms one element of development of an ecosystem-based approach to fisheries management. The requirement for this information has also been noted by the ICES Regional Ecosystem Study Group for the North Sea (ICES 2003).

Information on spawning can to some extent be estimated from the catches of mature adults but more precise data can be provided from egg surveys. This is because spawning may occur in regions that are not accessible to fishing gear and unlike adult fish, eggs do not actively avoid sampling gears. Despite the obvious importance of spawning in the life cycle of marine fishes there has never been a co-ordinated attempt to survey the ichthyoplankton of the whole North Sea. Previous studies have focussed on particular sectors e.g. the southern Bight and southern North Sea and composite maps of spawning locations have been derived by joining these fragments together or inferred from information on the distribution of mature fish.

1.1 International planning group and survey

ICES established the Planning Group on North Sea Cod and Plaice Egg Surveys (PGEGGS) in 2001. Participants in PGEGGS include institutes from England (CEFAS), The Netherlands (RIVO), Denmark (DIFRES), Norway (IMR), Germany (IFM) and Scotland (FRS). PGEGGS coordinated the plaice and cod egg survey carried out in 2004 with the following aims:

a) Investigating all areas of the North Sea for the distribution of cod and plaice eggs

b) Identifying and delimit areas with high concentrations of cod and plaice eggs

c) Tracing the sites of intensive cod and plaice spawning based on distributional information of egg stages and larval sizes.

d) To attempt to estimate egg production of plaice for regions where there is sufficient survey coverage (more than one survey covering the same region is required for this)

e) To describe where possible the distribution pattern of eggs/larvae of non-target species.

The survey itself was named PLACES (Plaice and Cod Egg Survey) to distinguish it from the activities of the planning group itself. Much of this report is based on the work of PGEGGS described in Fox et al. (2005a) and the PGEGGS reports (ICES 2003, 2004, 2005a).

1.2 Dutch contribution

The Dutch contribution to PLACES was funded by the Ministry of Agriculture, Nature and Food quality with the specific objectives:

(1) To create a map of cod spawning based on the distribution of stage 1 eggs.

(2) To create a map of plaice spawning from the distribution of stage 1 eggs.

(3) To further develop procedures for genetic determination of species.

The progress report delivered in July 2004 (Bolle et al., 2004) presented the preliminary results of the Dutch cruises only. The goals listed above could only be achieved by international collaboration. Therefore this final report integrates all data collected within the international PLACES/PGEGGS framework. The Dutch contribution has been of paramount importance for the success of the 2004 cod and plaice egg survey. The Netherlands collected and analysed 370 (38%) of all 973 samples and, together with Germany, covered the southern North Sea and eastern English Channel from late December 2003 to early March 2004.

This report primarily focuses on the Dutch objectives listed above, but also presents the spatial distributions of stage 1 eggs of whiting and haddock, which have been distinguished from cod eggs by genetic analysis. The distribution maps of other species and stages will be presented in the PGEGGS report to the ICES Living Resources Committee planned for June 2006.

The temporal coverage of the ichthyoplankton surveys in the southern North Sea enables an estimate of the egg production for plaice for 2004. These results are being worked up at present and will be presented in 2006 in the form of an ICES paper and/or a peer-reviewed paper.

2. Methods

2.1 Sampling

Because of the scale of the North Sea, covering this area in a survey conducted by one country is not feasible. The North Sea was therefore divided into sectors to be surveyed by different countries (Figure 1). Dedicated PLACES cruises were planned to coincide with spawning activity based on historical information about the timing of spawning. Additional sampling was also undertaken opportunistically on a number of cruises (International Herring Larval Survey and German GLOBEC) to improve the temporal and spatial coverage. The survey plans requested participants to attempt to standardise sampling gear on the Gulf VII design but because of logistic and financial constraints, a wider variety of gears was employed (Table 1). Plankton samplers were also fitted with CTDs to collect environmental data. Calibrations for the CTDs followed the in-house protocols of each institute. At each station, plankton was collected using a sampler deployed in a double-oblique manner to within 2 - 5m of the seabed at a towing speed of 3 - 4.5 knots. On shallow stations, multiple oblique tows were undertaken to ensure that a sufficient volume of water was filtered. On deep stations the samplers were deployed down to 100 m. Care was taken to ensure smooth dive profiles, filtering the same volume of water per unit depth.

2.2 On-board sorting

On non-PLACES dedicated cruises the whole plankton sample was fixed in 4% formalin [4% formaldehyde in distilled water buffered with 2.5% sodium acetate trihydrate (w/v)] for subsequent laboratory sorting. Gene-probe based identification of fish eggs cannot be reliably undertaken on formaldehyde fixed material so these cruises were used to provide data on plaice egg distribution only (Fox et al. 2005b).

On dedicated PLACES surveys it was planned to use a genetic method to positively identify the early stage cod-like eggs as those of cod, haddock or whiting. This method currently requires that cod-like eggs are pre-sorted from the fresh plankton sample into 70% ethanol which preserves high-quality DNA. Upon recovery, the plankton samples were immediately examined and 'cod-sized' eggs were removed. These eggs were measured using interactive image-analysis systems or calibrated eye-piece graticules. Eggs falling in the size range 1.1 to 1.75 mm diameter and not possessing oil globules or other characteristic features (such as the segmented yolk of sprat eggs, *Sprattus sprattus*) were classified as 'cod-like'. These eggs were assigned a developmental stage according to Thompson and Riley (1981) and transferred into

individually labelled tubes containing 1.5 ml of ethanol. Ship-board sorting was continued until up to 50-100 eggs had been removed from the sample.

The protocols called for the remainder of the plankton sample to be fixed in 4% acetate buffered formalin and returned to the participating institute for sorting and identification of the ichthyoplankton.

All countries followed this protocol except Denmark. As Denmark deployed bongo-nets, two plankton samples were collected per station. All the material in one cod-end was fixed in 4% formalin for subsequent laboratory sorting whilst cod-like eggs were sub-sampled into ethanol from the other cod-end.

2.3 Laboratory sorting

Fish eggs were subsequently sorted, identified and enumerated on the basis of size and appearance (Russell 1976). All eggs lacking oil globules and between 1.1 mm and 1.75 mm in diameter were classed as 'cod-like' eggs and were measured and assigned to development stage. Plaice eggs were identified on the basis of their size (> 1.75 mm diameter) the absence of a large perivitelline space (as in long rough dab, *Hippoglossoides platessoides*). Eggs lacking oil globules and smaller than 1.1 mm diameter were enumerated but not staged. On stations where there was a very high abundance of eggs smaller than 1.1 mm diameter, a Folsom splitter could be used for sub-sampling the smaller eggs (UNESCO 1968).

With regard to larvae, participants were requested to sort, identify, enumerate and measure all larvae using criteria from Russell (1976). Due to inexperience in identifying fish larvae, the Netherlands sorted out but did not identify larvae, with exception of the clupeid larvae collected during the Herring Larvae Surveys. Rather than investing a lot of time in acquiring expertise in larval identification, the Netherlands choose to prioritise increasing sampling levels. The remainder of the Dutch samples have been sent to DIFRES (Denmark) for identification of the larvae. The larval data are not presented in this report, but will be subject of a later ICES report or paper.

Plankton samples were sorted and identified by in-house staff except for material collected by Denmark which was analysed by the Institute of Oceanology, Sopot, Poland. Results were electronically entered using a standard data entry program and then collated into a central ACCESS database.

Table 1 Cruise dates and gear deployed

Country	Ship	Cruise PLACES ID (National ID)	Cruise type	Start	End	Hauls made	Gear	Plankton analysed	Cod-like eggs pre-sorted for geneprobes
Netherlands	Tridens II	4	Herring larval	15/12/03	18/12/03	77	Gulf III, 20 cm opening*, 270 μm mesh	All eggs	No
Netherlands	Tridens II	1	PLACES	12/01/04	16/01/04	66	53 cm Gulf VII, 28 cm opening, 270 μm mesh	All eggs	Yes
Germany	Alkor	1 (233)	GLOBEC	09/01/04	19/01/04	108	53 cm Gulf VII, 28 cm opening*, 270 μm mesh	Plaice eggs only	No
Netherlands	Tridens II	5	Herring larval	19/01/04	23/01/04	92	Gulf III, 20 cm opening*, 270 μm mesh	All eggs	No
Scotland	Scotia	2	IBTS	21/01/04	13/02/04	48	Ring-net, 48 cm opening*, 250μm mesh	All eggs and larvae	Yes
Netherlands	Tridens I	2	PLACES	11/02/04	16/02/04	69	53 cm Gulf VII, 28 cm opening*, 270 μm mesh	All eggs	Yes
Germany	Heinke	1 (203)	PLACES	18/02/04	19/02/04	52	Bongo, 60 cm opening*, 500 μm mesh	All eggs > 1.1 mm, all larvae	Yes
England	Corystes	1	PLACES	18/02/04	08/03/04	138	76 cm Gulf III, 40 cm opening*, 270 μm mesh	All eggs and larvae	Yes
Netherlands	Tridens II	3	PLACES	01/03/04	04/03/04	66	53 cm Gulf VII, 28 cm opening*, 270 μm mesh	All eggs	Yes
Denmark	Dana	3	PLACES	25/02/04	06/03/04	104	Bongo, 60 cm opening*, 330 μm mesh	All eggs, cod, plaice and sandeel larvae	Yes
Norway	Haakon Mosby	1 (606)	PLACES	08/03/04	23/03/04	99	Gulf III, 20 cm opening*, 330 μm mesh, Seabird CTD	All eggs and larvae	Yes
Germany	Alkor	2 (236)	PLACES	06/04/04	08/04/04	54	Bongo, 60 cm opening*, 500 μm mesh	All eggs > 1.1 mm, all larvae	None found

*Opening size indicates diameter of the sampler mouth

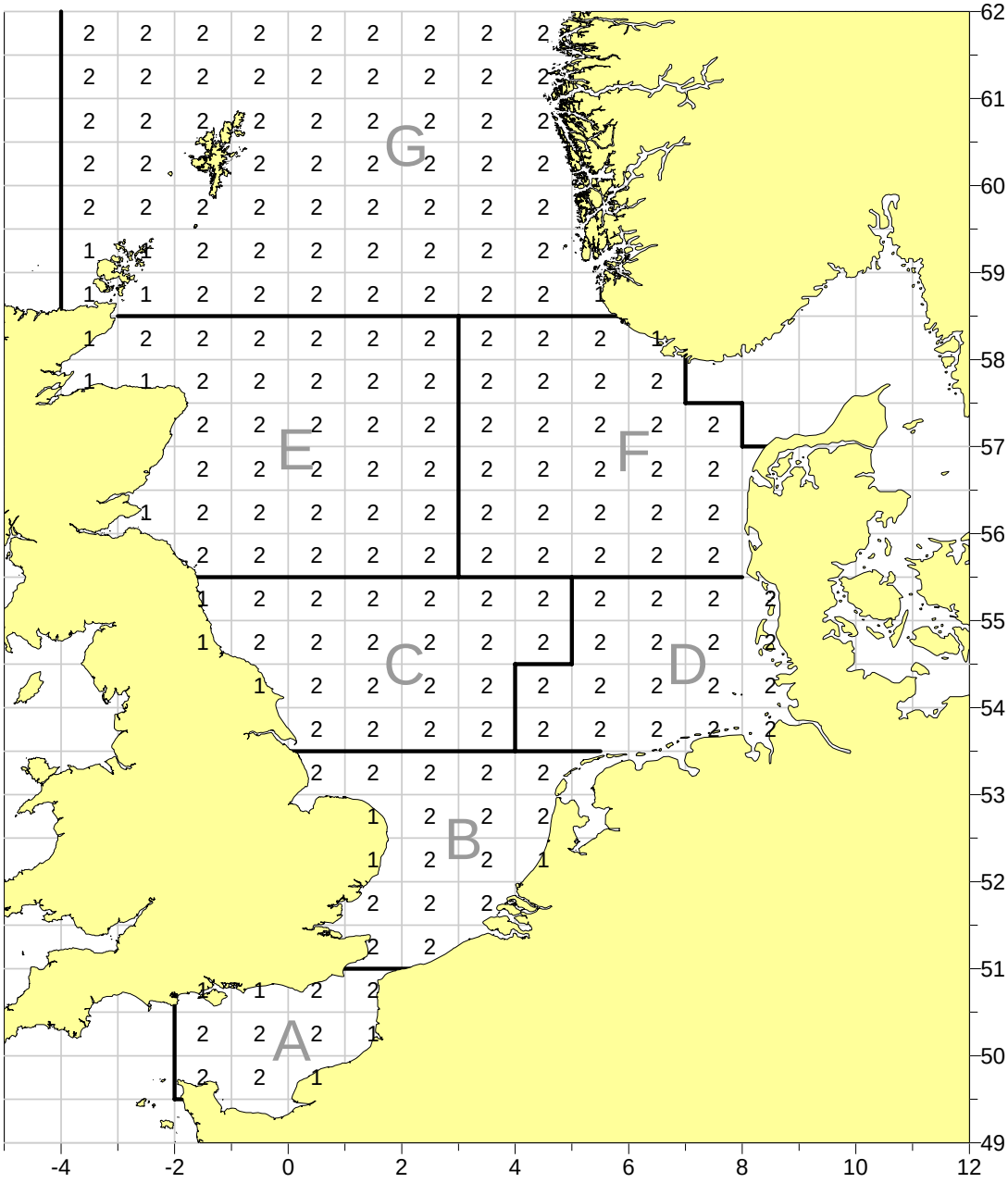

Figure 1. Division of the North Sea into regional sectors for the purposes of the PGEGGS/PLACES survey. The numbers indicate the planned number of plankton hauls per ICES rectangle. Sectors A + B + D: Netherlands and Germany; Sector C: Netherlands and England; Sector E: England; Sector F: Denmark, Sector G: Norway.

2.4 Genetic analysis

The early-stage eggs of cod are visually indistinguishable from those of haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*) and saithe (*Pollachius virens*). Because of this previous surveys have often only analysed late-stage eggs or have assumed that all the eggs falling into a particular size fraction were of one species. Biochemical methods have been suggested as one way around these problems and iso-electric focussing has been employed to distinguish 'cod-like' eggs in the Irish Sea (Heffernan et al. 2004). However, in practical terms the IEF method has some limitations since samples must be stored frozen or analysed immediately on board ship. In an attempt to improve molecular identification, Taylor et al. (2002) developed a highly sensitive TaqMan DNA-based method for identifying cod-like eggs and this method was employed to analyse samples from the 2004 ichthyoplankton survey.

Eggs for genetic typing were transported to the CEFAS Lowestoft laboratory (UK). Stage I eggs from hatchery spawned cod and haddock were embedded in the ethanol-preserved egg series collected at sea as blind standards for the genetic identification method. The genetic samples were then moved to the University of East Anglia where all the TaqMan analyses were performed.

The technical details of the genetic (TaqMan) method for distinguishing eggs of cod, haddock and whiting are described in Taylor et al. (2002) and previous application in the Irish Sea in Fox et al. (2005b). In summary, the technique is a PCR monitored in real-time by the release of up to three, fluorogenic dyes with unique emission spectra in a multiplex reaction. Each dye is linked to a species-specific probe and a quencher so that the release of the dye from the quencher results in an increase in detection signal proportional to the rate of amplification for that specific probe.

2.5 Treatment of the data

Absolute numbers of eggs were converted to numbers per cubic metre using estimates of the volumes of water filtered at each station derived from flow-meters carried on the plankton samplers. Numbers per cubic metre were then converted to numbers per m^2 of sea-surface by multiplying by the depth sampled. Flow-meter calibrations were based on in-house procedures for each institute. The data were then filtered to include only eggs at the early developmental stage (stage I).

The total abundance of stage I cod-like eggs was determined as the sum of those in the bulk formalin fixed fraction and the stage I eggs pre-sorted for genetic analysis on board (except for the Dana cruise where a bongo-net was used and eggs for genetic typing were sub-sampled from the second cod-end). At each station, the abundance of 'cod-like' eggs was apportioned

between cod, haddock, whiting by determining the ratio of each species by area by a GLM (multinominal GLM) and applying the spatial ratios to the un-speciated eggs. Because low numbers of stage I eggs were sub-sampled for genetic typing on some stations, the proportion of species at each station was calculated using TaqMan results from all the eggs sub-sampled at that station (Stages 1 through 5).

Maps of the results were prepared as bubble-charts using Surfer 8 and scaling the symbol area to be proportional to the egg abundance (Golden Software Incorp., Colorado, USA). For each species, the abundance estimates (numbers per m^2) of all cruises were combined to create one composite map.

2.6 Quality control

The accuracy of the pre-sorting undertaken at sea was assessed by comparison of frequency distributions of developmental stages for pre-sorted and laboratory-sorted eggs.

The reliability of the TaqMan egg identification method was assessed by consideration of percentages of blind-labelled standards samples positively identified and producing 'null' or 'failed' reactions.

Unfortunately, due to time and cost restraints it was not possible to carry out any inter-laboratory calibration of sorting, species identification and staging, despite the fact that PGEGGS acknowledged the necessity hereof (ICES 2003). Within RIVO, calibrations were carried out prior to the plankton analysis and quality controls were carried out after the plankton analysis were completed.

3. Results

3.1 Data collection

Sampling

All planned cruises were completed and it total 973 plankton hauls were made (Table 1). In some cases fewer stations were worked than planned owing to poor weather but good spatial and temporal coverage was still attained.

In terms of standardisation of sampling gears, Gulf VII high-speed samplers were employed on cruises undertaken by England and Netherlands. Norway had planned to borrow a Gulf VII but this failed to arrive in time so a Gulf III was used instead. Other cruises employed either bongo nets or Gulf IIIs, usually this gear was prescribed by other research programs on which PLACES sampling was piggy-backed, i.e. the choice of sampler was not at the discretion of PGEGGS. Volumes filtered by oblique-hauled samplers ranged from 6.3 to 848 cubic metres. As expected the larger gears filtered greater volumes of water and the Gulf VII sampler fitted with a 40 cm nosecone (Corystes) had the highest mean volume per opening area due to the venturi effect of the sloped nosecone (Brander et al. 1993, Nash et al. 1998). Tow lengths were not reported by all countries but in some cases may have been less than the minimum of 15 minutes recommended by the PLACES protocol on shallow stations. The vertical hauled ring-net (used only on Scotia cruise) sampled 8.7 – 14.9 cubic metres. This was considered to be too low a volume to provide meaningful ichthyoplankton abundance data and the data were excluded from further analyses.

Egg sorting and identification

All participants completed the required analysis of 'cod-like' and plaice eggs (Table 1). Some countries did not sort and enumerate eggs lacking oil globules and smaller than 1.1 mm in diameter but these would be of non-commercial or lower value species such as dab (*Limanda limanda*) and flounder (*Platichthys flesus*). These species were not a high priority for this survey.

Inter-laboratory calibrations and quality controls of sorting, identification and egg staging were not feasible. Within RIVO quality control showed an overall agreement of 93% in species identification. Figure 2 shows the bias in egg staging for all egg readers. Overall agreement in egg staging was 71% for all stages. For stages 1A and B overall agreement was 64 and 67% respectively.

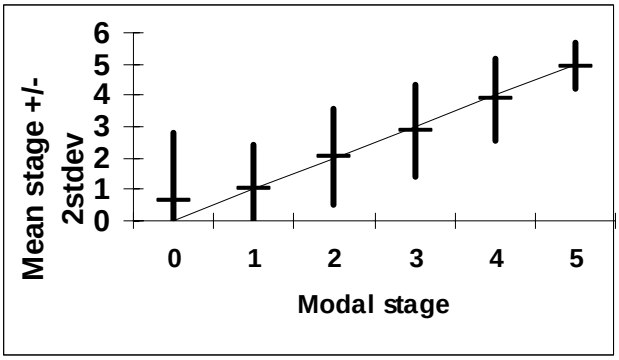

Figure 2. Estimated mean egg stage versus modal egg stage indicating a slight bias in the staging of stage IA eggs (in graph stage 1A =0, stage 1B=1, stage 2=2 etc.).

Genetic identification of cod-like eggs

Over the whole survey 9212 'cod-like' eggs were pre-sorted for subsequent genetic identification. The survey protocol called for cod-like eggs to be sub-sampled on every station. However, some stations were under-sampled. Generally on stations with a low to medium abundance of 'cod-like' eggs (< 500 eggs), participants were able to pre-sort more than 10% of the eggs from the total available for subsequent TaqMan analysis and on stations where cod-like eggs were very abundant up to 100 eggs were sub-sampled representing between 1 - 10% of the eggs available. The level of sub-sampling achieved on the Dana cruise was somewhat lower and this sector contained several stations where 'cod-like' eggs were found in the bulk formalin preserved sample but where eggs were not sub-sampled for genetic identification.

The frequency distribution of developmental stages in the pre-sorted sub-samples and in the remainder of the total plankton sample was generally in good agreement (Figure 3) although there appears to have been a bias towards pre-sorting too many later stage eggs. This is presumably because they are more visible due to the pigmented embryo and easier to pick out from the bulk plankton at sea. These results support the conclusion that pre-sorting at sea is capable of producing a representative sub-sample from the total eggs present providing extra care is taken to avoid under-sampling the transparent stage I eggs (Fox et al. 2005b). However because of the under-sampling of early stage eggs for gene-probing on some stations and the cost of genetic-identification, it would be preferable to focus all the pre-sorting and gene-probing effort on Stage I eggs in future studies aimed at mapping spawning areas. The exception would be if the abundance of later stages were required for determination of egg mortality rates (Dickey-Collas et al. 2003). If one wished to examine planktonic drift of eggs this could be determined by comparing stage I distributions determined using molecular identification with stage V distributions from conventional methods since by this stage cod and haddock can generally be identified from embryonic pigmentation.

Table 2. Reliability of TaqMan identification method based on analyses of blind-labelled hatchery spawned cod and haddock eggs.

	Geneprobe identity (number of eggs and percentage of total)			
	WHG	COD	HAD	Total
True identity				
COD	1 (0.33)	292 (96.37)	5 (1.65)	303
HAD	0	5 (1.61)	305 (98.07)	311

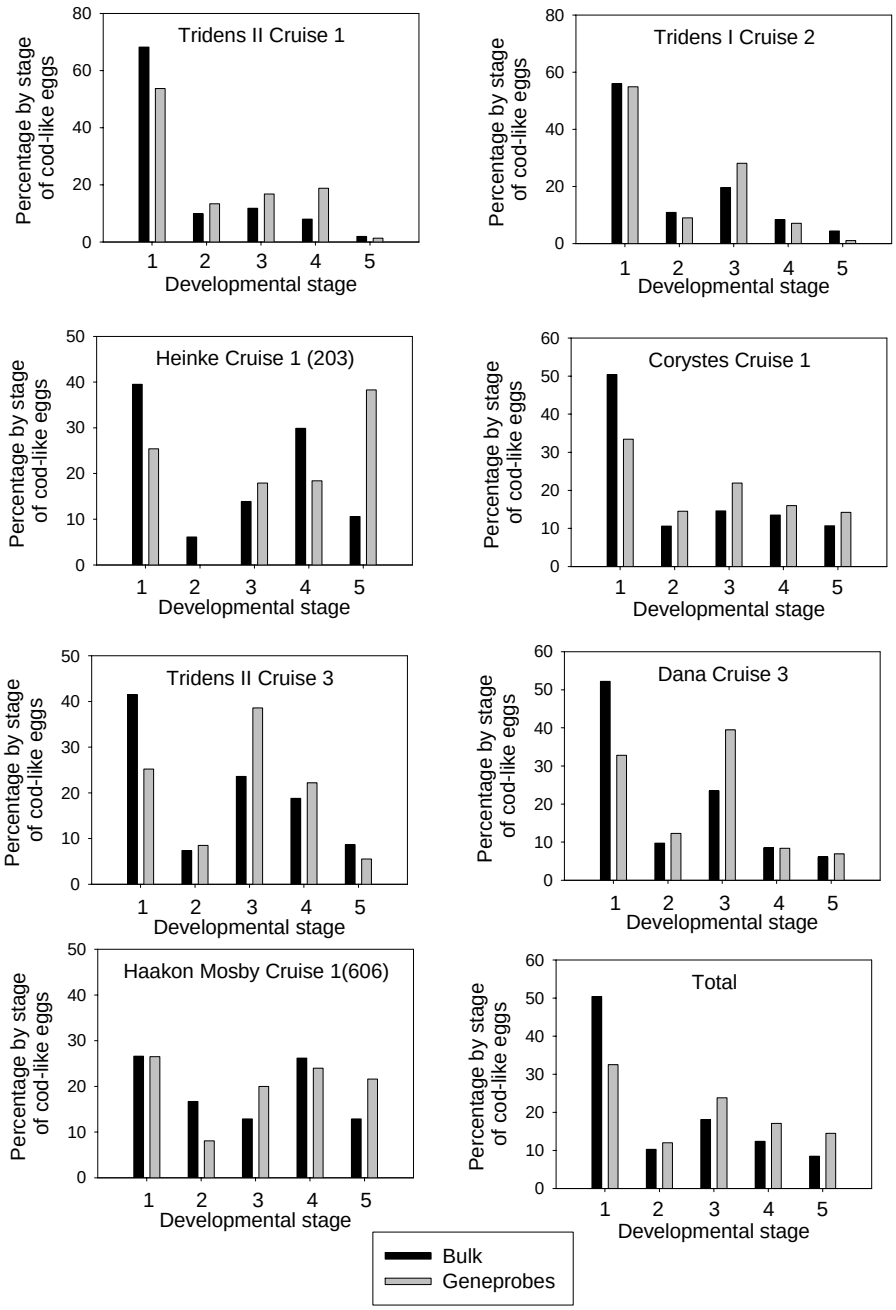

Figure 3. Comparisons of the distribution of cod-like eggs by developmental stage within the bulk formalin fixed portion of the plankton samples and in the sub-sample preserved in ethanol for subsequent genetic identification.

The results of TaqMan analysis of the 614 hatchery-spawned cod and haddock eggs included as blind standards indicated that although there was a small amount of cross-talk the TaqMan method has an accuracy in identification of > 95% (Table 2). Hatchery-spawned whiting eggs were not available for this study but should be included in any future application of the TaqMan or similar genetic-typing technique.

Of the 9212 'cod-like' eggs pre-sorted at sea around 5% were lost before processing and these records were re-allocated into the bulk fraction of the samples. In total 8865 eggs were analysed using the TaqMan. Of these, 32.4% were stage I, 12.1% stage 2, 23.9% stage 3, 17.1% stage 4 and 14.6% stage 5. Thus over the whole survey, 2872 stage I eggs were genetically identified.

The size range of field-sampled cod eggs (all stages) positively identified by TaqMan was 1.28 - 1.63 mm (95 percentiles of size distribution) and for haddock was 1.19 - 1.62 mm. There was an indication that a few cod and haddock eggs might be found below the 1.1 mm diameter cut-off used in this study but it is likely that these would be very limited in number (Figure 4). As expected the size ranges of cod and haddock eggs overlapped almost completely and were in good agreement with those quoted in Russell (1976). The maximum size of positively identified whiting eggs, 1.83 mm, was larger than literature data suggest, Russell (1976) quotes a min-max size range of 0.97 - 1.32mm. The data show that there was a considerable overlap between larger whiting eggs and smaller cod and haddock eggs. Because TaqMan probes have so far only been developed for cod, haddock and whiting, the assay can produce negative results due to the presence of eggs of other species. Eggs falling into this category tended to lie at the lower end of the size range (1.1 – 1.75 mm) pre-sorted at sea. The DNA from a few of these eggs was sequenced and they were identified as saithe (*Pollachius virens*). Over the whole survey, 17.6% of the 8865 'cod-like' eggs identified by TaqMan were cod, 48.3% were haddock, 22.2% whiting and 12.0% other species (Table 3). These ratios of species were applied across all samples (via the multi-nominal GLM) to obtain the distribution of stage 1 cod eggs.

Table 3. Frequency of species identified by TaqMan genetic probes across the whole survey

Stage	Species	Cod	Haddock	Whiting	Others	Total
Stage 1	n	533	1134	870	337	2874
	%	18.5	39.5	30.3	11.7	
Stage 2	n	243	492	223	111	1069
	%	22.7	46.0	20.9	10.4	
Stage 3	n	402	959	515	239	2115
	%	19.0	45.3	24.4	11.3	
Stage 4	n	192	853	236	235	1516
	%	12.7	56.3	15.6	15.5	
Stage 5	n	186	841	122	141	1290
	%	14.4	65.2	9.5	10.9	
Total	n	1157	4279	1966	1063	8865
	%	17.6	48.3	22.2	12.0	

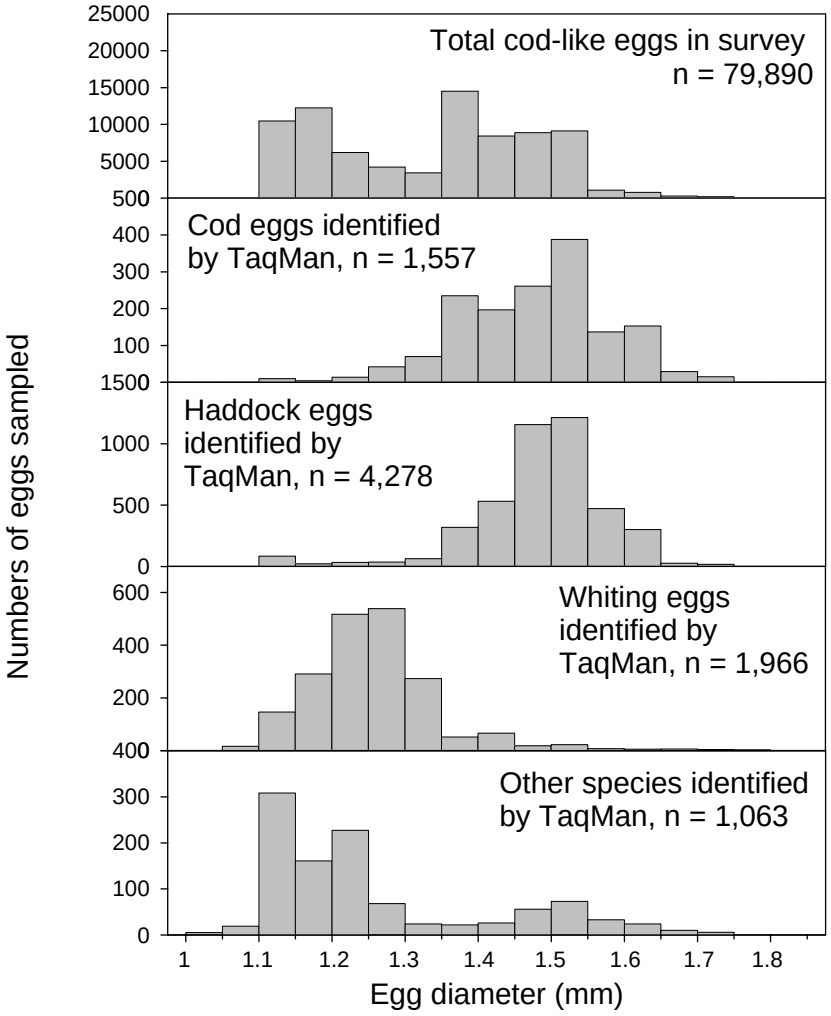

Figure 4. Size frequency distributions of the eggs positively identified using TaqMan probes compared with the size frequency distribution of cod-like eggs in the bulk plankton samples fixed in 4% formalin

3.2 Distribution of stage I plaice eggs

A composite map (Figure 5) of stage I plaice egg distributions was generated by combining data from six cruises: Alkor Cruise 1, Tridens-I Cruise 2, Heinke Cruise 1(203), Corystes Cruise 1, Dana Cruise 3 and Haakon-Mosby Cruise 1 (606). Other cruises either found very low numbers of plaice eggs or duplicated coverage of the selected cruises. Data from Sector F (Dana Cruise 3) are currently subject to revision as initially relatively high numbers of eggs from this sector were identified as plaice but comparison with results from the adjacent sector E suggested that the majority of these may have been mis-identified eggs of long-rough dab (*Hippoglossoides platessoides*). The original Dana data have been reduced in magnitude by a factor of 90% based on proportions of these two species in sector E at similar latitudes pending re-examination of the plankton samples.

Although this composite gives excellent spatial coverage, the timing of the more northerly cruises may have been a little late to capture the peak of plaice egg production. Based on extensive historical data from the Southern Bight, Simpson (1959) reported that the peak of egg production occurred in January or early February and based on more limited data Simpson (1959) stated that all the northern spawning areas were active by February. The more northerly PLACES surveys (Dana cruise 3, 25 Feb – 6 March, and Haakon Mosby Cruise 1, 8 – 23 March) would therefore probably have caught the end of any spawning although Simpson (1959) reported that plaice eggs could be found off the Moray Firth until May.

The composite map (Figure 5) shows remarkable similarity to results presented by (Simpson 1959) based on surveys from the 1930s to 1950s except for the more northward extension of spawning around the eastern end of the Dogger Bank (Figure 6). According to Simpson (1959) this area was not important for plaice spawning in the 1930s – 1950s although earlier reports indicate that prior to 1910 plaice eggs could be found here (Masterman 1911). It is not clear whether these changes have resulted from stock size differences (the stock was apparently low in the 1930s), differences in survey coverage or differences in data treatment. In 2004 the highest concentrations of eggs were found in the eastern channel, German Bight and southern edge of the Dogger Bank. North of the Dogger Bank plaice eggs were scarce excepting isolated patches off Flamborough Head, off the Firth of Forth, the Moray Firth and to the east of the Shetland Isles. Again all these areas are well known as plaice spawning grounds from historical records. The long-term stability of plaice spawning locations appears to be a common feature with similar results being found for the Irish Sea (Fox et al. 2000). This stability is probably a result of these locations lying at the up-stream ends of predictable hydrodynamic transport routes that carry the eggs and larvae to suitable nursery areas (Cushing 1990, Fox et al. 2005a).

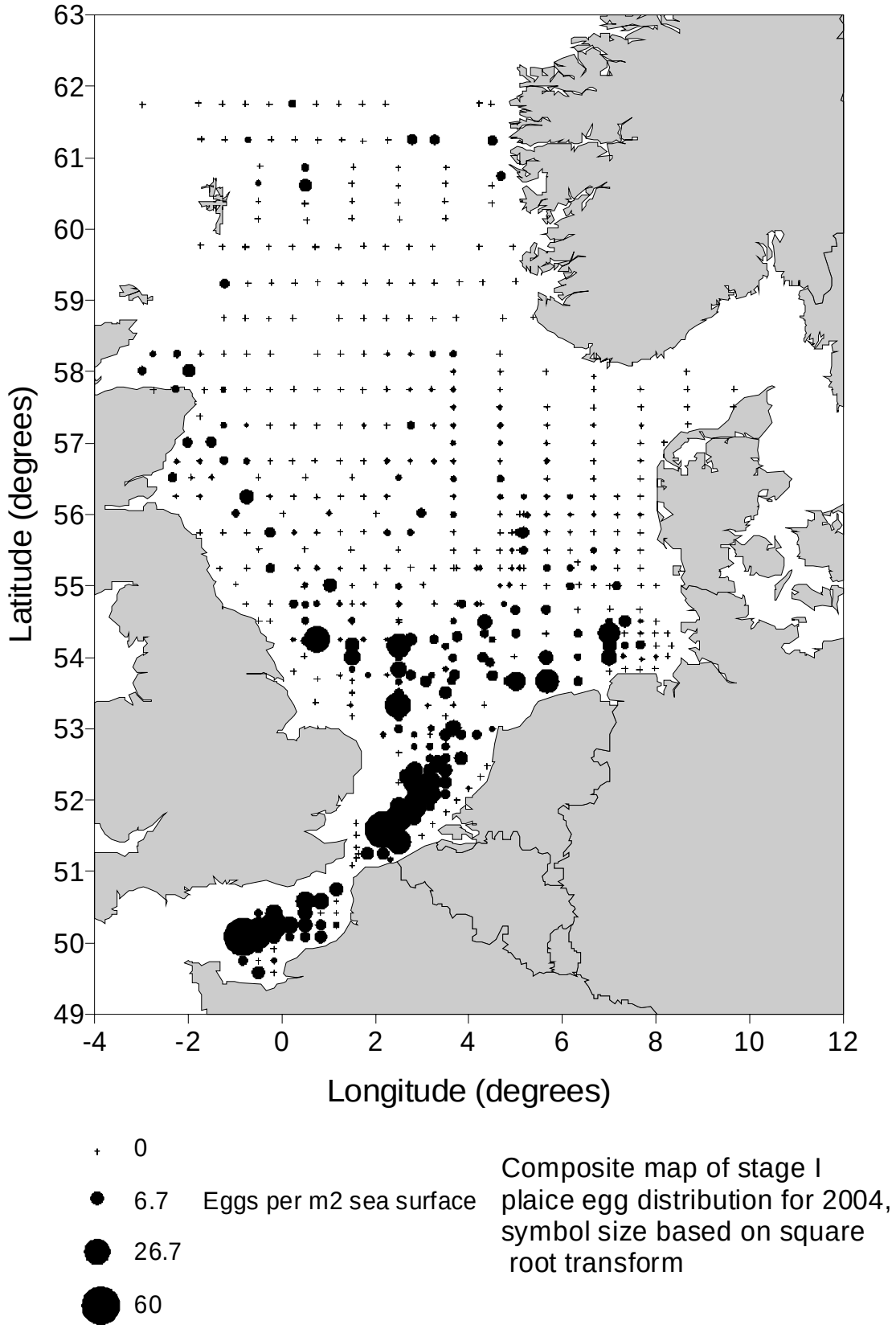

Figure 5. Composite map of the spawning locations of plaice from the 2004 North Sea ichthyoplankton survey. Note that data from sector F are subject to revision (see text).

Another issue to consider is the accuracy of plaice spawning locations determined from egg surveys. At the water temperatures recorded during the ichthyoplankton surveys (3.5-8.5°C) plaice eggs take between 6.5 to 2.5 days to reach the end of stage 1 (Ryland & Nichols 1975). Assuming spawning is relatively continuous the centres of density of stage I eggs should therefore be close to the sites of spawning although up to three days drift and dispersion may have occurred. Simpson (1959) suggested that stage I eggs might drift by up to 25 miles in this time in the southern Bight and dispersal could be greater if conditions were stormy. However, recent work on the dispersal of plaice eggs and larvae in the southern North Sea (Bolle et al., 2005) showed that the displacement during the egg stage is limited.

The additional surveys undertaken, particularly in the southern North Sea, during 2004 will enable an estimate of egg production to be produced for this region. The results are being worked up at present and should be ready in 2006. This will be of value for assessing the status of the North Sea plaice stock for which the spawning stock biomass (SSB) is currently thought to be below the precautionary reference point of 230.000 tonnes based on conventional assessments. The egg production estimate for 2004 can be compared to those for the years 1987-1989 (Heessen and Rijnsdorp 1989, Van der Land 1990, 1991), in which the conventional assessments estimated SSB at a much higher level than at present (ICES 2005b). This comparison provides an evaluation of trends in SSB that is free from the assumptions underlying fisheries-based assessment methods.

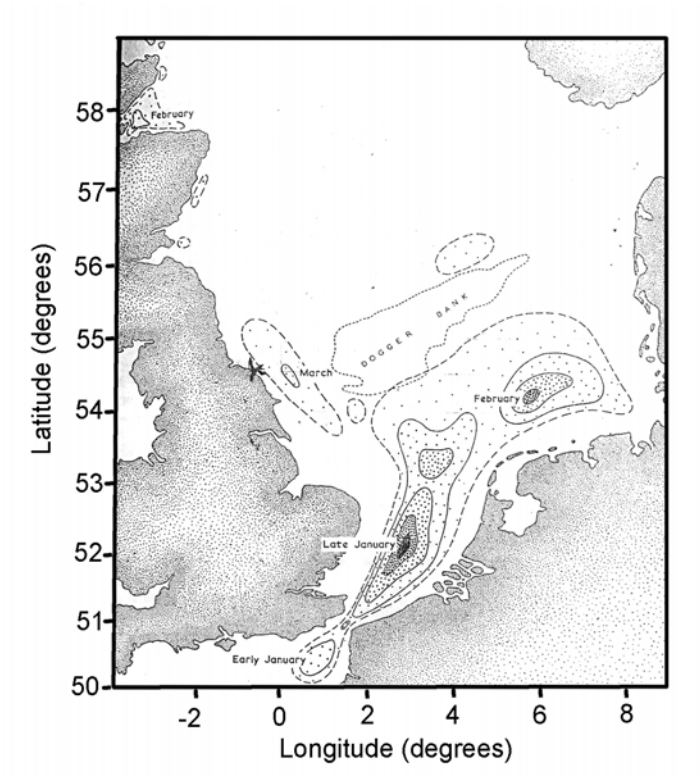

Figure 6. The spawning areas and times of North Sea plaice according to Simpson (1959).

3.3 Distribution of stage I cod eggs

The main concentrations of stage I cod eggs were found around the southern and eastern edges of the Dogger Bank with another patch in the German Bight (Figure 8). This was in accordance with results in Heessen and Rijnsdorp (1989). Rather low abundances of cod eggs were found north of 57°N. In trying to interpret these data one is hindered by the lack of comprehensive historical surveys. However, the pattern for 2004 does bear resemblance to the partial composite produced by Daan (1978) based on data after 1945 (Figure 7). That composite contained an un-surveyed region which was partially covered by Harding and Nichols in 1976 (published in 1987). They found concentrations of stage I eggs off the western edge of the Dogger Bank which were assumed to be cod although our data on size distributions of eggs identified by TaqMan suggest that at least a proportion of these eggs may have been whiting. The 2004 icthyoplankton survey shows a decline of spawning activity in the Southern Bight compared to the historical data, however the spatial and temporal coverage of sampling (suitable for gene-probing) in the Southern Bight was limited.

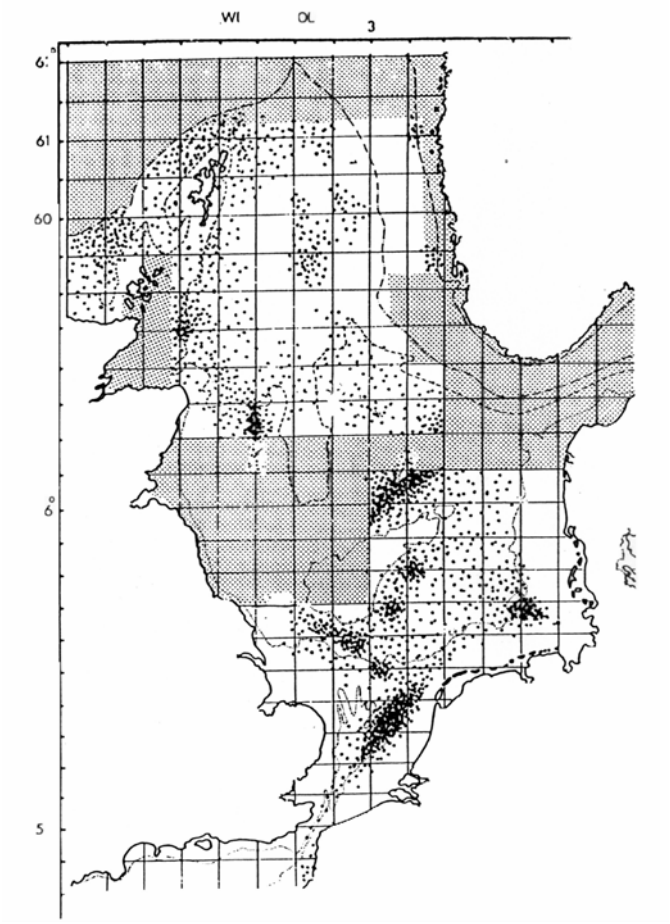

Figure 7. Spawning areas of cod in the North Sea according to information after 1945 from Daan (1978). Note that the shaded areas were not surveyed at the time

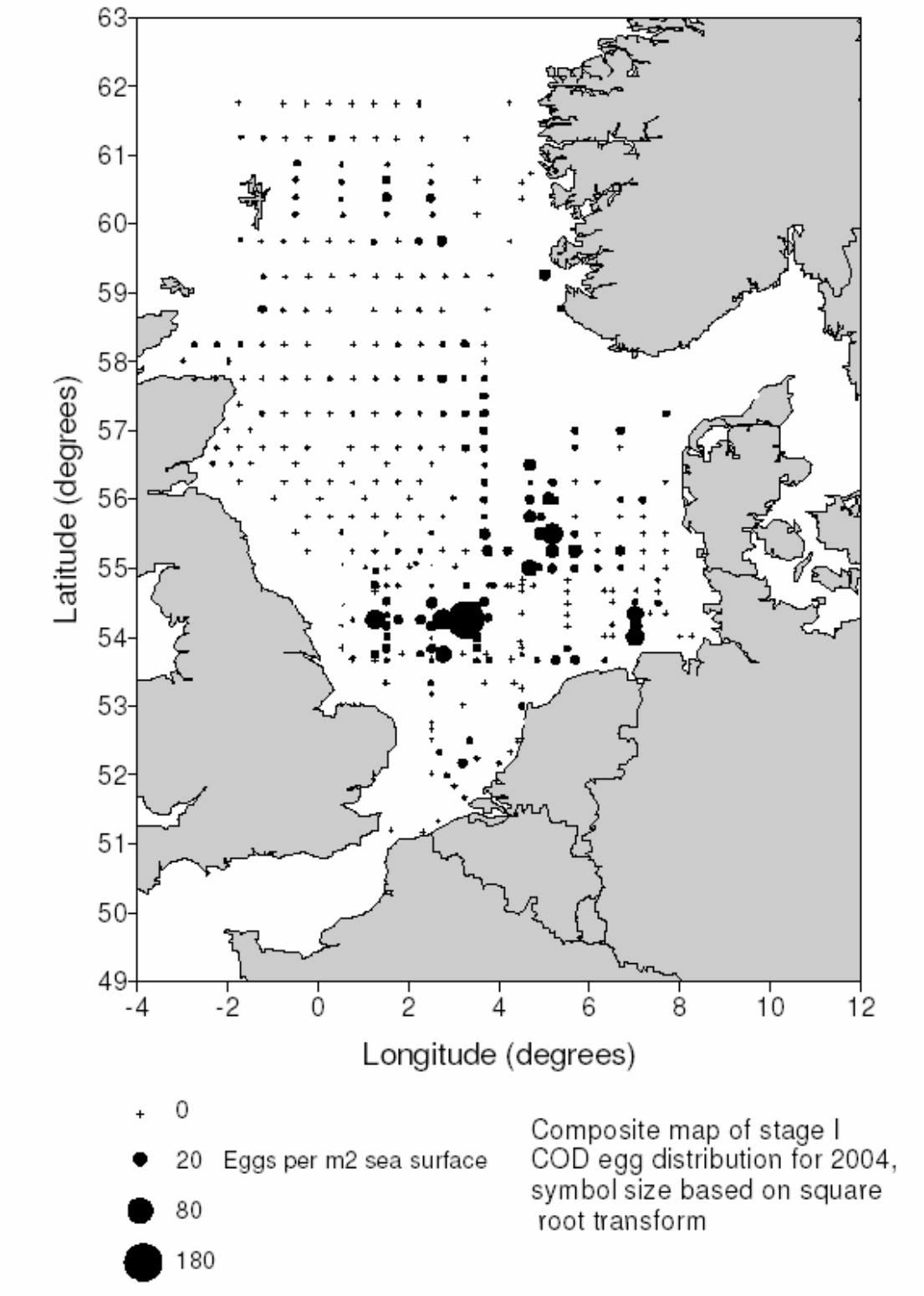

Figure 8. Composite map of the distribution of stage I cod eggs based on the distribution of stage 1 'cod-like' eggs scaled on a station-by-station basis by species proportions determined using TaqMan analysis of all stage 'cod-like' eggs sub-sampled and applied via a mutli-nominal GLM.

Although the North Sea cod is treated as a unit stock for management purposes there is some evidence from genetics and tagging that it may be composed of at least three sub-stocks (Blanchard et al. 2005a). Data from annual trawl surveys conducted in August-September suggests that the summer distribution of cod in the North Sea has shifted north in recent years (Blanchard et al. 2005b, Perry et al. 2005). Since there appears to be a negative link between sea temperatures during the first six months of the year and overall North Sea cod recruitment (O'Brien et al. 2000), it is tempting to think that these distribution changes might also be linked to the recent warming of the North Sea. The analyses by Blanchard et al. (2005b) and Perry et al. (2005) treat North Sea cod as a single stock, but apparent geographic shifts in population abundance could also be the result of changes in sub-stock abundance caused by differential fishing pressure or population dynamics responses of the sub-stocks to environmental change at a local scale.

The 2004 icthyoplankton survey results demonstrate that, despite apparent changes in summer-time cod distributions, the overall spawning pattern does not appear to have changed substantially since at least the 1940s, except perhaps for the Southern Bight. Most findings on North Sea cod distribution in relation to environmental change have used data from surveys conducted in the summer. Cod distribution survey data around the time of spawning (Jan-Feb) is also available from the ICES International Bottom Trawl Surveys. Although results for recent years still show some 3+ cod occurring to the south and east of the Dogger Bank, the bulk of the population (both numerically and in terms of biomass) appears to be north of latitude 57°N. From this information we expected to see higher levels of egg production in the northern North Sea compared with the south, but the 2004 ichthyoplankton survey results do not appear to show this. Care must be taken in comparing spawning levels in the two regions since with a single survey we could have missed the peak of egg production in the north. However, the surveys were designed to coincide with the expected spawning times based on historical data. These results raise questions about where and when the cod in the northern North Sea are spawning. Although it is possible that individual cod could be moving south to spawn there is no evidence for movements of this scale in tagging records (Righton, D., CEFAS pers. comm.). Since the spawning grounds do not appear to have moved in relation to the observed changes in distribution of adult cod in recent years, this might mean that cod spawning areas for specific sub-stocks are geographically fixed to recurrent hydrodynamic patterns or other landscape features. If this is so, specific sub-stocks of North Sea cod may have less flexibility to geographically relocate this stage of their life cycle in response to environmental change than is often supposed for mobile marine organisms. The results also raise questions about whether a high abundance of cod north of 57°N automatically leads to higher egg production (and this potential recruitment) in this region.

3.4 Distribution of stage I haddock and whiting eggs

Abundances of haddock eggs in the 2004 survey (Figure 9) were highest in the north-western to central-northern North Sea. According to Gibb et al. (2004) the peak of spawning occurs in mid-March. The two surveys covering this region (Corystes Cruise 1 and Haakon Mosby Cruise 1(606)) should therefore have occurred just before the peak of spawning. Historical data suggest that haddock spawning areas show high inter-annual variability (Gibb et al. 2004) with the main concentration being to the west of the Shetland Isles. This pattern was also observed by Heath et al. (1994) based on the abundance of late stage haddock eggs. The 2004 ichthyoplankton survey results show haddock spawning over a considerably larger area and this may reflect the healthy status of this stock (spawning stock biomass at 460.000 tonnes in 2003).

The spawning period of whiting extends from February to May with the peak in April (Gibb et al. 2004). The 2004 ichthyoplankton surveys were not designed to specifically target this species and would therefore have only coincided with the start of spawning. Because of this and the fact that only 'cod-like' eggs over 1.1 mm were analysed (according to Russell (1976) the size range for whiting eggs is 0.97 –1.32 mm) the composite map produced for whiting egg distribution cannot be considered a complete picture (Figure 10). It is thought that there are two whiting sub-stocks in the North Sea, north and south of the Dogger Bank. The distribution of whiting eggs seen in 2004 was consistent with this view with concentrations to the south-west of the Dogger Bank, no eggs in the central North Sea but then smaller numbers to the east of Shetland Isles. Again it must be emphasised that the relative abundances of eggs between these areas cannot be taken as evidence of the relative importance of these spawning grounds since the peak of whiting egg production does not occur until later in the year.

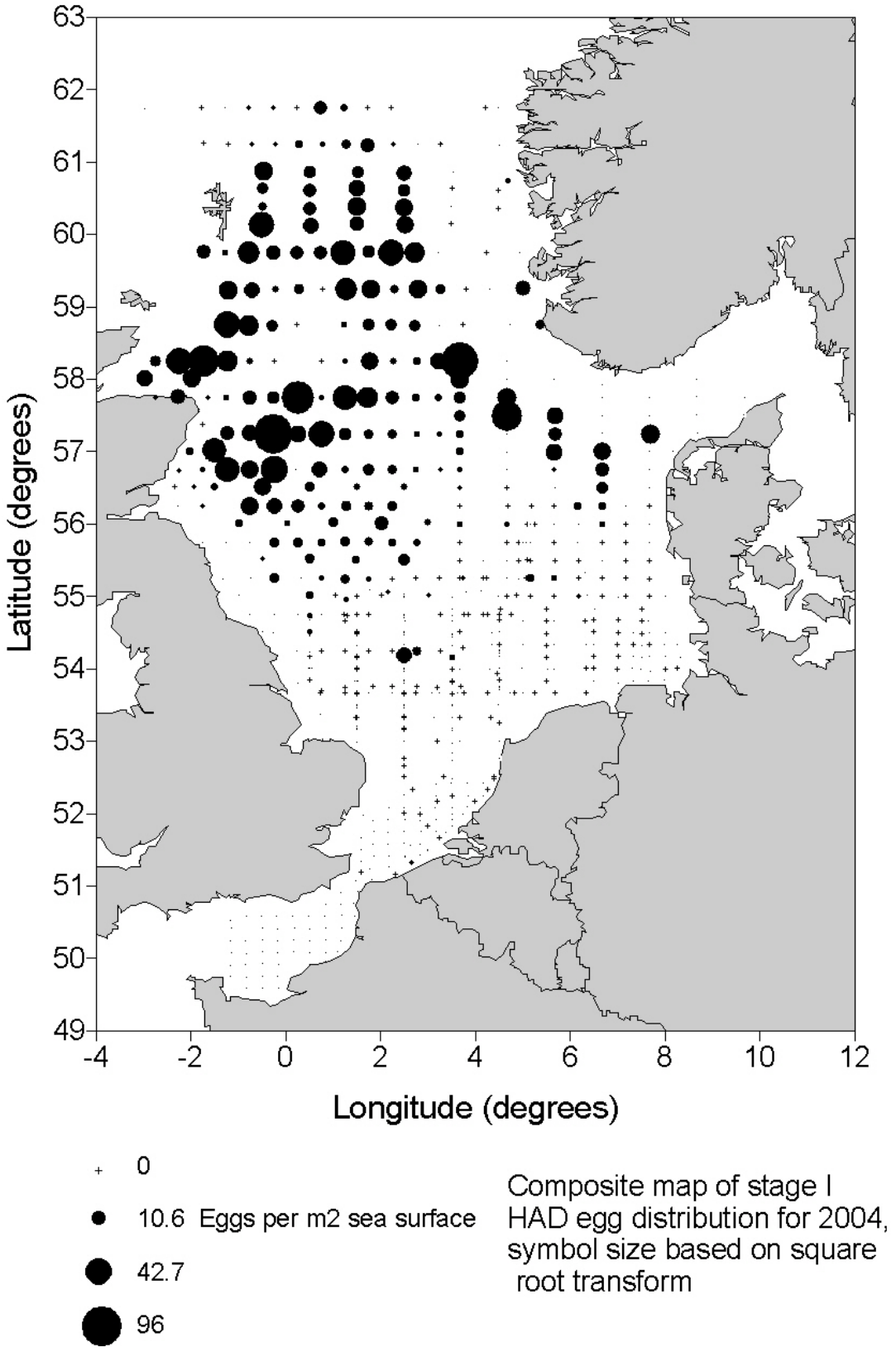

Figure 9 Composite map of the distribution of stage I haddock eggs based on the distribution of stage 1 'cod-like' eggs scaled on a station-by-station basis by species proportions determined using TaqMan analysis of all stage 'cod-like' eggs sub-sampled and applied via a mutli-nominal GLM.

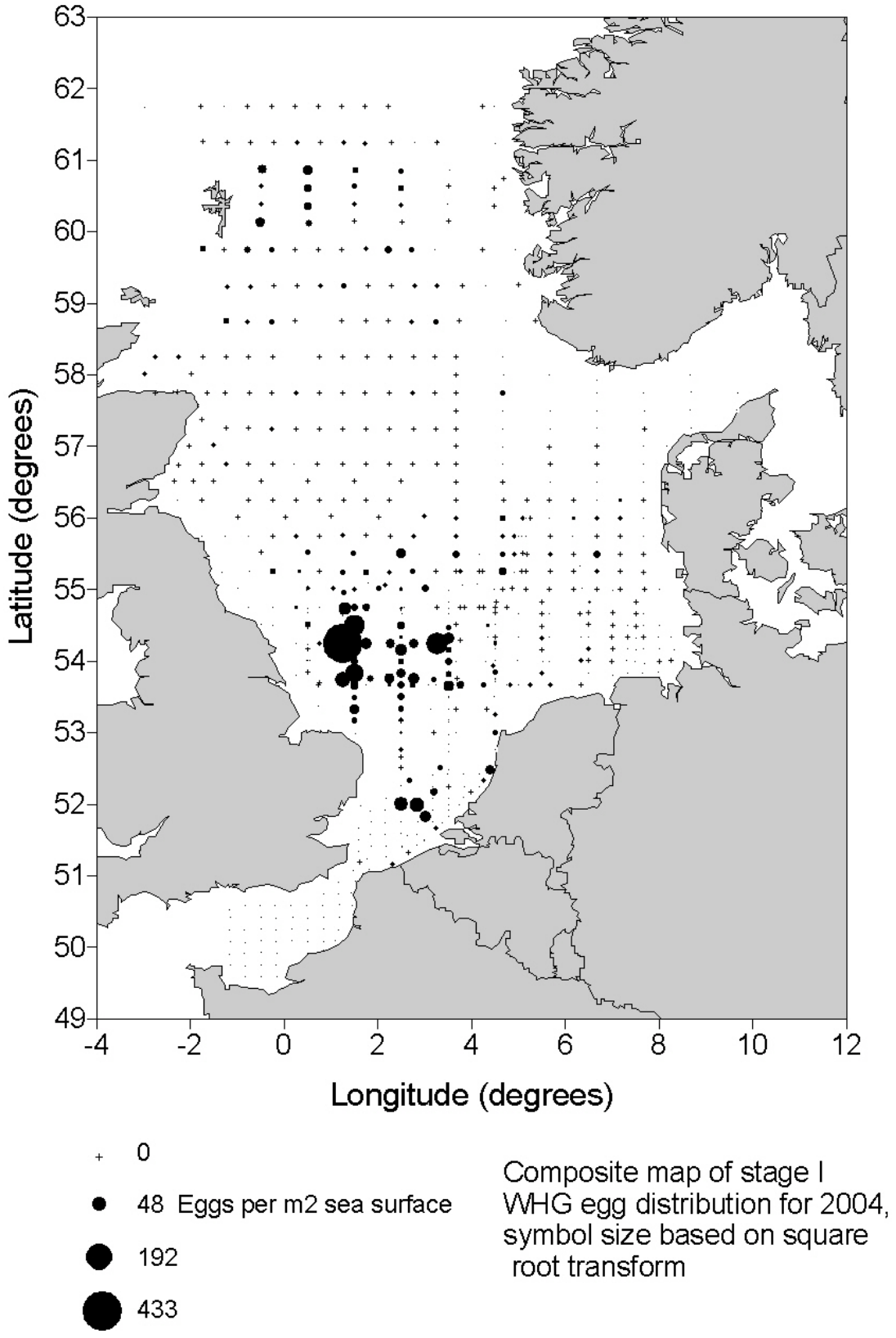

Figure 10. Composite map of the distribution of stage I whiting eggs based on the distribution of stage 1 'cod-like' eggs (>1.1mm diameter) scaled on a station-by-station basis by species proportions determined using TaqMan analysis of all stage 'cod-like' eggs sub-sampled and applied via a mutli-nominal GLM.

4. Discussion

The 2004 North Sea ichthyoplankton survey was the first attempt to cover the whole North Sea. Because it involved many institutes there were inevitably some problems with achieving a standardised sampling program. If the exercise is repeated in the future, every effort should be made to further standardise equipment (for example using standardised plankton samplers should reduce concerns about differences with calibrations of water volumes filtered between gears). Ideally staff should be exchanged between institutes to ensure sampling, identifications and egg staging is carried out in as standardised manner as possible.

There is a lack of experience with sorting and identification of fish eggs and larvae in many European fisheries institutes. If ichthyoplankton surveys are to become a more widely used tool in European fisheries management, this will need to be addressed through improved training and mobility programs.

In some cases there were clearly problems with sub-sampling adequate numbers of stage I eggs for genetic identification. As mentioned previously, we recommend that in any future surveys designed to map spawning grounds one should concentrate sub-sampling efforts on stage I eggs as opposed to sub-sampling all developmental stages for genetic identification.

The 2004 North Sea ichthyoplankton survey was successful in covering the entire North Sea at least once. Some sectors were surveyed several times and this will allow an estimate of plaice egg production to be produced for these areas. However, the northern North Sea was surveyed only once and in the Southern Bight only one dedicated PLACES cruise was carried out in which samples were collected for genetic identification. With this single-cruise approach we may have missed the peak of cod egg production, but this seems unlikely because both cruises were carried out in the expected period of peak spawning based on historical data.

The unexpected findings concerning the lack of cod eggs in the northern North Sea provide a strong impetus to repeat this survey to confirm these results within a reasonable timeframe (perhaps 3-5 years). Because of the issue of potentially missing the peak of cod spawning it would be recommended that the whole North Sea be surveyed at least twice (Jan-Feb and Feb-Mar). Whether there is sufficient justification for repeating this survey on a more regular basis remains to be debated.

Our finding that the spawning area of haddock is now much more extensive than previously reported is extremely interesting in relation to changes in the abundance of the stock. From a scientific and policy advice point of view, the fact that we have observed such an expansion could justify mapping of spawning areas on a more regular basis.

As well as providing up-dated maps of spawning locations, ichthyoplankton surveys can also be used to produce assessments of stock status that are free from the assumptions underlying fisheries-based assessment methods (Armstrong et al. 2001). The major problem with applying the annual egg production assessment method on the scale of the North Sea would be cost and logistics. A major advantage of the multi-survey approach is that one is much less likely to miss the peak of egg production and one can see how the spawning areas change as the season progresses (Fox et al. 2000). To reduce the costs it might be opted to conduct egg production methods within more constrained regions of ICES area IV such as the southern North Sea every few years. This would likely be acceptable for species whose spawning is largely confined to this area e.g. plaice but would be problematic for species such as cod since one would be ignoring a large fraction of the management unit.

5. Conclusions and recommendations

- Application of genetic techniques proved to be necessary to be able to distinguish between cod, haddock, whiting and saithe eggs, which have overlapping distributions in egg diameter.
- Cod were found to spawn in previously documented locations, although with relative less spawning in the Southern Bight. No relative increase in spawning in the northern North Sea was found.
- Plaice were found to spawn in similar locations as described in the past, although with relatively more spawning in the German Bight.
- The spatial and temporal coverage in the southern North Sea is sufficient to enable an estimation of the SSB of plaice in 2004 using the annual egg production method.
- To confirm the spatial distribution pattern of cod spawning, we recommend repeating the North Sea ichthyoplankton survey in 3-5 years time and covering the whole North Sea at least twice.
- To obtain fisheries-independent assessments of the stock status, we recommend carrying out ichthyoplankton surveys every 3 years and covering sub-regions within the North Sea at least 3-4 times.

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