ICES PGEGGS Report 2005

ICES Living Resources Committee
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REF. D

Report of the Planning Group on North Sea Cod and Plaice Egg Surveys in the North Sea (PGEGGS)

10–12 May 2005

Lowestoft, UK

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Conseil International pour l'Exploration de la Mer

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Contents

1 Introduction

The ToR of the Planning Group on North Sea Cod and Plaice Egg Surveys in the North Sea (PGEGGS) called for the design of coordinated ichthyoplankton surveys in the North Sea to describe the spawning areas of cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*). The first and second meetings of PGEGGS considered the policy drivers behind this activity and conducted a literature review of results from historical studies on cod and plaice spawning. The second and third meetings formulated a plan for coordinated surveys to take place in 2004 leading to requests for practical support in the form of ship time and staff time. The actual surveys were conducted from January through late March 2004. Initial data were available by May 2005 and the planned meeting was brought forward to allow more of the participants to attend. The initial data were considered at the meeting held in Lowestoft but the report on the meeting was delayed to allow the final compilation of data to be completed and a more complete set of results to be presented.

2 Terms of reference

The **Planning Group on North Sea Cod and Plaice Egg Surveys in the North Sea** [PGEGGS] (Chair: Clive Fox, UK will meet in Lowestoft from 6 June to 10 June 2005 to:

a) compile data from the surveys undertaken in 2004;

b) produce survey report for the 2005 Annual Science Conference ;

c) plan for a Workshop where detailed spatio-temporal analyses of the data from the surveys will be completed and where a feasibility study of undertaking stock biomass assessments of main North Sea commercial stocks using egg production methods in future years will be undertaken.

PGEGGS will report by 1 September 2005 for the attention of the Living Resources and the Resource Management Committees.

3 Participants

A complete list of participants is given at Annex I of this report.

4 Overall aims

At the PGEGGS meeting convened in Kiel (ICES CM 2004/G:03, Ref. D), six aims for the planned North Sea survey were set out:

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

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

c) Trace 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 for regions where there is sufficient survey coverage

e) Correlate the distribution patterns of eggs and larvae to hydrographic features and investigate potential physical/biological linkages

f) To describe where possible the distribution pattern of eggs/larvae of non-target (not plaice or cod) species

5 Overall progress

Participants reported that all sampling had gone largely according to plan except for a minor problem for Norway who had planned to use a Gulf VII sampler borrowed from Port Erin. Unfortunately this did not arrive in time so sampling was undertaken using a Gulf III.

Poor weather resulted in fewer stations being worked than planned in the northern North Sea but good spatial coverage was still obtained.

Levels of pre-sorting of eggs for genetic identification varied between participants and cruises. The overall numbers of eggs pre-sorted were rather low in some cases due to shortages of trained staff. This has caused some problems with re-allocating eggs to species on a station-by-station basis. Aggregation of the stations by strata is being investigated as a way to overcome this.

The Norwegian pre-sorted eggs were also mislaid in transit to Lowestoft when the shipping company responsible went bankrupt. However, after much effort the samples were found and received safely in Lowestoft.

All other pre-sorted eggs were received safely in Lowestoft, checked and sent to UEA (Norwich) for genetic analysis.

All results from the bulk analysis of formalin fixed plankton and from the genetic analysis of ‘cod-like’ eggs pre-sorted at sea have been completed. The data have been compiled into a central database held at CEFAS, Lowestoft. A copy of the database has been sent to all PGEGGS participants. These data have been used to produce a series of initial maps and analyses that are presented in this report.

In relation to the six overall aims laid out above:

a) Investigate all areas of the North Sea for the distribution of cod and plaice eggs – Completed.

b) Identify and delimit areas with high concentrations of cod and plaice eggs – Initial maps presented in this report, delimitation of areas of high concentrations will be based on spatial analyses conducted at the November 2005 workshop.

c) Trace the sites of intensive cod and plaice spawning based on distributional information of egg stages and larval sizes – see aim (b).

d) To attempt to estimate egg production for regions where there is sufficient survey coverage – To be undertaken for plaice in the Southern Bight.

e) Correlate the distribution patterns of eggs and larvae to hydrographic features and investigate potential physical/biological linkages – Analyses of this aspect are ongoing.

f) To describe where possible the distribution pattern of eggs/larvae of non-target (not plaice or cod) species – the distribution of the non-target species will be mapped during autumn 2005 using the available data.

6 Initial results

Full details of the methods used and the initial results for early stage eggs are given in a paper for the Annual Science Conference (ICES CM2005/AA:04). This is attached as Annex 3 to this report.

The results have also been analysed to produce summary maps of all the developmental stages for plaice, cod, haddock and whiting egg distributions (Annex 4).

7 Communications

The group proposed a number of potential publications arising from this work. It was felt that various individuals were most interested in taking the lead on different aspects:

a) A short communication to a high impact journal focussed on cod spawning (Clive Fox);

b) A more detailed scientific paper covering cod, haddock and whiting – this has been covered as a paper prepared for the ICES 2005 Annual Science Conference and is attached as Annex 3 (Clive Fox);

c) Possible CEFAS Data Report on the raw plankton data for all species identified. This was seen as a useful means to publish raw data in a citeable format (Clive Fox);

d) Production estimates of plaice (Mark Dickey-Collas & Cindy van Damme);

e) Cod production in relation to spawning stock (Gerd Kraus);

f) Distribution of eggs and larvae in relation to hydrography (Peter Munk);

g) Distribution of sandeel larvae (Peter Munk and Petter Fossum).

8 Workshop

ToR c) requires that PGEGGS hold a workshop to undertake detailed spatial analysis of the data and to consider the possibility of undertaking such surveys in the North Sea in the future. It is planned to hold such a meeting in November 2005 in Copenhagen. Additional statistical support for this activity will be provided by CEFAS.

9 Conclusions

The 2004 ichthyoplankton surveys were successful in covering the whole North Sea and mapping the occurrence of early stage eggs of cod and plaice. In addition new data on the spawning locations of haddock have been produced.

The dataset contains additional information on hydrography and the distribution of eggs and larvae of non-target species.

As well as progressing the analysis of the data beyond the initial stages presented here, the November meeting of PGEGGS will consider whether repeating the surveys in the future is justified and produce recommendations for the planning of any such survey.

10 Recommendations

The **Planning Group on North Sea Cod and Plaice Egg Surveys in the North Sea** [PGEGGS] (Chair: Clive Fox, UK) will meet in Charlottenlund, Denmark from 14 November to 16 November 2005 to:

a) undertake statistical analyses of data from the PLACES 2004 surveys;

b) to consider the feasibility of undertaking stock biomass assessments for North Sea commercial stocks using egg production methods;

c) produce recommendations on whether further North Sea egg surveys are required and guidance on their design in light of the experiences in 2004.

Supporting Information

Priority:	The work of PGEGGS has a high priority given concerns about the current status of North Sea cod and plaice stocks and the need to produce scientifically credible advice for policy fomulation.
Scientific Justification and relation to Action Plan:	Action Plan: 1.2.1, 1.2.2, 1.8, 1.10 Terms of reference a) The rationale for establishing a coordinated international North Sea ichthyoplankton survey in 2004 has been presented in the report of PGEGGS which met in IJmuiden from 24–26 June 2003. Detailed survey plans were presented in the report of the meeting in Kiel from 11–12 November 2003. Initial analysis of data took place at a meeting in Lowestoft, 10–12 May 2005 and results will be presented at the 2005 ICES ASC, Aberdeen. We are now close to completing the data analysis stage and the proposed workshop will keep progress on track. Term of reference b) and c) Monitoring spawning areas of main fish species has been recommended as a high priority for Ecosystem Based Approach to Management by the Bergen Declaration Meeting of Scientific Experts. The surveys conducted in 2004 are designed to initiate this process for the North Sea. However longer term monitoring will require evaluation and planning of what is feasible in the light of the results from the 2004 survey. The workshop is designed to provide this evaluation.
Resource Requirements:	No specific resource requirements beyond the need for members to prepare for and participate in work by correspondence.
Participants:	Attendees at the last PGEGGS meeting held in Lowestoft in May 2005 plus representation from FRS, Scotland
Secretariat Facilities:	None
Financial:	No financial implications
Linkages To Advisory Committees:	Data are required by the ICES Working Group on the Assessment of Demersal Stocks in the North Sea and Skagerrak, and REGNS.
Linkages To other Committees or Groups:	No formal linkages.
Linkages to other Organisations:	No formal linkages
Secretariat Marginal Cost Share:	

Annex 1: List of participants

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Annex 2: Action Plan Audit

Year	Committee Acronym	Committee name	Expert Group	Reference to other committees	Expert Group report (ICES Code)	Resolution No.		
2004/2005	LRC	Living Resources Committee	PGEGGS	D	2005/G:11	2G09		
Action Plan	**Action Required**	**ToR's**	**ToR**	**Satisfactory Progress**	**No Progress**	**Unsatisfactory Progress**	**Output (link to relevant report)**	**Comments (e.g., delays, problems, other types of progress, needs, etc.**
No.	**Text**	**Text**	**Ref. (a, b, c)**	**S**	**0**	**U**	**Report code and section**	**Text**
1.2.1	Understand and quantify the biology and life history, stock structure, dynamics, and trophic relationships of commercially and ecologically important species.	compile data from the surveys undertaken in 2004;	a)	S				
1.2.2	Quantify the changes in spatio-temporal distribution of the stocks of important species in relation to environmental change, using survey and commercial data.	compile data from the surveys undertaken in 2004;	a)	S				
1.8	Implement a North Sea-oriented monitoring programme that incorporates oceanographic and fisheries data.	compile data from the surveys undertaken in 2004;	a)	S				
1.10	Develop better tools and training opportunities for monitoring and observation of physical, chemical and biological properties of marine ecosystems.	compile data from the surveys undertaken in 2004;	a)	S				
1.2.1	Understand and quantify the biology and life history, stock structure, dynamics, and trophic relationships of commercially and ecologically important species.	produce a survey report for the 2005 Annual Science Conference;	b)	S				
1.2.2	Quantify the changes in spatio-temporal distribution of the stocks of important species in relation to environmental change, using survey and commercial data.	produce a survey report for the 2005 Annual Science Conference;	b)	S				
1.8	Implement a North Sea-oriented monitoring programme that incorporates oceanographic and fisheries data.	produce a survey report for the 2005 Annual Science Conference;	b)	S				
1.10	Develop better tools and training opportunities for monitoring and observation of physical, chemical and biological properties of marine ecosystems.	produce a survey report for the 2005 Annual Science Conference;	b)	S				
1.2.1	Understand and quantify the biology and life history, stock structure, dynamics, and trophic relationships of commercially and ecologically important species.	plan for a Workshop where detailed spatio-temporal analyses of the data from the surveys will be completed and where a feasibility study of undertaking stock biomass assessments of main North Sea commercial stocks using egg production methods in future years will be undertaken.	c)	S				Workshop to be from 14-16 November 2005
1.2.2	Quantify the changes in spatio-temporal distribution of the stocks of important species in relation to environmental change, using survey and commercial data.	plan for a Workshop where detailed spatio-temporal analyses of the data from the surveys will be completed and where a feasibility study of undertaking stock biomass assessments of main North Sea commercial stocks using egg production methods in future years will be undertaken.	c)	S				
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NOT TO BE QUOTED WITHOUT PERMISSION OF THE AUTHORS

Annex 3: Initial results from the 2004 ichthyoplankton survey of the North Sea

ICES CM2005:AA04

Initial results from the 2004 ichthyoplankton survey of the North Sea

Clive Fox , Taylor M., Dickey-Collas, M., van Damme C.J.G., Bolle, L., Daan, N., Rohlf, N., Kraus, G., Munk, P., Fossum, P., Bailey, N.

Abstract

In 2004 an international consortium comprising England, Scotland, Netherlands, Germany, Denmark and Norway conducted an ichthyoplankton survey covering the whole of the North Sea in order to comprehensively survey cod and plaice spawning areas. At each station, 'cod-like' eggs were pre-sorted at sea from the plankton sample and preserved in ethanol for subsequent analysis using species-specific genetic probes. The remainder of the plankton sample was fixed in formalin and ichthyoplankton subsequently sorted and identified using traditional visual methods. The results showed stage I plaice spawning to be located in the traditional areas reported from the literature although with evidence of a more northward extension up the eastern edge of the Dogger Bank compared to data from the 1930s. The distribution of stage I cod eggs also conformed to historical patterns being most abundant around the southern and eastern edge of the Dogger Bank, in the German Bight, off the Moray Firth and to the east of the Shetland Isles. 'Cod-like' eggs were also found in southern Bight but this area was not as well sub-sampled for genetic analyses. Most of the 'cod-like' eggs on stations that were sub-sampled adequately in this region were shown to be whiting or other species. Data was also produced on the distribution of haddock eggs which were found over a wider region of the north-western North Sea than shown in historical maps. Whiting eggs were found south of the Dogger Bank and to the east of the Shetland Isles but were absent from the central North Sea. These results are discussed in relation to historical patterns of spawning and recent changes in the abundance of the North Sea plaice, cod and haddock stocks.

Keywords: cod, plaice, spawning, ichthyoplankton, North Sea

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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 coordinated 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 melding these fragments together or inferred from information on the distribution of mature fish.

In response, ICES established the Planning Group on North Sea Cod and Plaice Egg Surveys (PGEGGS) in 2001. The TOR of PGEGGS focused on two species of major concern namely, cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*).

PGEGGS therefore set out to design an initial survey to take place 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) Correlate the distributional patterns of eggs and larvae to hydrographic features, and investigate potential physical/biological linkages;

f) 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.

Materials and Methods

The early stage eggs of cod are visually indistinguishable from those of haddock (*Melanogrammus aeglefinus*), larger whiting (*Merlangius merlangus*) eggs and eggs of some other species. 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.

Field sampling

Because of the scale of the North Sea, covering this area in a survey conducted by one country is not practical (Figure 1). The North Sea was therefore divided into sectors to be surveyed by different countries (Figure 2). 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 logistical constraints a wider variety of gears was employed (Table 2). 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 m 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.

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. TaqMan 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 ethanol which preserves high-quality DNA. Upon recovery, the plankton sample was therefore transferred into a jug and kept cool on ice. Aliquots of the sample were examined in Pyrex pie-dishes and 'cod-sized' eggs removed using wide-bored pipettes. These eggs were transferred into drops of seawater on a Petri dish and 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 *et al.* (1981) and transferred into individually labelled tubes containing 1.5 ml of ethanol. Ship-board sorting was continued until up to 100 eggs had been removed from the sample.

All countries followed this protocol excepting Denmark and Scotland. Denmark deployed bongo-nets thus retrieving two plankton samples per station. For Danish samples, all the material in one cod-end was fixed in 4% formalin [4% formaldehyde in distilled water buffered with 2.5% sodium acetate trihydrate (w/v)] for subsequent laboratory sorting whilst cod-like eggs were sub-sampled into ethanol from the other cod-end. Scotland deployed a ring-net but few eggs or larvae were caught and the data are not included in this report.

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 icthyoplankton.

Laboratory sorting and identification of icthyoplankton

Fish eggs and larvae were subsequently to be 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) and thick chorion. 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 cod and plaice larvae using criteria from Russell (1976). The larval data are not presented in this paper but will be subject of a later report. If resources allowed, participants were also asked to identify larvae other than those of cod and plaice. 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.

Application of TaqMan probes to pre-sorted eggs

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.

Total DNA was extracted from individual eggs in 96-well plates using Proteinase K digestion and either the Bilatest magnetic bead extraction kit (Bilatec AG, Germany) running on a Roboseq 4204 S robot (MWG), or salt precipitation using a manual protocol modified from Aljanabi & Martinez (1997). PCR was undertaken using 1 µl (~50ng) of extracted DNA per well amplified with 200nM of each species-specific probe (COD-P, FAM labelled, HAD-P, TET labelled, and WHI-P, VIC labelled), 300nM of the GAD-F and GAD-R primers, 8.3 µl of TaqMan Universal PCR Master Mix (ROX passive reference) (Applied Biosystems) and 4.9µl tissue culture H_2O (Sigma). Plates were run under real-time conditions on three dye layers with eight 'No Template Controls' (NTCs) and three positives (DNA extracted from eggs from hatchery cod, haddock and whiting) per 96 well plate. The assay was run using the default cycling conditions. Post PCR, the results were analysed using the Sequence Detection Software version 1.71 (Applied Biosystems). The ΔR_n values for each cycle and dye layer were then exported to MS Excel, and processed further as described in Taylor *et al*. (2002).

Samples which had been manually extracted and failed to react with the TaqMan probes were amplified using universal teleost cytochrome B primers GLU (L)-TGACTTGAAGAACCAC/TCGTTG-3' (Palumbi 1996) and CB2-(H)-AAAC TGCAGCCCCTCAGAATGATATTTGTCCTCA-3' (Kocher *et al*., 1989). PCR was performed in an MJ Research PTC-200 thermal-cycler under the following conditions: 94°C, 120 s, followed by 30 cycles of 94°C for 30 s; 47°C for 30 s; 72°C for 45 s, followed by 72°C for 10 min. Ten µl reaction mixes consisted of 1 µl (≈ 20 ng) template DNA, 0.5 µm of each primer, 5µl of 2x PCR mastermix

(ABGene), and 2µl H_2O. PCR products were resolved on 1.2% agarose gels run in 0.5x TBE buffer and stained with ethidium bromide. Samples that produced amplification products were classed as 'null' reactions i.e. not cod, haddock or whiting but another species for which specific probes have not yet been developed. Samples that produced no amplification products were classified as 'failed extractions'.

Treatment of the data

The accuracy of the sub-sampling undertaken at sea was assessed by comparison of egg diameter and developmental stage frequency distributions in the sub-samples and in the subsequent laboratory analyses of the remaining formalin fixed eggs. Secondly, 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.

Absolute numbers of eggs in both the bulk formalin fixed portions of the samples and in the geneprobe sub-samples were converted to numbers per cubic metre using estimates of the volumes of water filtered at each station derived from flowmeters 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. Flowmeter 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 sub-sampled for genetic analysis at each station (except for Dana Cruise 1 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 and other species on the basis of the ratio of these species determined from the TaqMan analyses of the pre-sorted eggs from that location. 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).

Results and discussion

Field sampling

All planned cruises were completed and a total 973 plankton hauls were made. 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.

Ichthyoplankton sorting and identification

All participants completed the required analysis of 'cod-like' and plaice eggs (Table 2). 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. For dedicated PLACES surveys, most countries were also able to sort, enumerate and measure larvae of at least the target species, cod and plaice. There is therefore good spatial coverage for plaice and 'cod-like' eggs and complete larval data for sectors C, D, E and G and partial larval data for sector F (Figure 2). The larval data are not presented here.

Distribution of stage I plaice eggs

A composite map (Figure 3) 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 subject to revision. 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 Mar – 23 Mar) 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 3) 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 4). According to Simpson (1959) this area was not important for plaice spawning in the 1930s – 1950s although earlier reports indicate that prior to 1910 (Figure 5) 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).

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 (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 suggested that stage I eggs might drift by up to 25 miles in this time in the southern Bight although dispersal could be greater if conditions were stormy (Simpson 1959).

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 in due course. This will be of value for assessing the status of the stock for which the spawning stock biomass is currently thought to be below the precautionary reference point of 230 kilo tonnes based on conventional assessments.

Genetic identification of cod-like eggs

Over the whole survey 9,212 ‘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, due to staffing levels this was not achieved on all cruises and 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 (Table 3, Figure 6) 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.

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 4). 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 9,212 ‘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 8,865 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, 2,872 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 7). 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 8,865 ‘cod-like’ eggs identified by TaqMan were cod, 48.3% were haddock, 22.2% whiting and 12.0% other species (Table 5).

Distribution of stage I 'cod-like' eggs

Stage I 'cod-like' eggs were abundant in the southern Bight and along the southern edge of the Dogger Bank (Figure 8). Lower concentrations were found in the German Bight, off the Scottish east coast and to the east of the Shetland Isles and at the mouth of the Skaggerak. A single high abundance station occurred at the extreme northern edge of the survey grid. With the exception of the eggs in the Southern Bight we were able to use the TaqMan identifications to assign these eggs as cod, haddock, whiting of other species. The concentration of cod-like eggs in the Southern Bight was found in samples collected for the January herring larval survey (Tridens II, Cruise 5) where sub-sorting of 'cod-like' eggs from these samples at sea was not possible. Data from this cruise is therefore excluded from the composite maps of cod, haddock and whiting eggs.

Distribution of stage I cod, haddock and whiting eggs

Because on many stations the numbers of stage I eggs pre-sorted and analysed by TaqMan was considered too low to reliably allocate species proportions, proportions of cod, haddock, whiting and others were computed on a station-by-station basis using all the eggs analysed by TaqMan (i.e. Stages 1 through 5). Stations on which it was judged that there was still an inadequate number to reliably apportion the bulk 'cod-like' eggs to species were then excluded. This resulted in some reduction in reliable survey coverage compared with the overall coverage, particularly in sectors B and F. Basing the species proportions on TaqMan analysed eggs of all stages may have led to some over-estimation of the spatial extent of the spawning grounds since later stage eggs would be dispersed and advected away from their origins. A stratum-based approach using only stage I data by 2-degree boxes was also tried. Although this increased the numbers of stage 1 eggs analysed to > 30 for all strata (including those in sector F), it led to some significant distortions in the pattern of cod and haddock egg concentrations when compared with maps prepared on a station-by-station basis. This occurred because the relative proportions of the different species can change quite rapidly at spatial scales smaller than the 2^o resolution. It may therefore be preferable to model the proportions of the species present using smaller strata or smooth functions based on egg stage I TaqMan results and we plan to investigate this shortly. In retrospect it would have been preferable to focus all the sub-sampling effort on stage I eggs only and this would be recommended for future exercises designed to map spawning areas.

Figures 9–12 present composite maps of the distribution of stage I cod, haddock, whiting and other species eggs based on the proportions of these species derived from all egg stages analysed using TaqMan. The lack of sub-sampling of the high abundance of 'cod-like' eggs found in the southern Bight on Tridens II Cruise 5 has already been mentioned but on the basis of the few stations in this region that were sampled a few weeks later, whiting appeared to be the dominant species. According to Brander (1994) the peak of spawning in the Southern Bight occurs around year-day 40 and since this is coincident with Tridens I Cruise 2 we might conclude that cod egg production in this region is probably now very limited. More intensive sampling is however required to confirm this. The main concentrations of stage I cod eggs were found around the southern and eastern edges of the Dogger Bank (in accord with results in Heessen and Rijnsdorp (1989), Figure 13) with another patch in the German Bight. Rather low abundances of cod eggs were found north of 57^o. 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 14). That composite contained an un-surveyed region which was partially covered by Harding and Nichols (1987) in 1976. 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 suggests that at least a proportion of these eggs may have been whiting.

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 does appear 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. However, 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 (excepting perhaps for the southern Bight for which we have limited information). As mentioned above most findings on North Sea cod distribution in relation to environmental change have used data from surveys conducted in the summer (presumably because it is thought that increasing summer temperatures will be more limiting whilst winter temperatures will always be within physiological tolerances). Cod distribution survey data around the time of spawning (Jan-Feb) is also available from the ICES International Bottom Trawl Surveys. Results for recent years still show some 3+ cod occurring to the south and east of the Dogger Bank although the bulk of the population (both numerically and in terms of biomass) appears to be north of latitude 57^{o}N (Figure 15). 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 north. However, the surveys were designed to coincide with the expected spawning times based on historical data and 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 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^{o}N automatically leads to higher egg production (and this potential recruitment) in this region.

Abundances of haddock eggs in the 2004 survey (Figure 10) 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 11). 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.

As mentioned previously TaqMan probes have so far been developed only for cod, haddock and whiting and therefore other species can present a negative result suing this method (Fox *et al*., 2005b). There were relatively low numbers of such eggs in the southern North Sea but larger numbers at the northern edge of the survey. The DNA from some of these eggs was sequenced and they were shown to be saithe (*Pollachius virens*). The occurrence of large numbers of saithe eggs in this region is fully consistent with the behaviour of the adults since they spawn in deep water (100–200 m) from January to April (Russell 1976, Wheeler 1978).

Standardisation of gears and protocols

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 is carried out in as standardised manner as possible. In some cases there were clearly problems with sub-sampling adequate numbers of stage I eggs for genetic identification. CEFAS experience suggests that this can be done successfully but it does require adequate, experienced staff (Fox *et al*., 2005b). As mentioned previously we would 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.

Many of these problems would be solved if molecular identification method could be applied successfully and reliably to eggs which had been fixed in formalin. Although we have been unsuccessful in getting the TaqMan method to work reliably with DNA from formalin preserved samples there are several recent papers claiming that genetic methods can be applied to formalin-fixed biological samples (Kirby & Lindley 2005; Perez *et al*., 2005). The success rates claimed are certainly lower than with high-quality DNA (Kirby and Lindley (2005) quote a success rate of 65% whilst Perez *et al.* (2005) quote an 85% success rate) but such success rates would probably be acceptable compared with the disadvantages of pre-sorting eggs at sea. The advantages of being able to identify formalin-fixed eggs would be that the genetic analysis could be applied after conventional laboratory-based sorting, that plankton sampling could be undertaken from fishing vessels as well as research vessels leading to greater involvement of the industry, that one could analyse 'cod-like' eggs collected during other surveys such as the herring larval survey without altering their protocols and that the staffing costs of field-work would be much reduced. One reason why the TaqMan approach may fail with DNA from formalin-fixed samples is it discriminates between species based on a few nucleotide differences (Taylor *et al*., 2002). This may mean it is especially sensitive to DNA template degradation and that size-fragment based methods may be more successful. The potential advantages of being able to work with formalin-fixed samples are so strong that despite the initial setbacks more work should be undertaken on applying molecular methods to these samples.

Finally there is a lack of experience with sorting and identification of fish eggs and larvae in many European fisheries institutes. If icthyoplankton 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.

Spatial and temporal coverage

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. The 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 three 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, icthyoplankton 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). In 2006, it is planned to begin such an exercise for the Irish Sea for the purpose of monitoring the recovery of the VIIa cod stock. The major problem with applying the annual egg production assessment method on the scale of the North Sea would be cost and logistics. The method to be applied in Area VIIa requires good coverage of the whole spawning period (it is planned to use around 5 or more surveys) and it is unlikely that one could conduct a similar exercise across the whole of Area IV because of the much greater sea area. 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). Despite the cost it might be possible to conduct egg production methods within more constrained regions of 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.

Based on the results presented here and the points mentioned above, the next meeting of PGEGGS will draw-up recommendations for future North Sea ichthyoplankton surveys.

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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

Table 2 Water volumes filtered

Country	Ship	Cruise PLACES ID (National ID)	Gear	Water volume filtered per station (m^3)				
				n	Min	Max	Mean	Std dev
Netherlands	Tridens II	4	Gulf III, 20 cm opening*, 270 µm mesh	77	14.5	87.0	47.5	17.9
Netherlands	Tridens II	1	53 cm Gulf VII, 28 cm opening, 270 µm mesh	66	39.9	255.7	137.6	44.9
Germany	Alkor	1 (233)	53 cm Gulf VII, 28 cm opening*, 270 µm mesh	108	6.3	67.8	30.1	14.4
Netherlands	Tridens II	5	Gulf III, 20 cm opening*, 270 µm mesh	92	21.3	112.1	53.8	17.8
Scotland	Scotia	2	Ring-net, 48 cm opening*, 250µm mesh	48	8.7	23.7	14.9	3.8
Netherlands	Tridens I	2	53 cm Gulf VII, 28 cm opening*, 270 µm mesh	69	21.7	233.7	70.8	43.1
Germany	Heinke	1 (203)	Bongo, 60 cm opening*, 500 µm mesh	52	24.2	195.0	113.6	40.7
England	Corystes	1	76 cm Gulf IV, 40 cm opening*, 270 µm mesh	138	211.8	848.7	420.5	94.5
Netherlands	Tridens II	3	53 cm Gulf VII, 28 cm opening*, 270 µm mesh	66	55.0	188.5	87.4	22.9
Denmark	Dana	3	Bongo, 60 cm opening*, 330 µm mesh	239	306.4	917.7	561.3	124.9
Norway	Haakon Mosby	1 (606)	Gulf III, 20 cm opening*, 330 µm mesh, Seabird CTD	99	31.4	146.3	72.3	24.3
Germany	Alkor	2 (236)	Bongo, 60 cm opening*, 500 µm mesh	54	35.8	265.2	125.5	55.7

Table 3 Percentages of cod-like eggs by developmental stage in the sub-samples preserved in ethanol for geneprobe analysis and in the bulk formalin fixed portions of the plankton samples

Ship	Cruise	Sample	Unstaged	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Total
Tridens II	1	Geneprobes (n)	0	80	20	25	22	2	149
		Geneprobes(%)	0	53.7	13.4	16.8	14.8	1.3	
		Bulk (n)	1	289	42	50	34	8	424
		Bulk (%)	0.2	68.2	9.9	11.8	8.0	1.9	
Tridens I	2	Geneprobes (n)	0	232	38	119	30	4	423
		Geneprobes(%)	0	54.9	9.0	28.1	7.1	1.0	
		Bulk (n)	10	784	153	274	117	61	1399
		Bulk (%)	0.7	56.0	10.9	19.6	8.4	4.4	
Heinke	1 (203)	Geneprobes (n)	0	51	0	36	37	77	201
		Geneprobes(%)	0	25.4	0	17.9	18.4	38.3	
		Bulk (n)	0	527	82	186	399	142	1336
		Bulk (%)	0	39.5	6.1	13.9	29.9	10.6	
Corystes	1	Geneprobes (n)	0	1734	753	1138	828	736	5189
		Geneprobes(%)	0	33.4	14.5	21.9	16.0	14.2	
		Bulk (n)	51	17973	3794	5186	4829	3820	35653
		Bulk (%)	0.1	50.4	10.6	14.6	13.5	10.7	
Tridens II	3	Geneprobes (n)	0	124	42	190	109	27	492
		Geneprobes(%)	0	25.2	8.5	38.6	22.2	5.5	
		Bulk (n)	2	1762	313	1002	798	368	4245
		Bulk (%)	0.1	41.5	7.4	23.6	18.8	8.7	
Dana	3	Geneprobes (n)	1	229	86	276	59	48	699
		Geneprobes(%)	0.1	32.8	12.3	39.5	8.4	6.9	
		Bulk (n)	0	11478	2138	5156	1873	1337	21982
		Bulk (%)	0	52.2	9.7	23.5	8.5	6.1	
Haakon Mosby	1 (606)	Geneprobes (n)	0	546	166	409	494	444	2059
		Geneprobes(%)	0	26.5	8.1	20.0	24.0	21.6	
		Bulk (n)	1	425	267	206	418	206	1523
		Bulk (%)	0.1	26.6	16.7	12.9	26.2	12.9	
Total		Geneprobes (n)	1	2996	1105	2193	1579	1338	9212
		Geneprobes(%)	0	32.5	12.0	23.8	17.1	14.5	
		Bulk (n)	90	35670	7250	12827	8786	6022	70719
		Bulk (%)	0.1	50.4	10.3	18.1	12.4	8.5	

Table 4 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

Table 5 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	

Figures

1. Locations of major areas of the North Sea, Skagerrak and Channel (from OSPAR, North Sea Quality Status Report, 2000).
2. Division of the North Sea into regional sectors for the purposes of the PGESSG/PLACES survey – numbers indicate the planned number of plankton hauls per ICES rectangle: Sectors A + B – Netherlands and Germany; D – Germany; Sectors C+E – England; Sector F – Denmark, Sector G – Norway.
3. 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).
4. The spawning areas and times of North Sea plaice according to Simpson (1959).
5. The spawning areas of North Sea plaice for the early 1900s according to Masterman (1911)
6. 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
7. 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.
8. Composite map of the distribution of 'cod-like' eggs from the 2004 North Sea ichthyoplankton survey.
9. 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 on that station. Stations on which an inadequate number of cod-like eggs were sub-sampled have been excluded.
10. 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 on that station. Stations on which an inadequate number of cod-like eggs were sub-sampled have been excluded.
11. Composite map of the distribution of stage I whiting 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 on that station. Stations on which an inadequate number of cod-like eggs were sub-sampled have been excluded.
12. Composite map of the distribution of stage I other species 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 on that station. Stations on which an inadequate number of cod-like eggs were sub-sampled have been excluded.
13. Distribution of stage I and 2 cod eggs based on data from Heessen and Rijnsdorp (1989). Filled symbols are proportional to the accumulated egg production during January-February 1988. Note that the criteria for classifying eggs as cod were not stated.
14. 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.
15. Relative density of cod aged 3 and older from the IBTS Q1 survey series for recent years (2001, 2002 and 2003). NOTE that the bubble size has been scaled on a logarithmic basis to the catch data to emphasise rectangles with low catches. Left hand plots = density in numbers per hour; right hand plots = density in biomass (kg) per hour.

Figure 1


Tampen
Sørfjorden
Haakonsvern
Shetland Islands
Hardangerfjord
Bømlafjord
Oslofjord
Glomma
Frierfjorden
Breviksfjord
Väderöarna
Fladen Ground
Norwegian Trench
Lista
Kristiansandfjord
Skagerrak
Cromarty Firth
Moray Firth
Skagen
Laesø
Dee
Limfjord
Fladen
Devil's Hole
Kattegat
Tay
Forth
Hvide Sande
Nymindegab
Wear
Tyne
Dogger Bank
Amrum
Tees
Sellafield
Flamborough Head
Oyster Grounds
German Bight
Great Ouse
Norderney
Mellum
Elbe
Borkum
Humber
Wadden Sea
Ems
Dornumersiel
Weser
HAMBURG
Frisian Front
Terschelling
Jade Bay
BREMERHAVEN
Wash
AMSTERDAM
Alde
Crouch
Haringvliet
ROTTERDAM
Flemish Banks
Eastern Scheldt
LONDON
Thames
Western Scheldt
ANTWERP
Meuse
Scheldt
Dover Strait
Rhine
Tamar
Channel
Cap de la Hague
LE HAVRE
Seine
Rance
I
II
III
IV
V

Figure 2

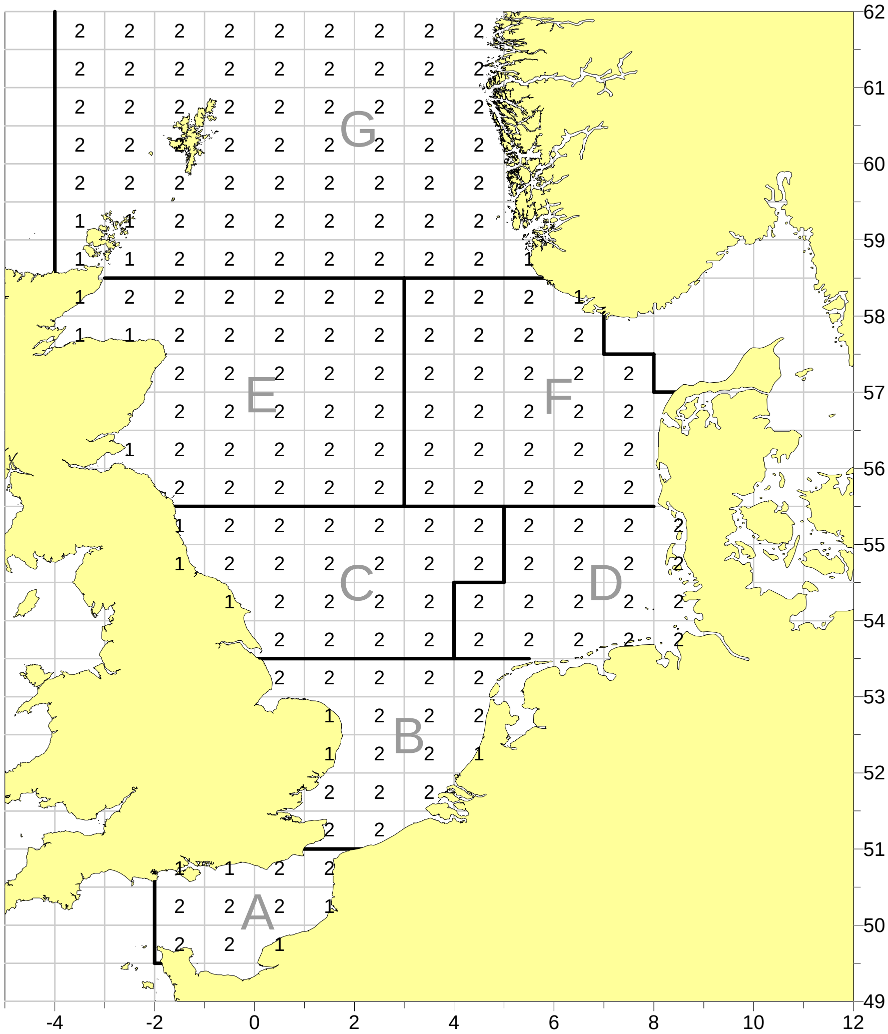
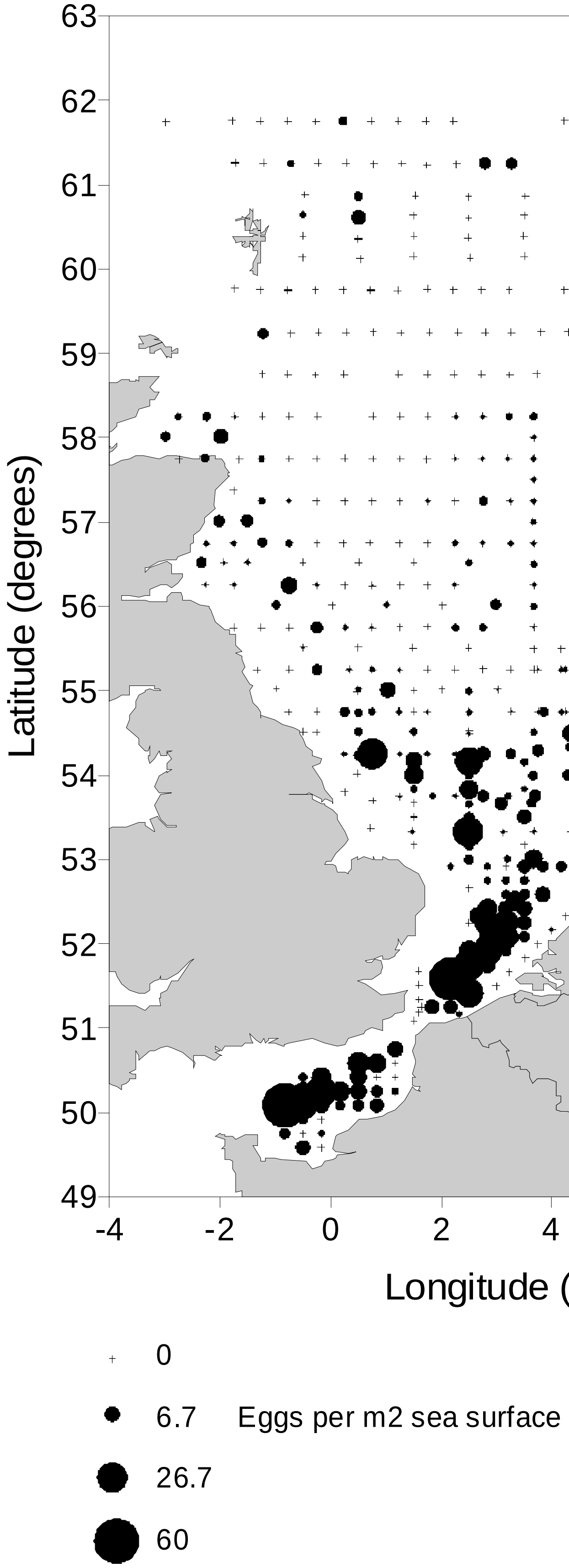
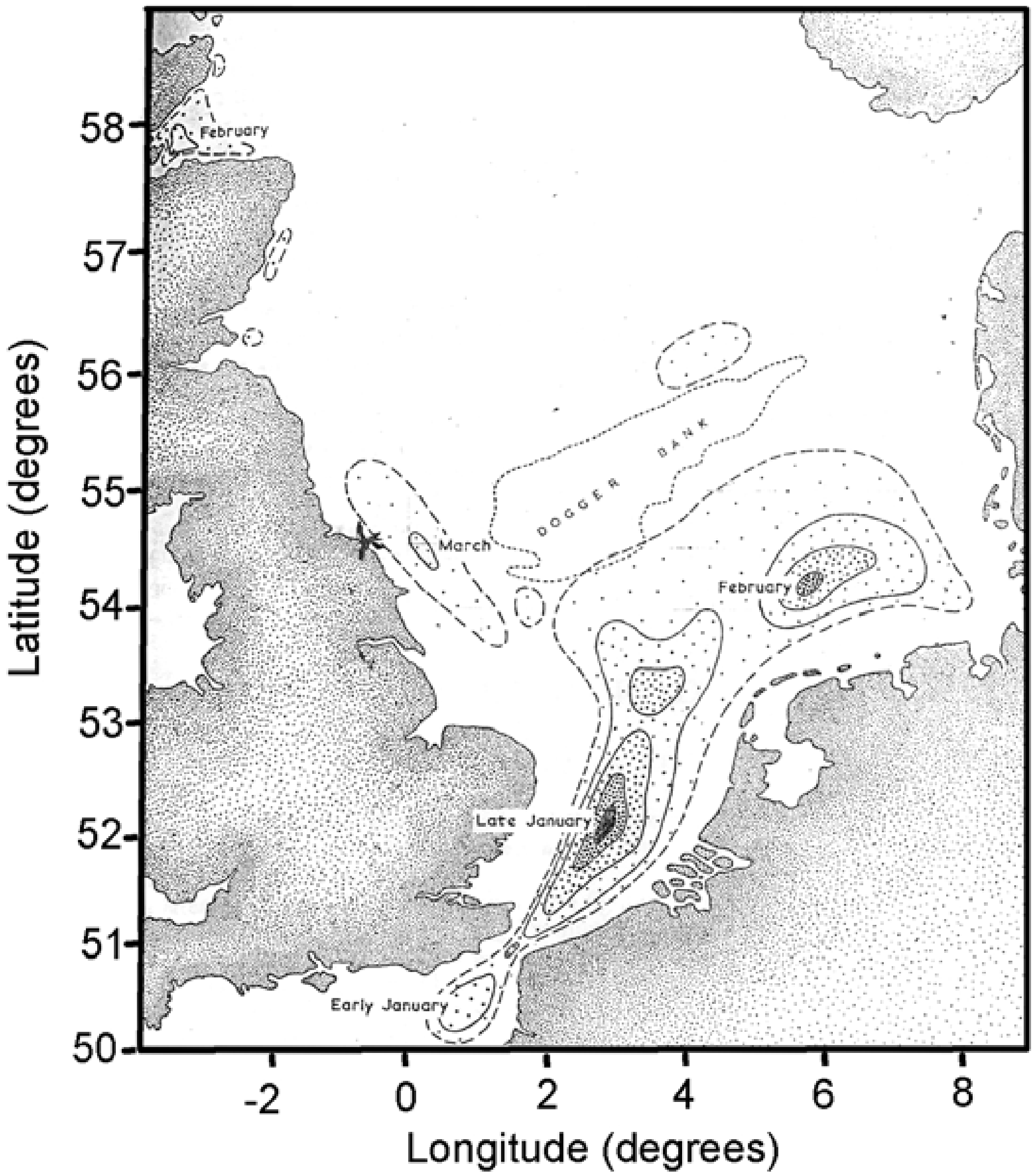


Figure 3



Composite map of stage I plaice egg distribution for 2004, symbol size based on square root transform

Figure 4



58
57
56
55
54
53
52
51
50
Latitude (degrees)
-2
0
2
4
6
8
Longitude (degrees)
February
March
DOGGER BANK
February
Late January
Early January

Figure 5

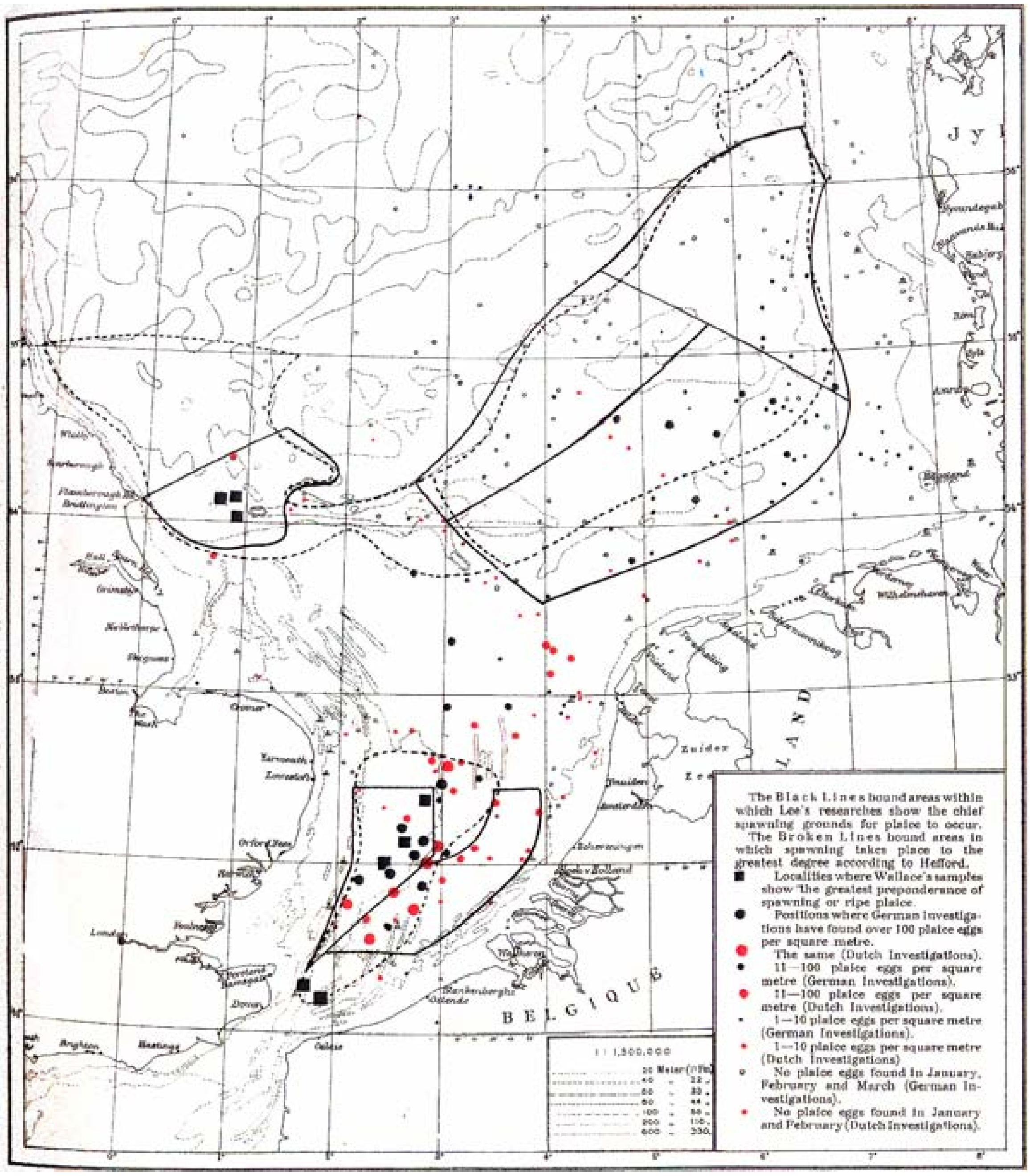

The Black Lines bound areas within which Lee's researches show the chief spawning grounds for plaice to occur.
The Broken Lines bound areas in which spawning takes place to the greatest degree according to Hefford.
Localities where Wallace's samples show the greatest preponderance of spawning or ripe plaice.
Positions where German investigations have found over 100 plaice eggs per square metre.
The same (Dutch Investigations).
11—100 plaice eggs per square metre (German Investigations).
11—100 plaice eggs per square metre (Dutch Investigations).
1—10 plaice eggs per square metre (German Investigations).
1—10 plaice eggs per square metre (Dutch Investigations)
No plaice eggs found in January, February and March (German Investigations).
No plaice eggs found in January and February (Dutch Investigations).
1 : 1,500,000
B E L G I Q U E
Zuider
Yarmouth
Lowestoft
Cromer
Dover
Calais
Ostende
Brighton
Hastings
Boston
Scarborough
Grimsby

Figure 6

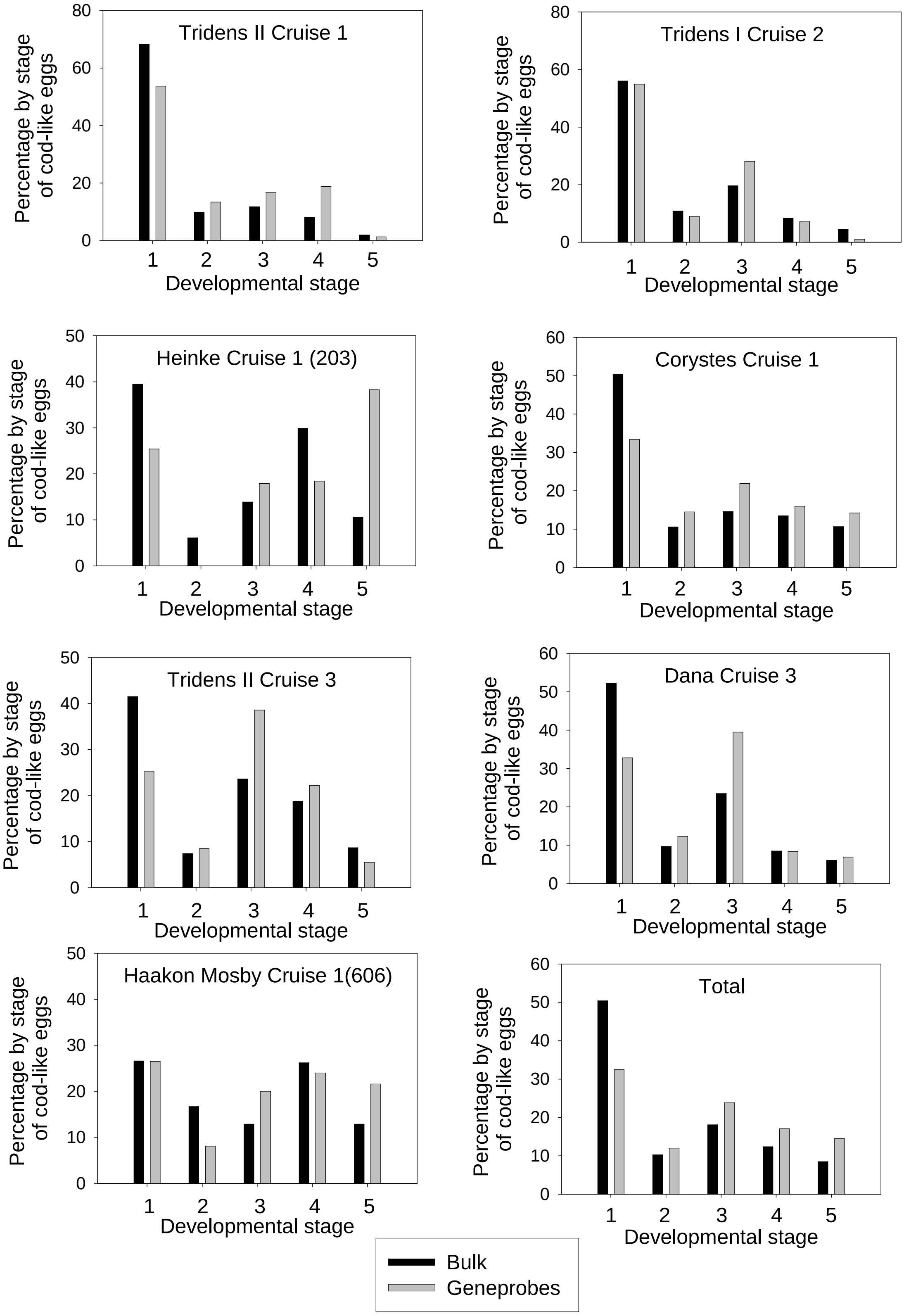

Tridens II Cruise 1
Tridens I Cruise 2
Heinke Cruise 1 (203)
Corystes Cruise 1
Tridens II Cruise 3
Dana Cruise 3
Haakon Mosby Cruise 1(606)
Total
Percentage by stage of cod-like eggs
Developmental stage
Bulk
Geneprobes

Figure 7

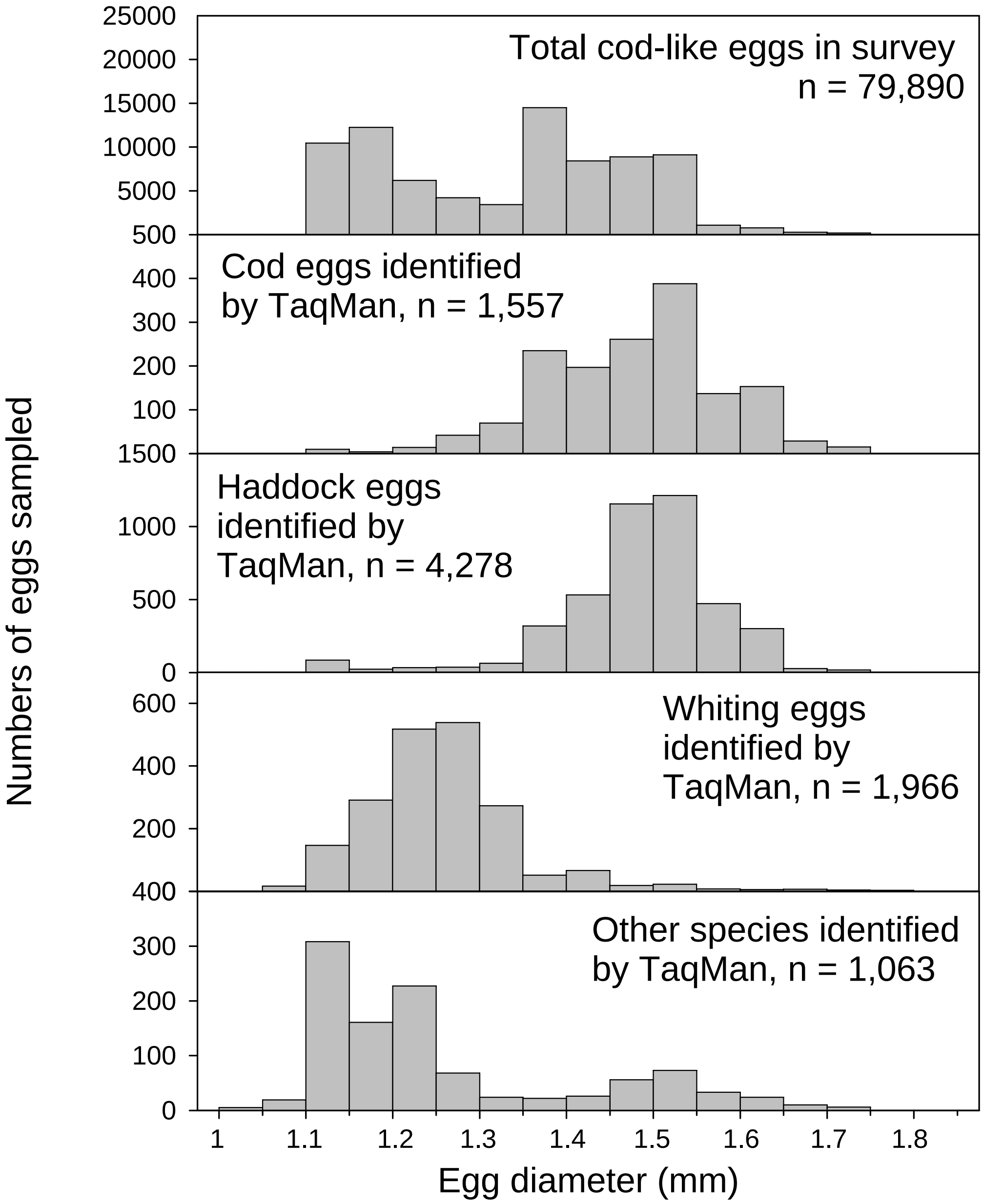
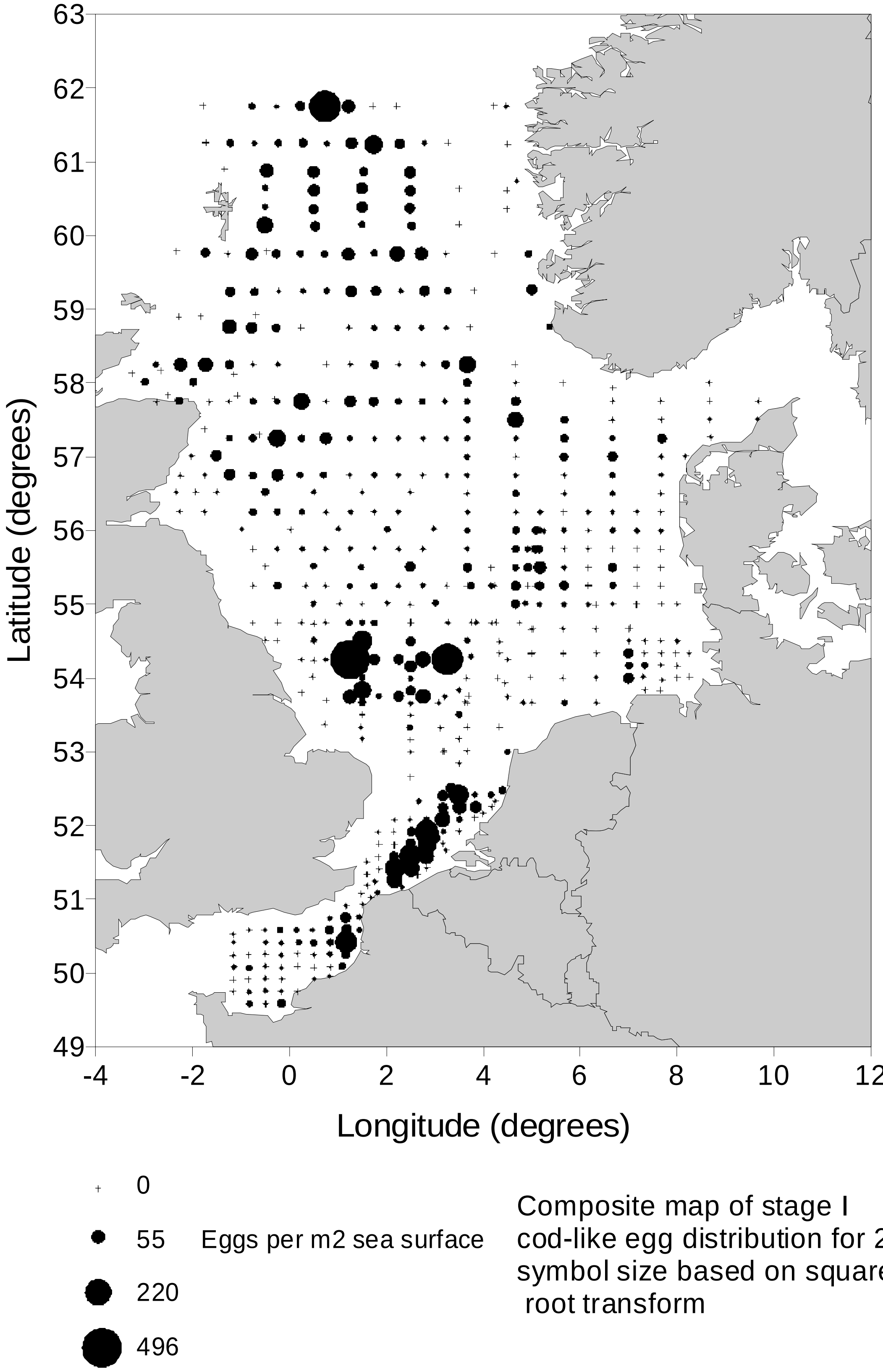
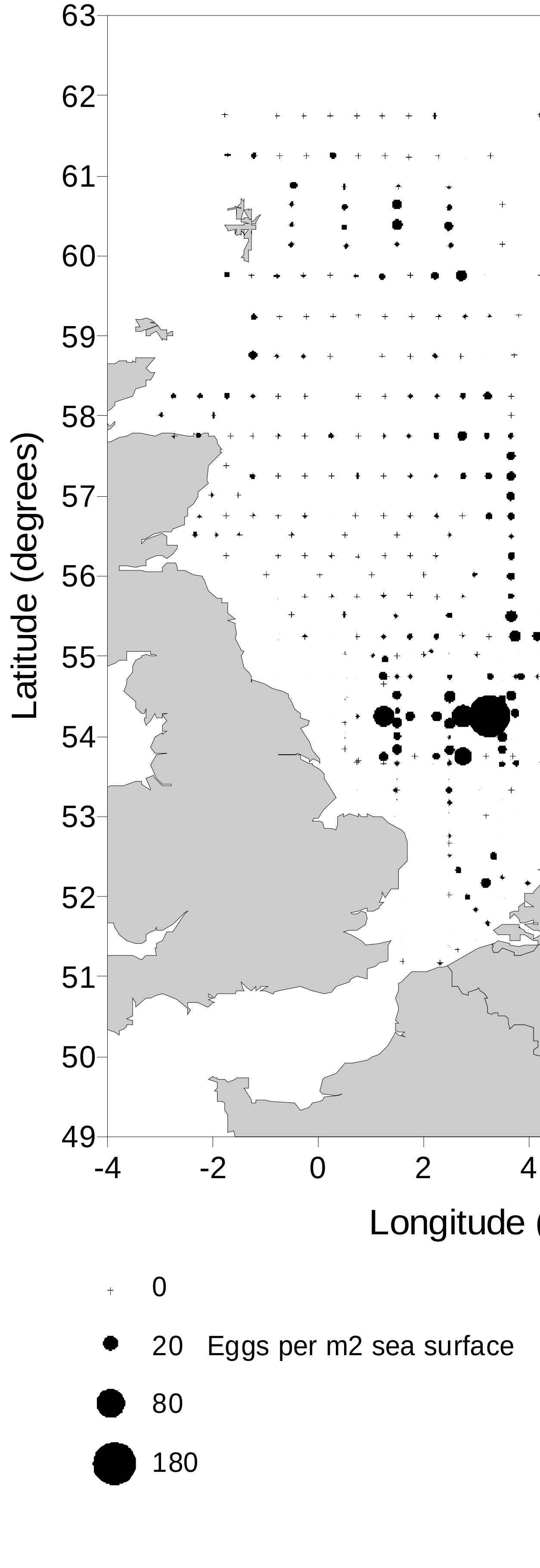

Total cod-like eggs in survey
n = 79,890
Cod eggs identified
by TaqMan, n = 1,557
Haddock eggs
identified by
TaqMan, n = 4,278
Whiting eggs
identified by
TaqMan, n = 1,966
Other species identified
by TaqMan, n = 1,063
Numbers of eggs sampled
Egg diameter (mm)
25000
20000
15000
10000
5000
500
400
300
200
100
1500
1000
500
0
600
400
200
400
300
200
100
0
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8

Figure 8



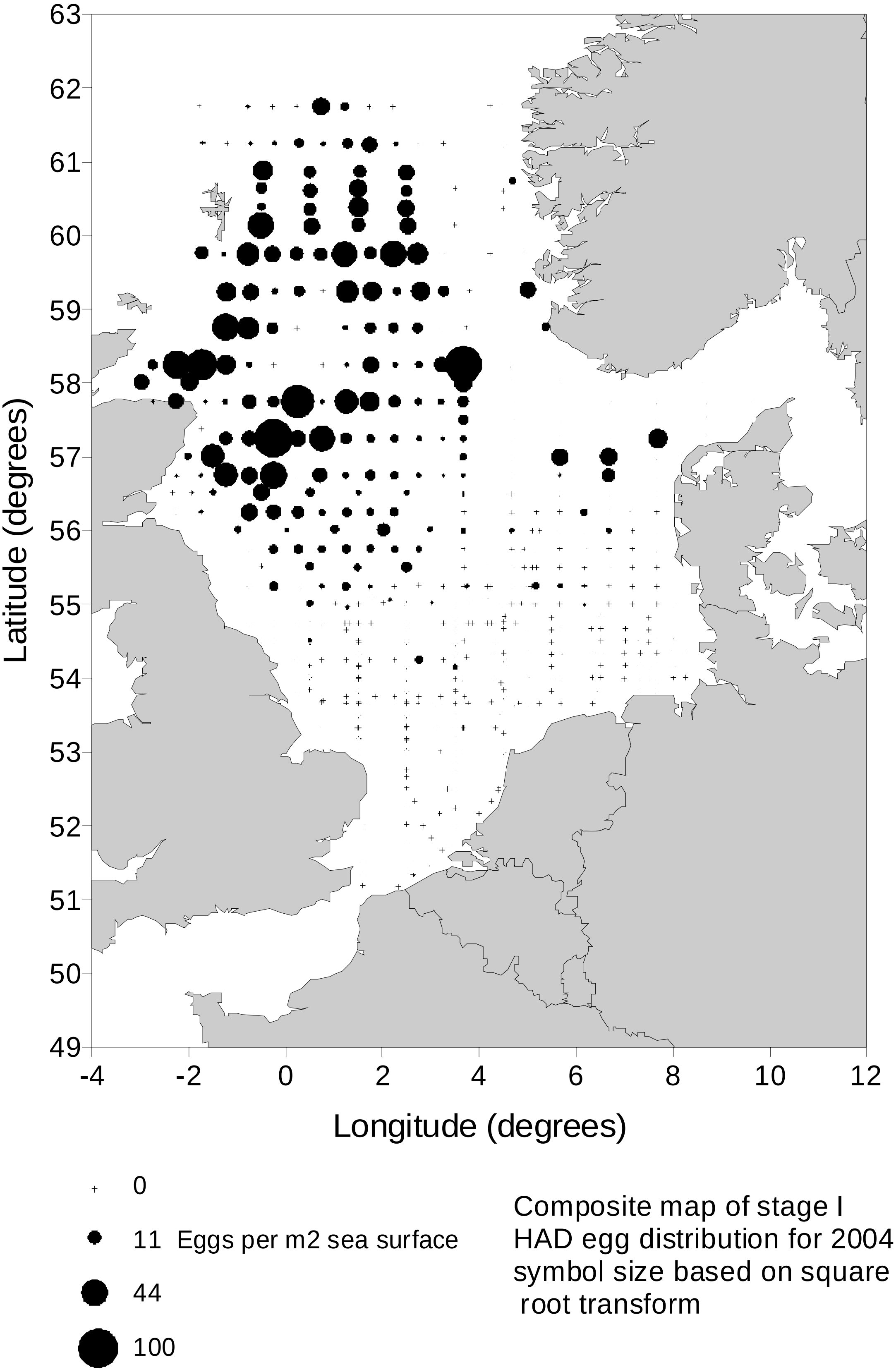
Composite map of stage I cod-like egg distribution for 2004, symbol size based on square root transform

Figure 9



Composite map of stage I COD egg distribution for 2004, symbol size based on square root transform

Figure 10



Composite map of stage I HAD egg distribution for 2004, symbol size based on square root transform

Figure 11

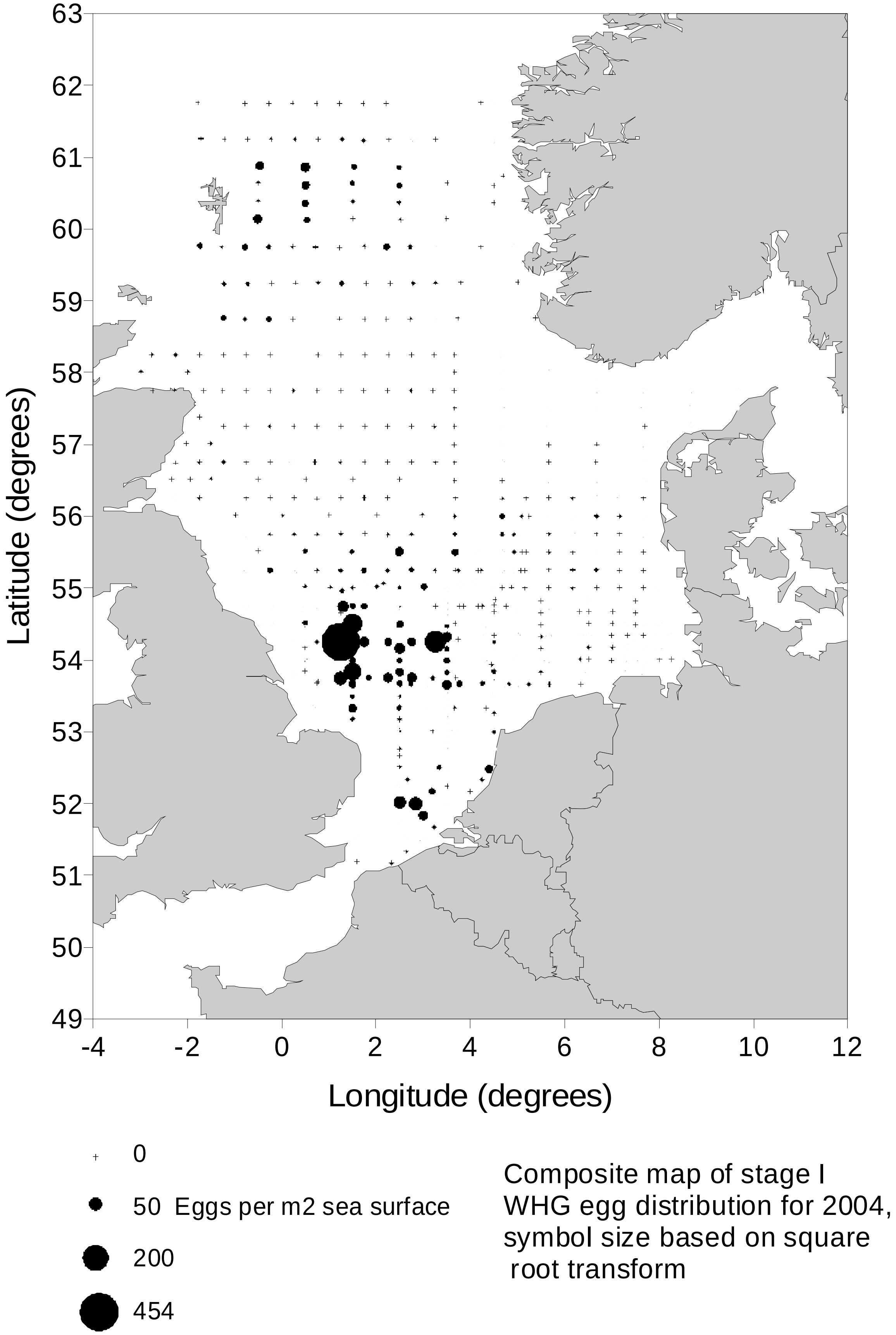
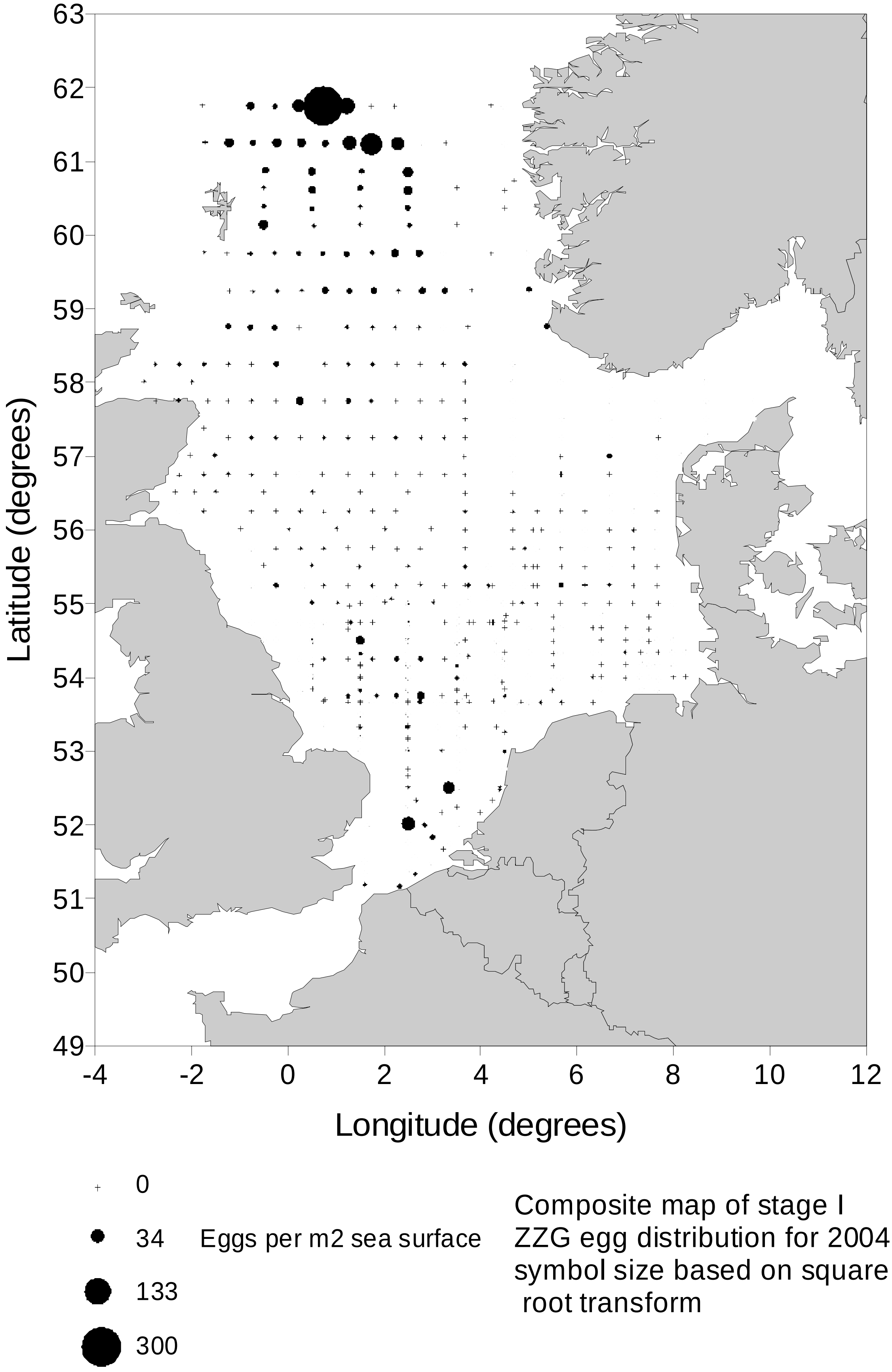

Latitude (degrees)
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
-4
-2
0
2
4
6
8
10
12
Longitude (degrees)
0
50 Eggs per m2 sea surface
200
454
Composite map of stage I
WHG egg distribution for 2004,
symbol size based on square
root transform

Figure 12



Composite map of stage I ZZG egg distribution for 2004, symbol size based on square root transform

Figure 13

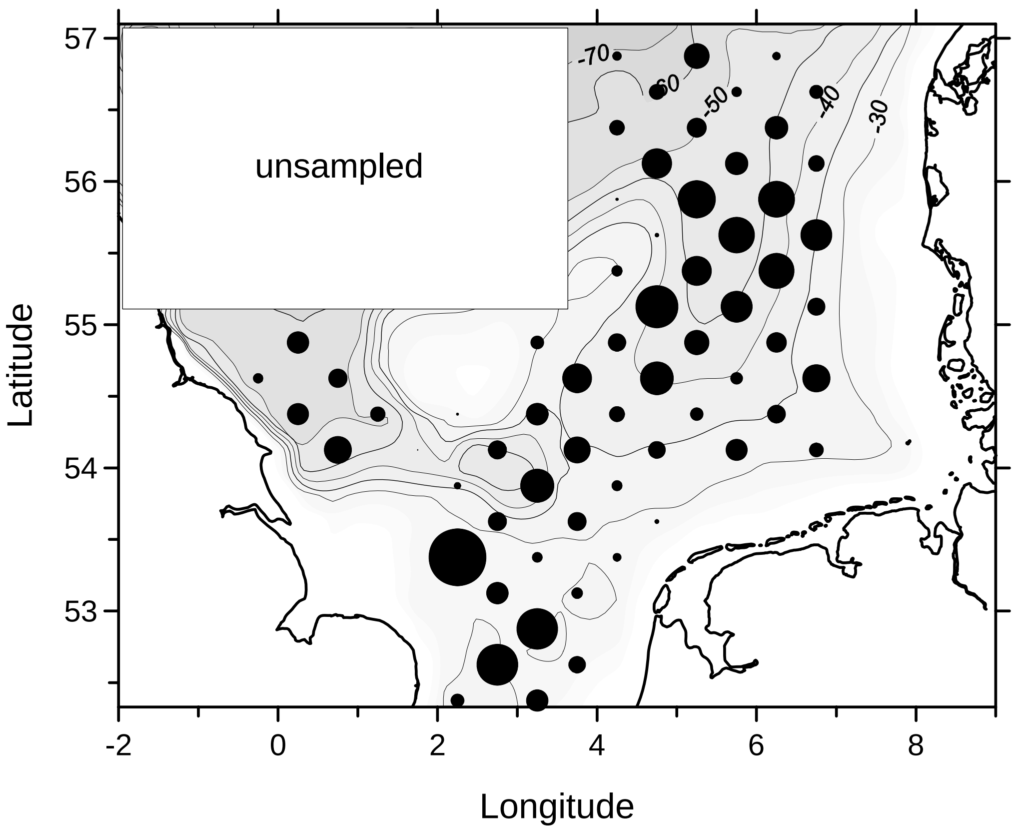

unsampled
-70
-60
-50
-40
-30
57
56
55
54
53
Latitude
-2
0
2
4
6
8
Longitude

Figure 14

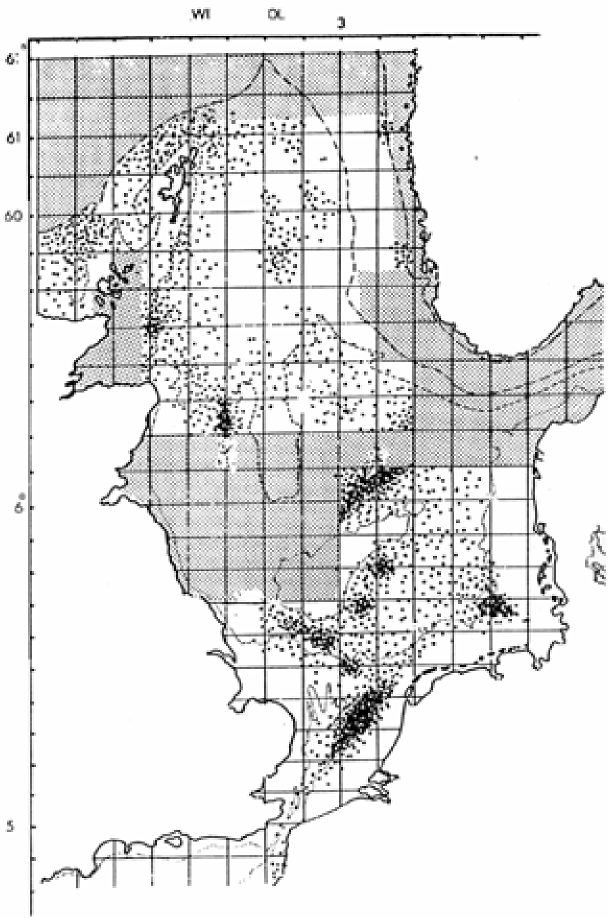
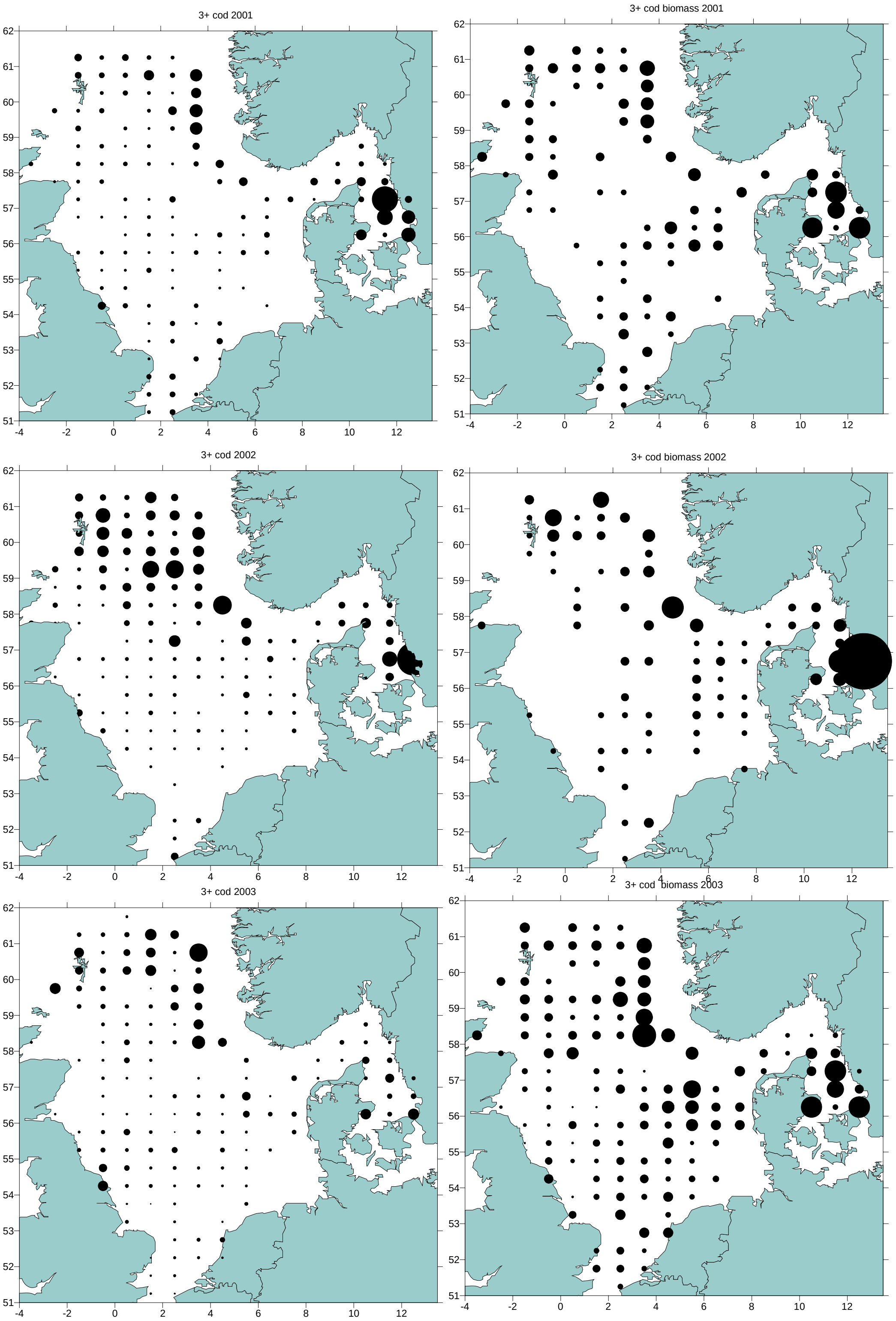
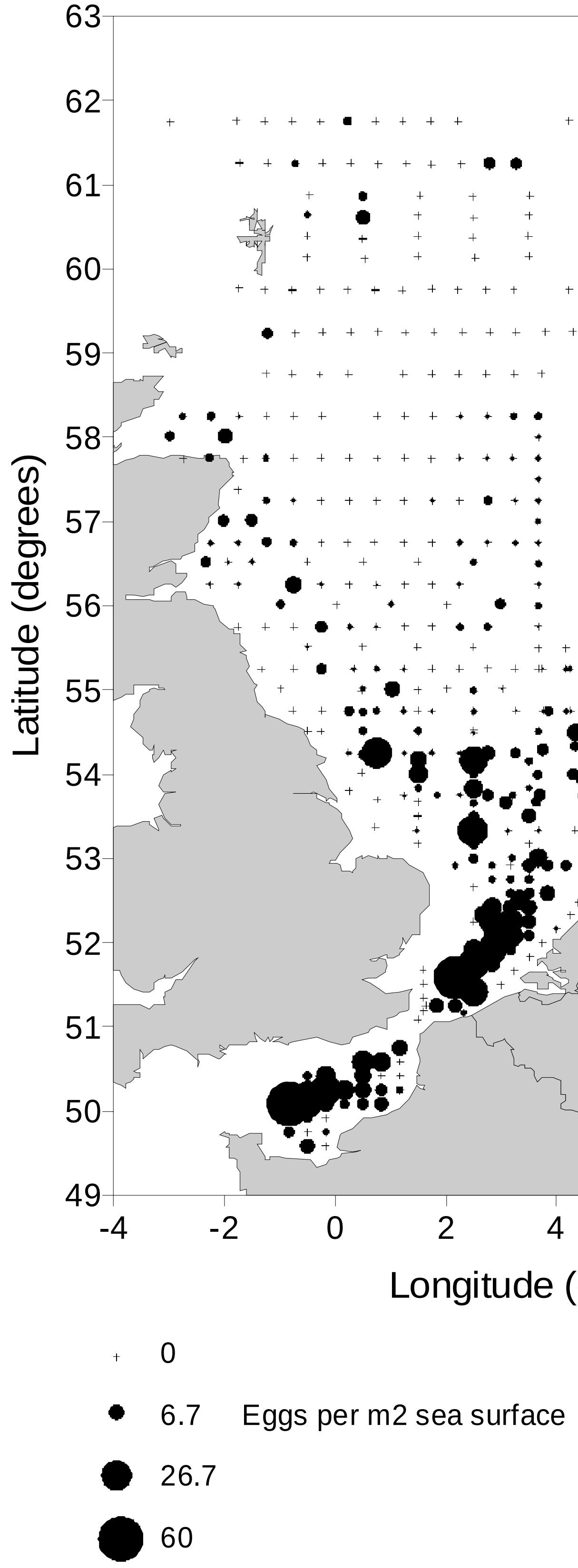


Figure 15

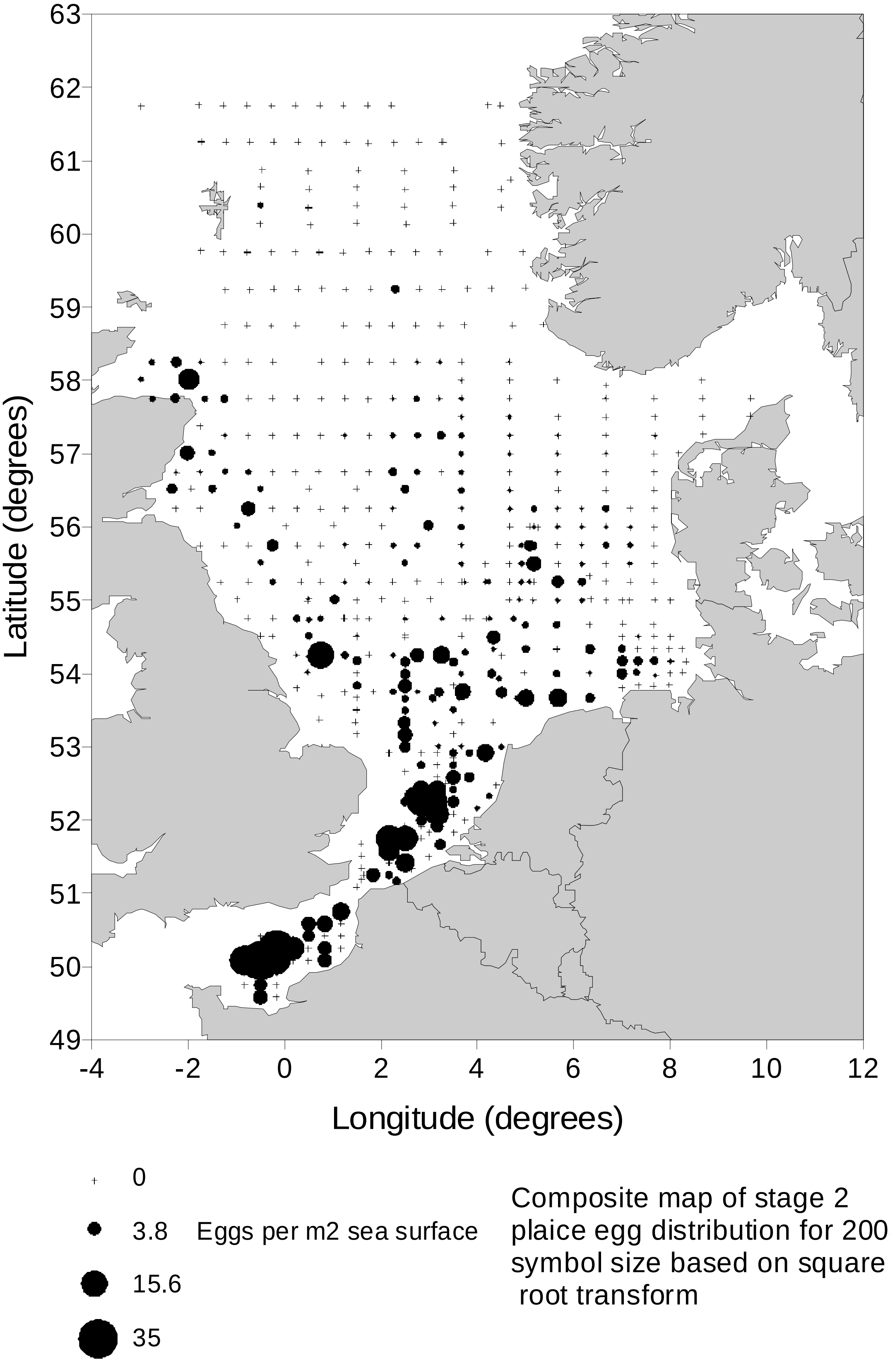

3+ cod 2001
3+ cod biomass 2001
3+ cod 2002
3+ cod biomass 2002
3+ cod 2003
3+ cod biomass 2003
62
61
60
59
58
57
56
55
54
53
52
51
-4
-2
0
2
4
6
8
10
12

Annex 4: Distribution maps for all developmental stages of plaice, cod, haddock, whiting and other cod-like eggs

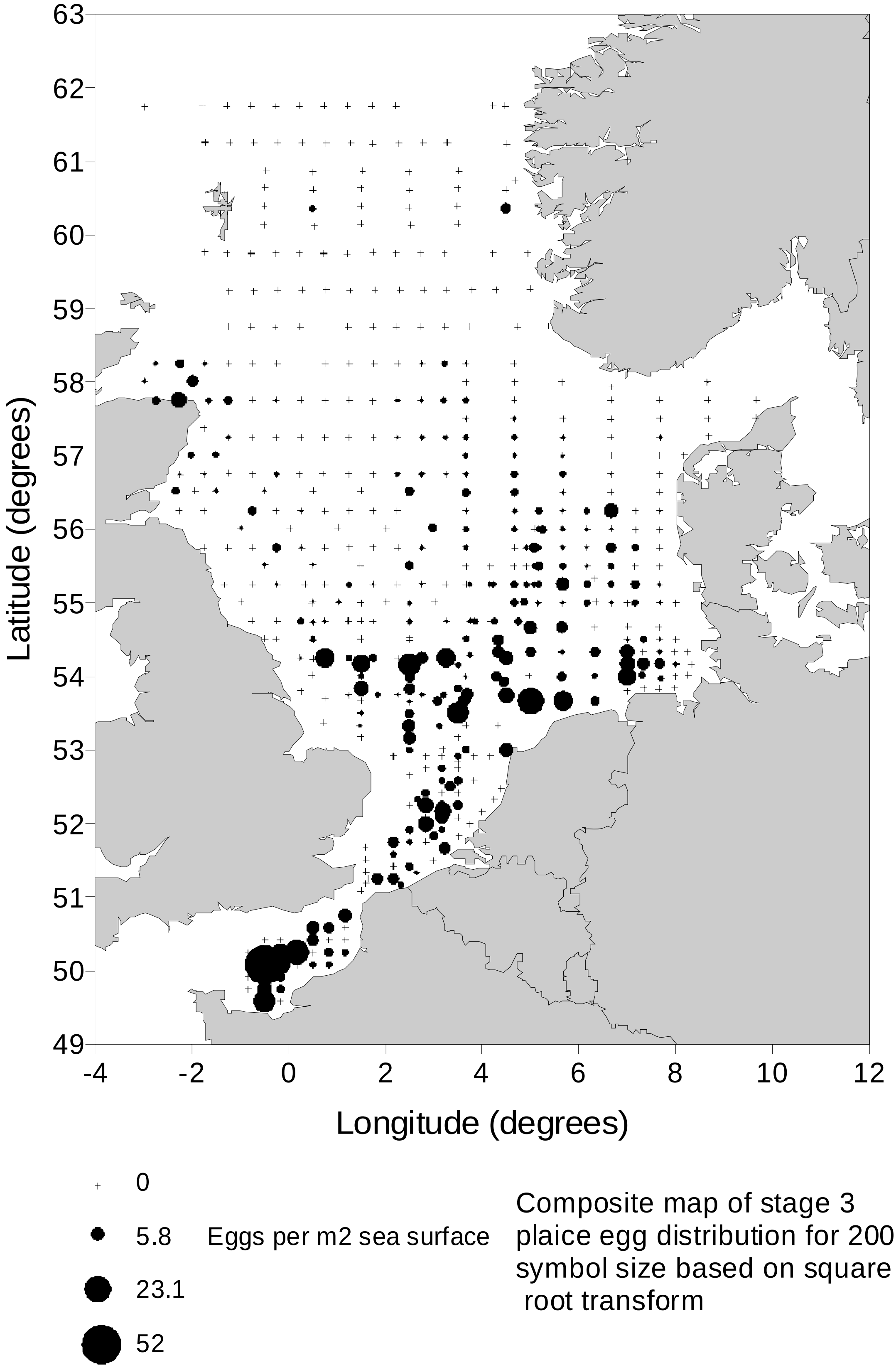
Species are coded as follows PLE = plaice; COD = Cod; HAD=Haddock; WHG = Whiting; ZZG = other species with cod-like eggs



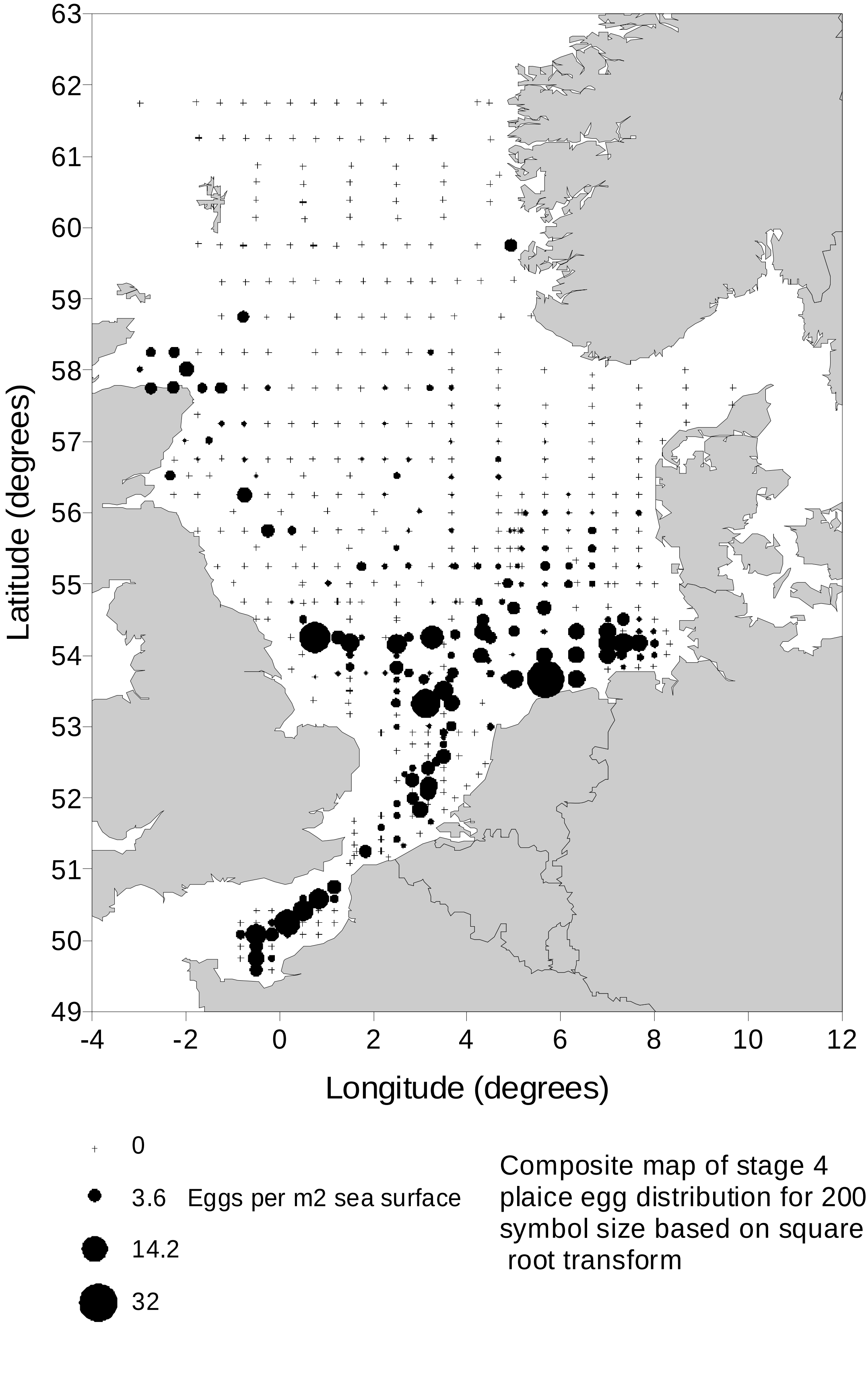
Composite map of stage I plaice egg distribution for 2004, symbol size based on square root transform



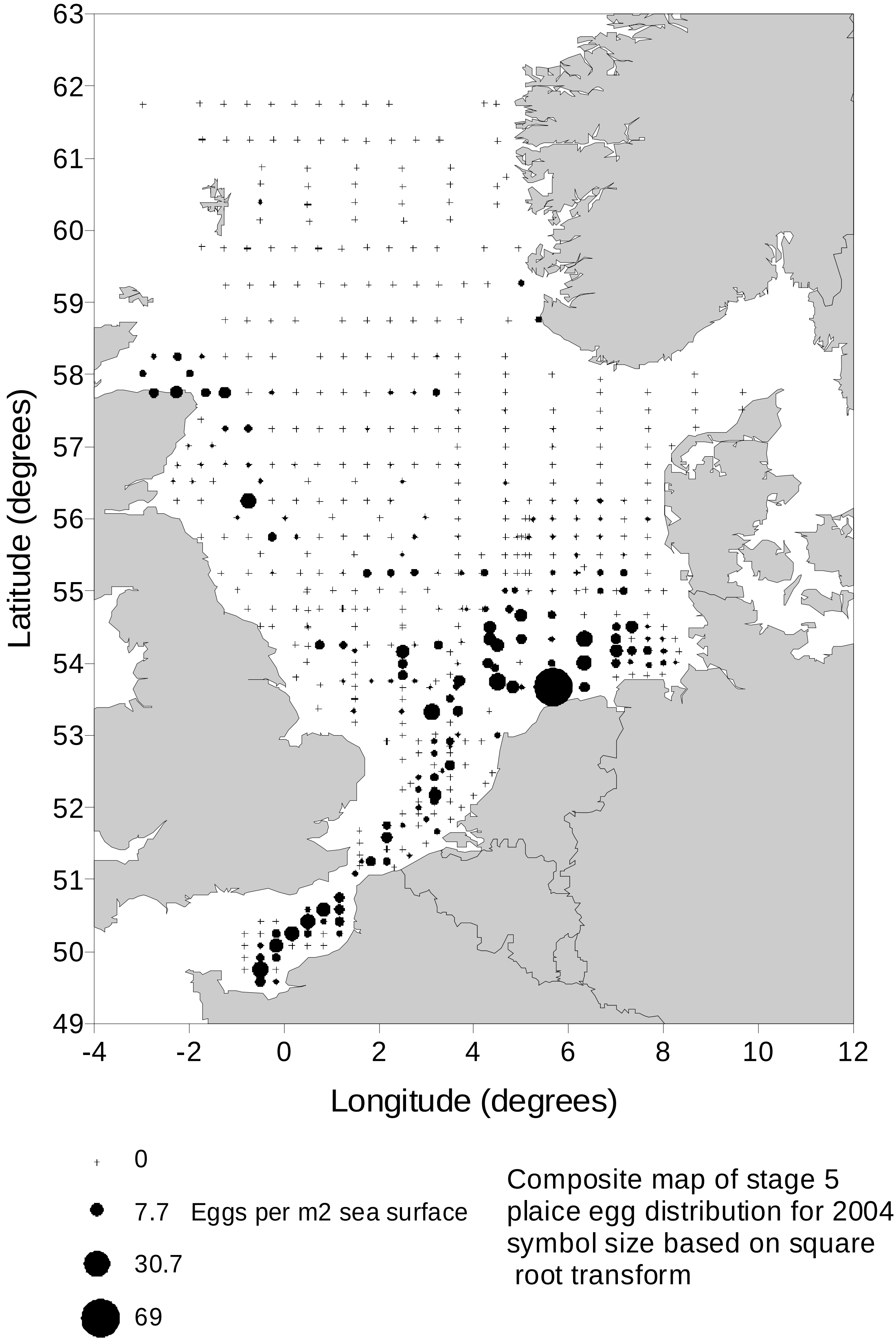
Composite map of stage 2 plaice egg distribution for 2004, symbol size based on square root transform



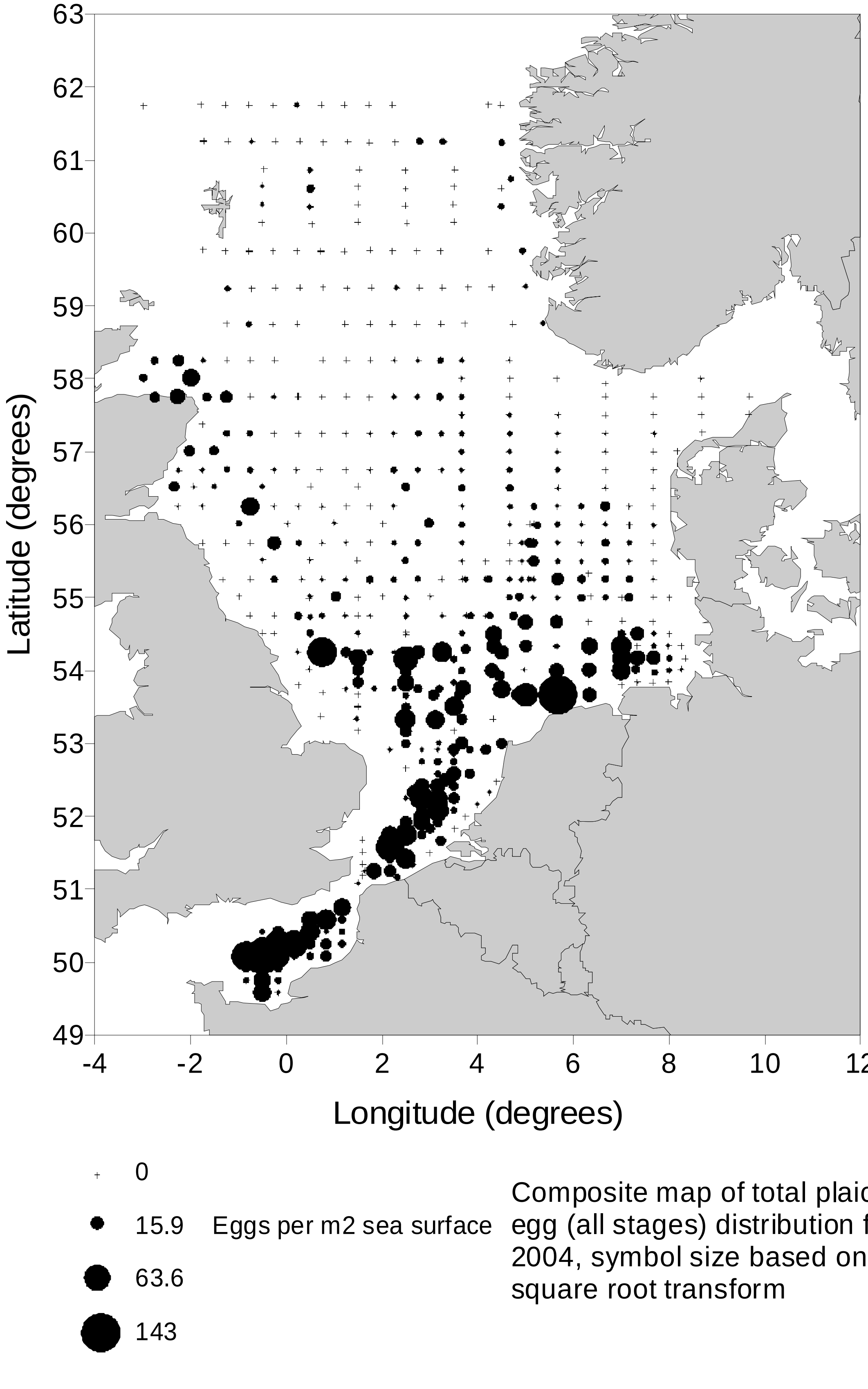
Composite map of stage 3 plaice egg distribution for 2004, symbol size based on square root transform



Composite map of stage 4 plaice egg distribution for 2004, symbol size based on square root transform

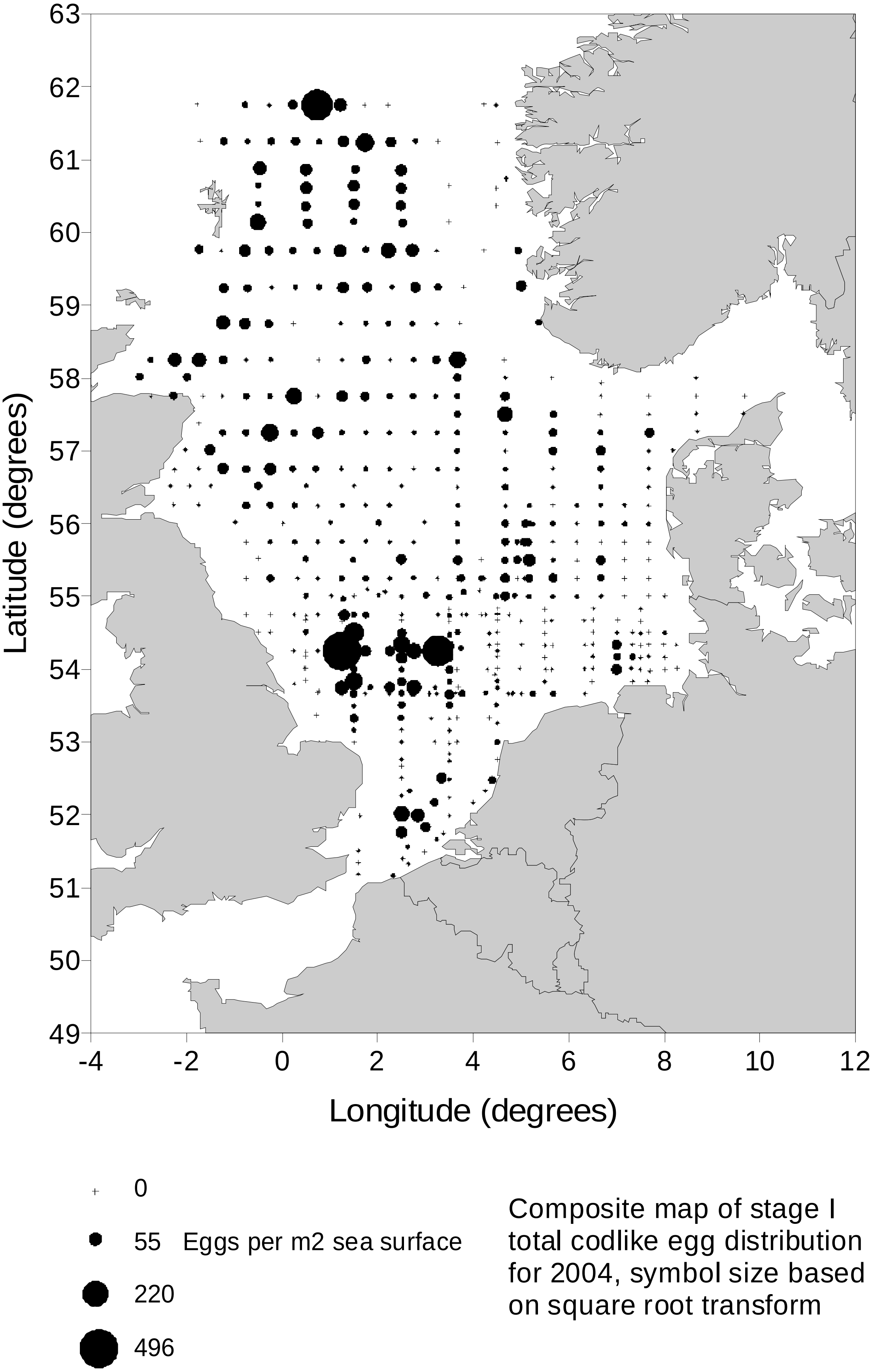


Composite map of stage 5 plaice egg distribution for 2004, symbol size based on square root transform

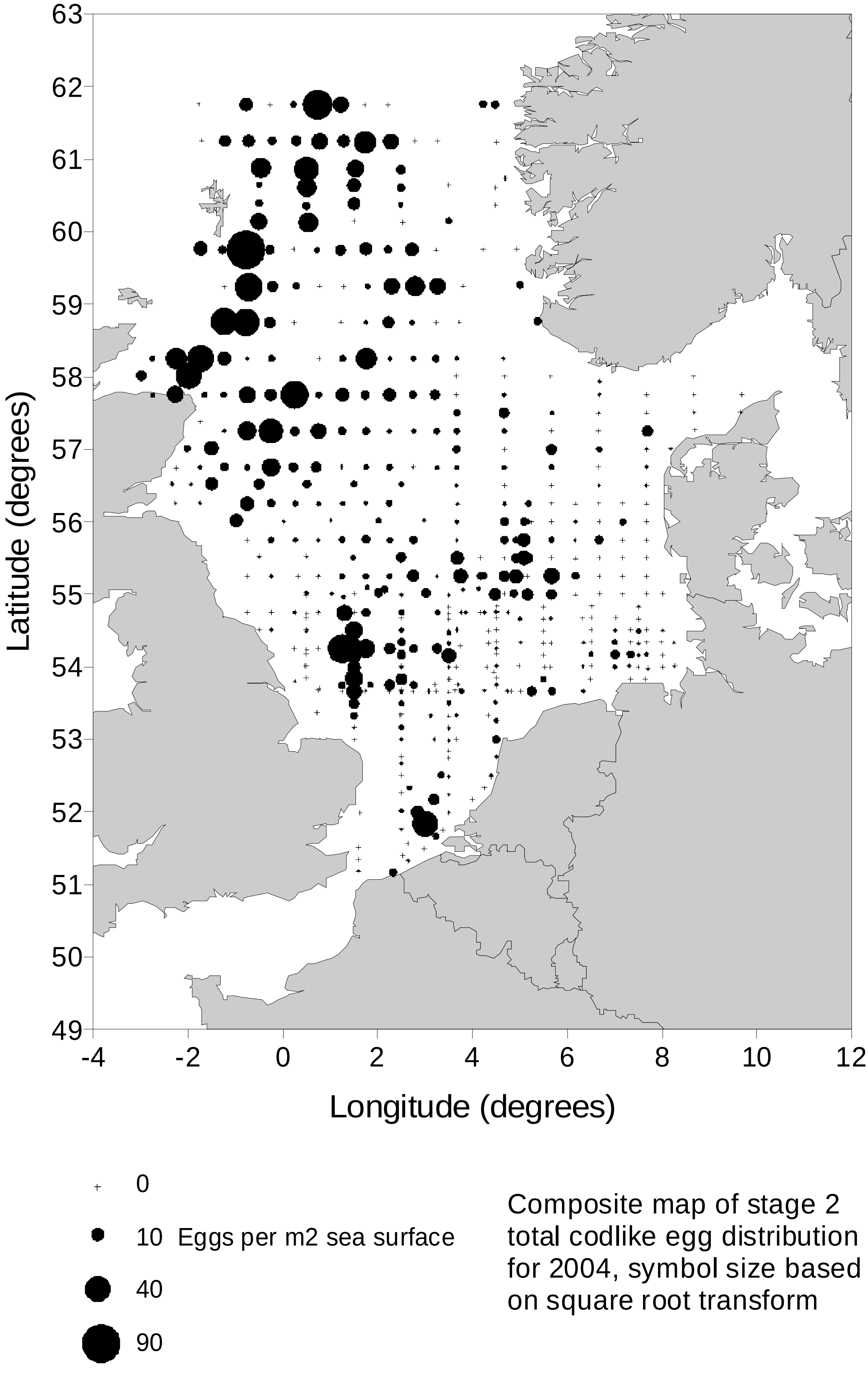


Composite map of total plaice egg (all stages) distribution for 2004, symbol size based on square root transform

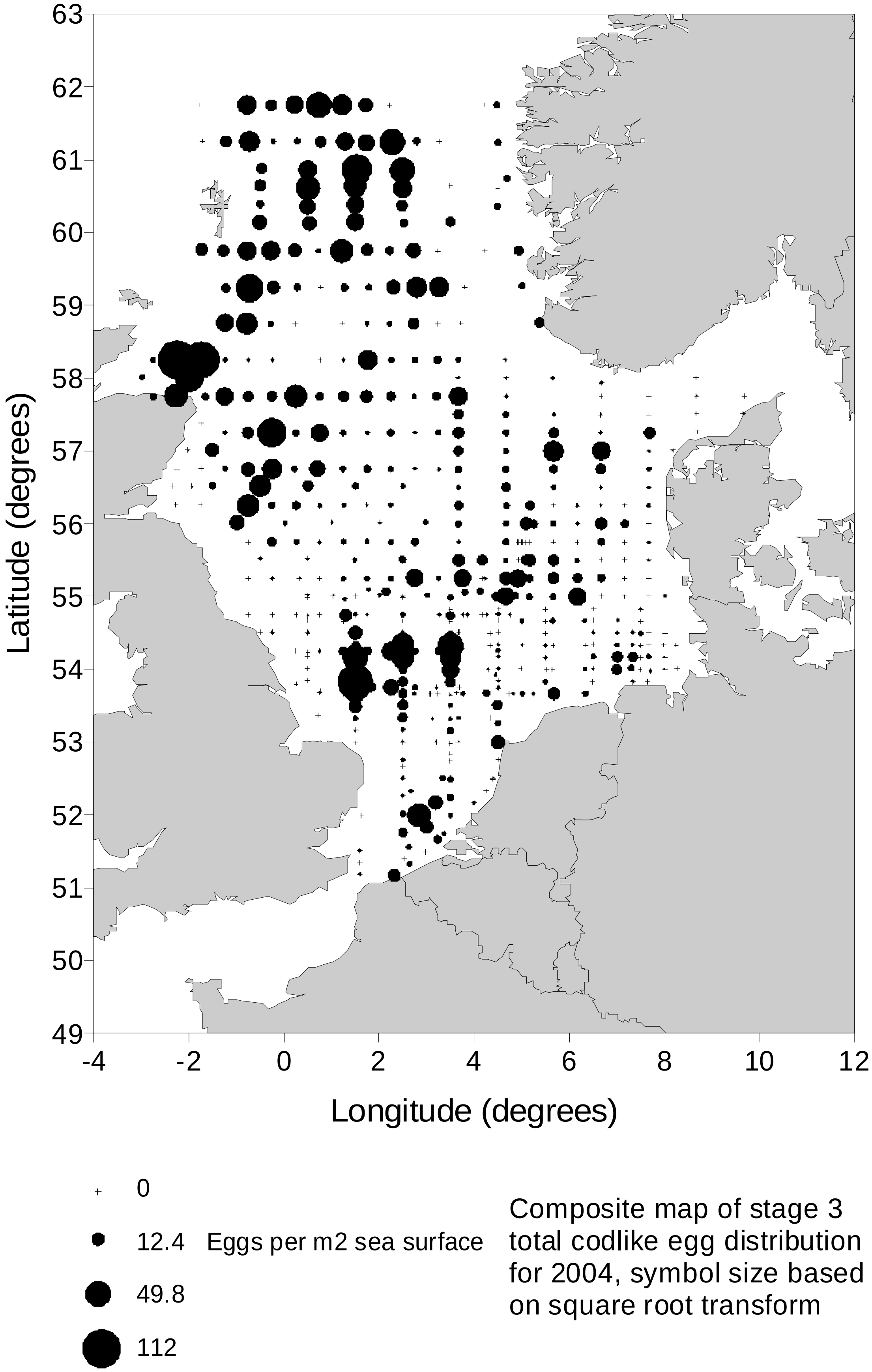
Distribution maps of total cod-like eggs (> 1.1 mm and < 1.75 mm diameter lacking oil globules) in the North Sea in 2004



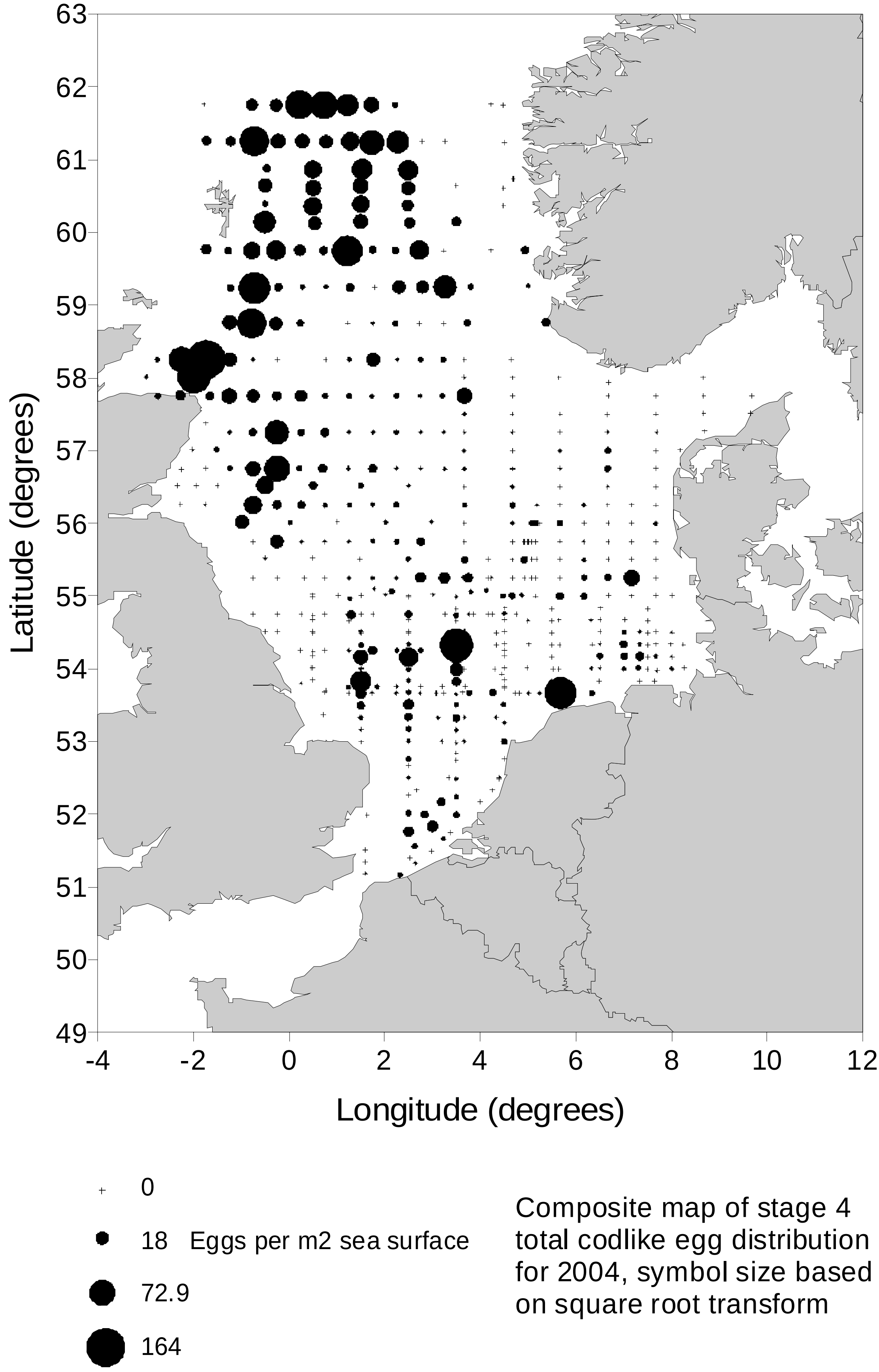
Composite map of stage I total codlike egg distribution for 2004, symbol size based on square root transform



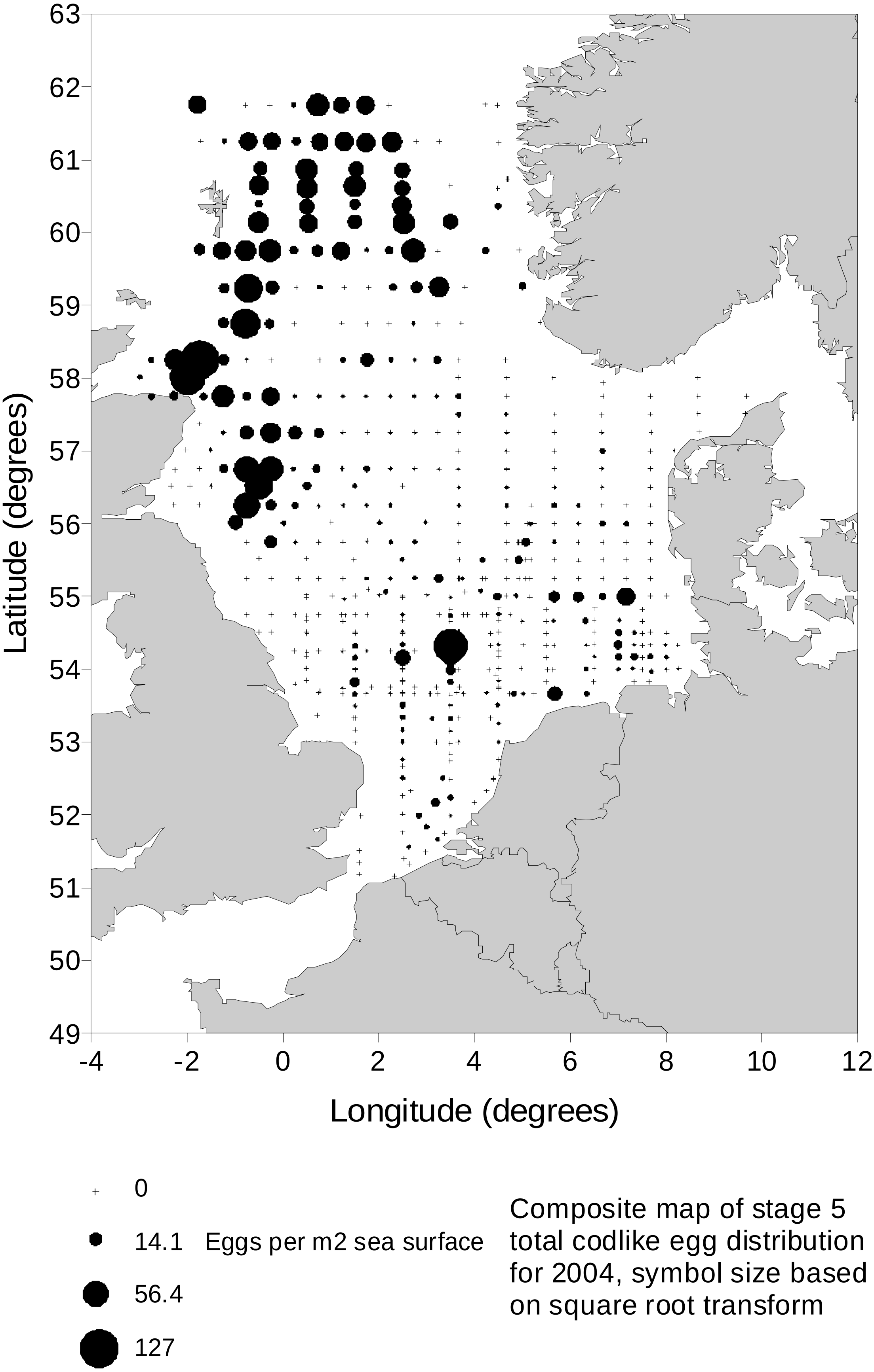
Composite map of stage 2 total codlike egg distribution for 2004, symbol size based on square root transform



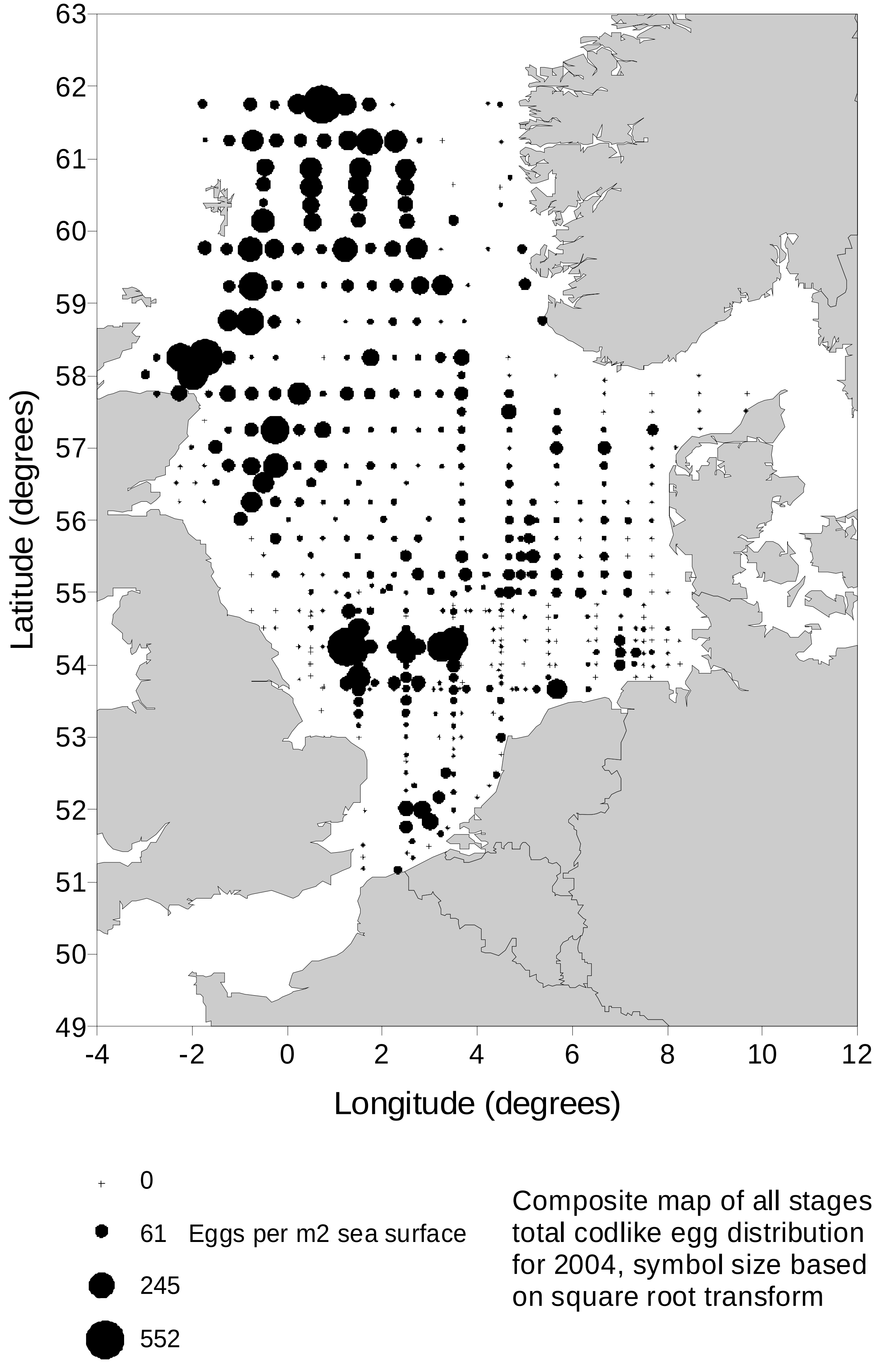
Composite map of stage 3 total codlike egg distribution for 2004, symbol size based on square root transform



Composite map of stage 4 total codlike egg distribution for 2004, symbol size based on square root transform



Composite map of stage 5 total codlike egg distribution for 2004, symbol size based on square root transform



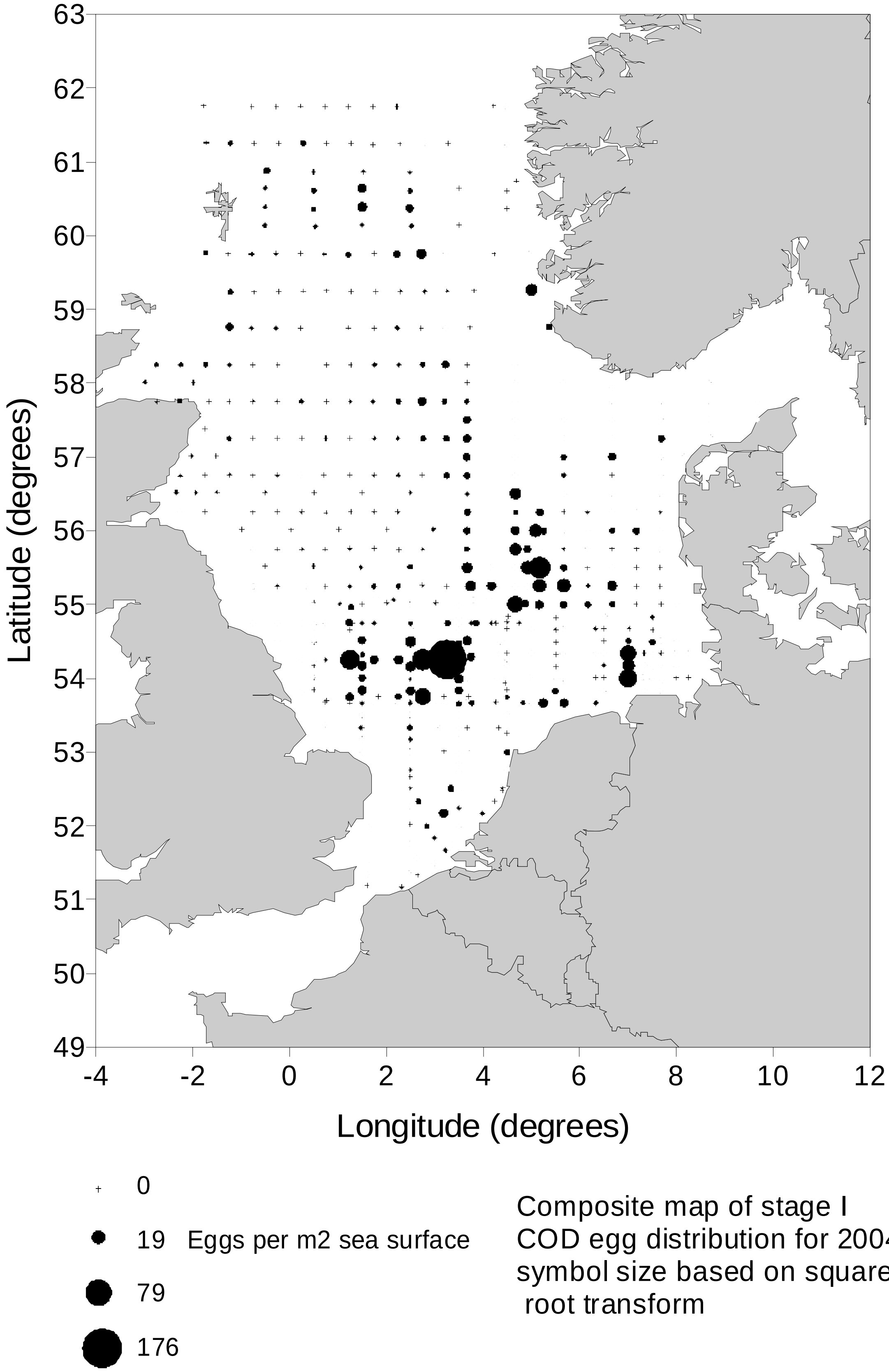
Composite map of all stages total codlike egg distribution for 2004, symbol size based on square root transform

Distribution maps of cod eggs in the North Sea in 2004

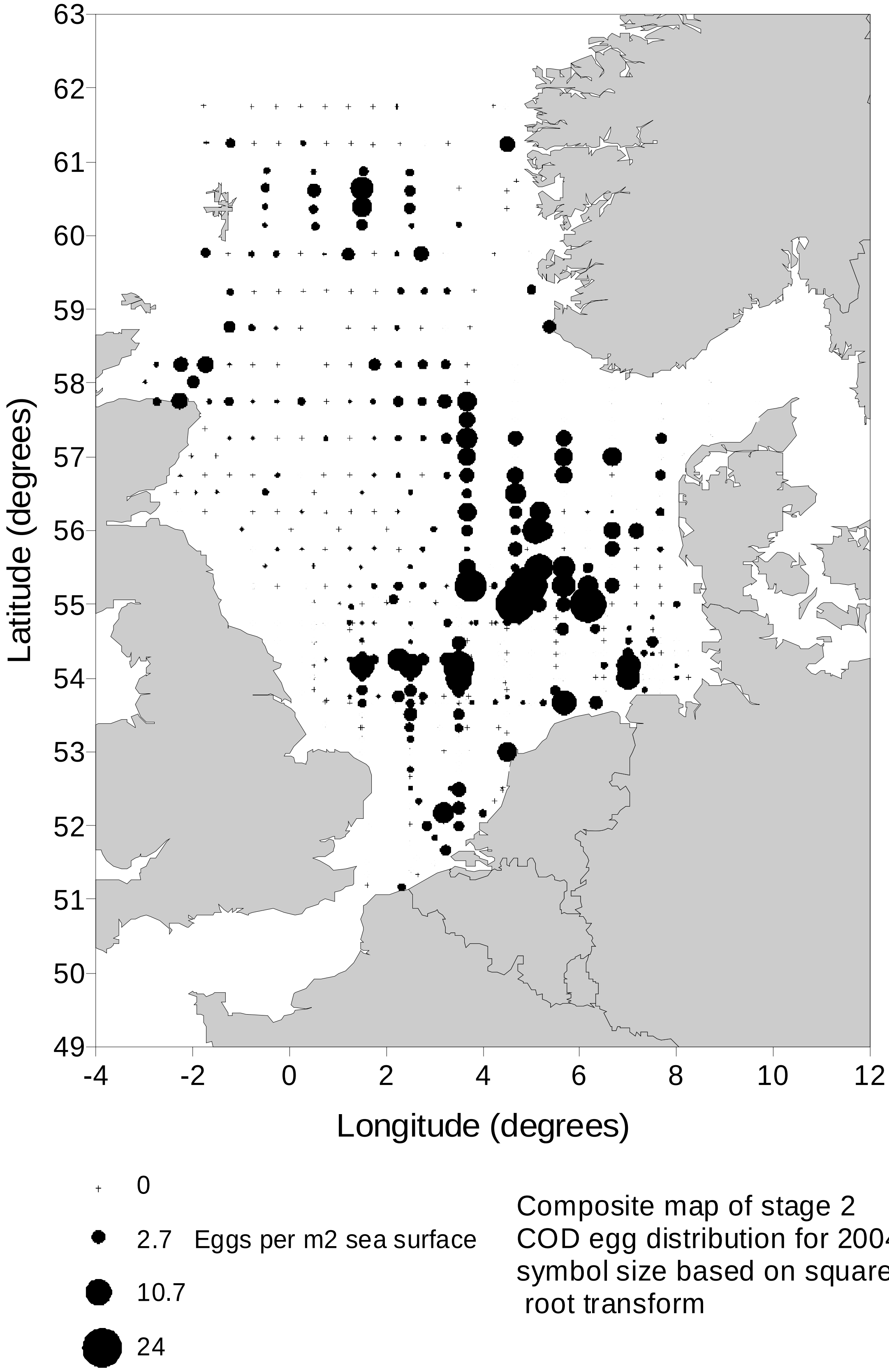
The abundance of total cod-like eggs was reduced in proportion to the ratio cod to haddock plus whiting plus other unidentified species as determined using genetic probes on a sub-sample of the eggs.

Because too few eggs of specific stages were sub-sampled on some stations, the ratio of species on each station was based on all the eggs sub-sampled for TaqMan analysis (i.e. all developmental stages).

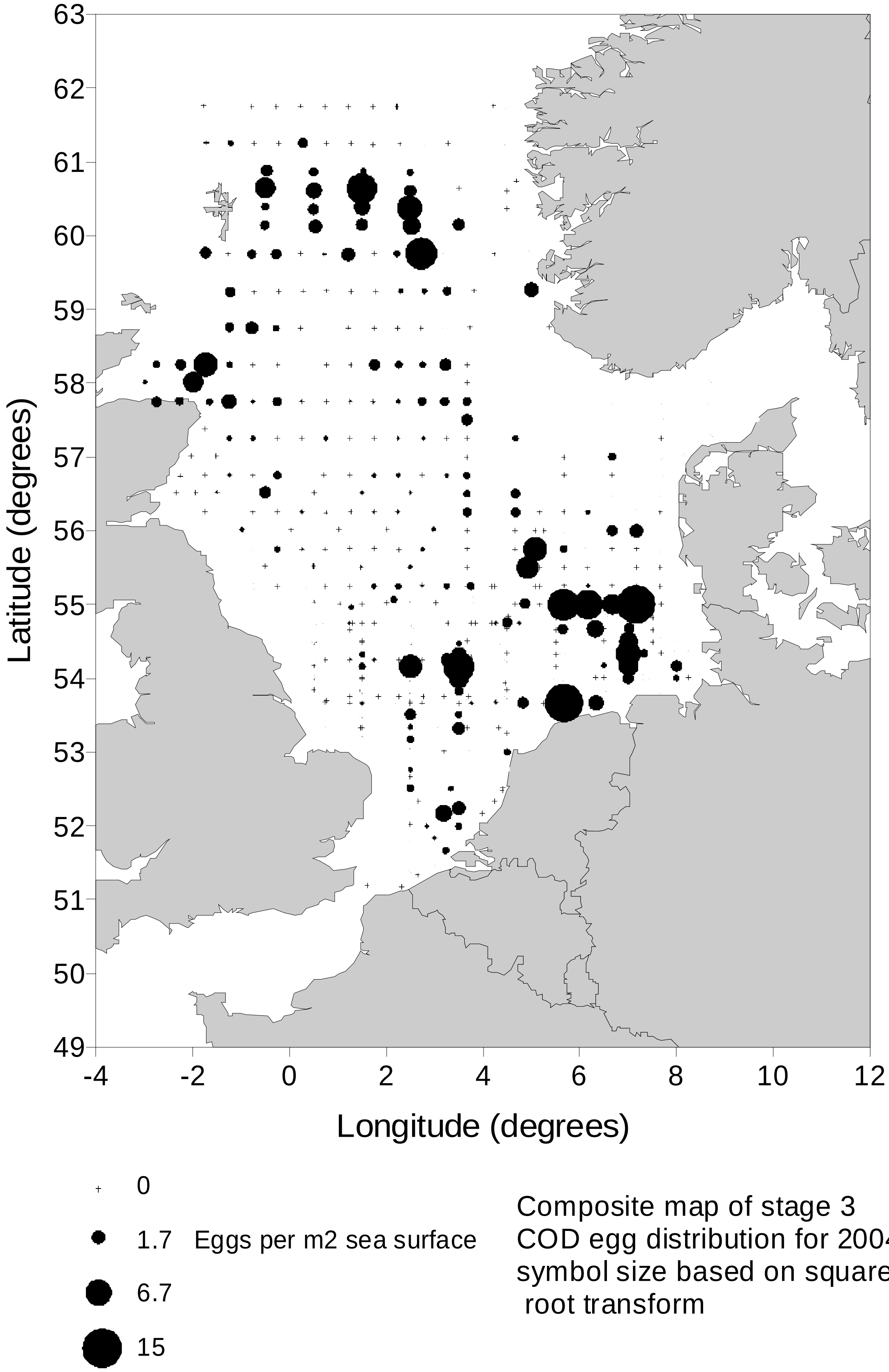
Stations where cod-like eggs were recorded but insufficient cod-like eggs were sub-sampled for genetic analysis have been excluded.



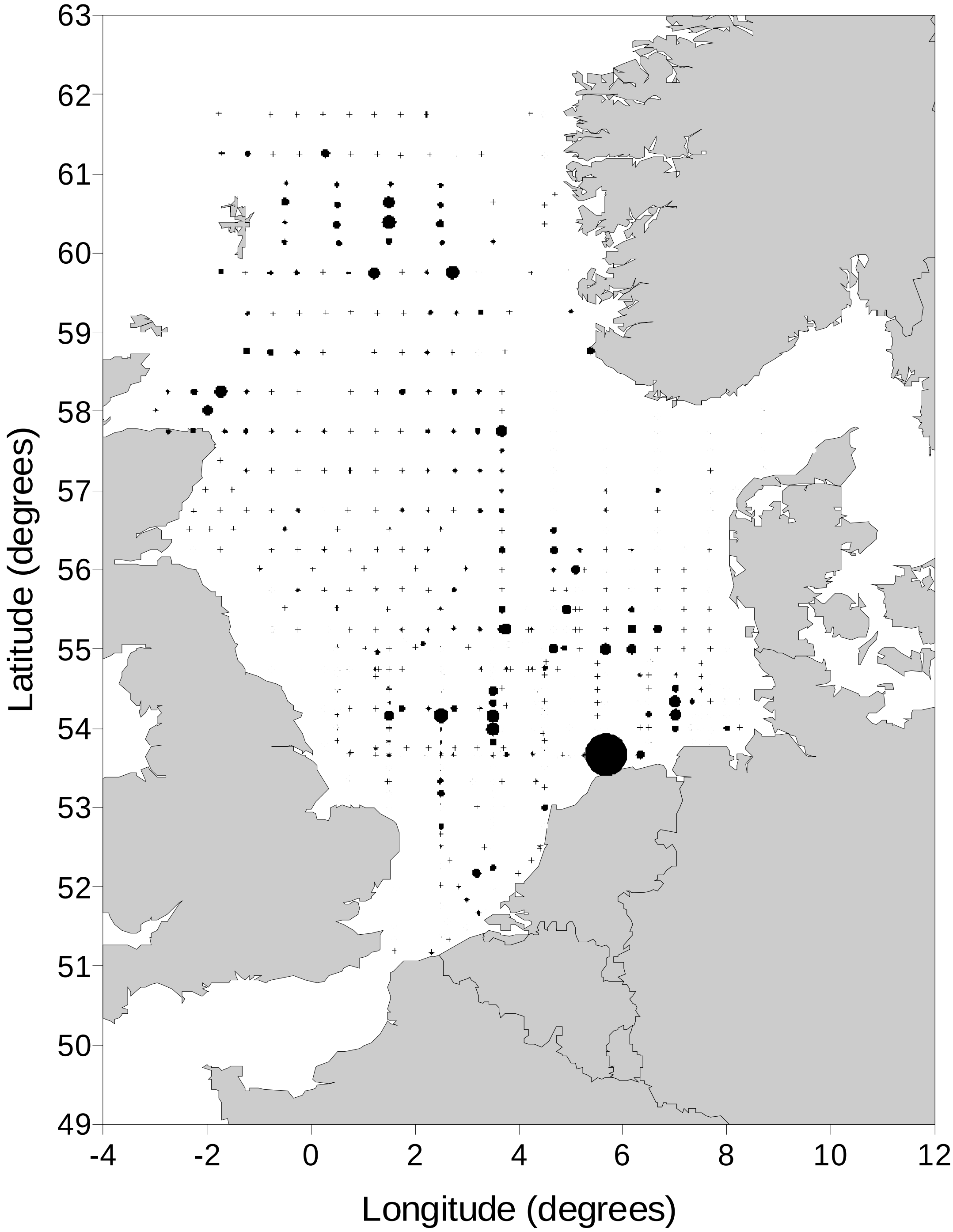
Composite map of stage I COD egg distribution for 2004, symbol size based on square root transform



Composite map of stage 2 COD egg distribution for 2004, symbol size based on square root transform

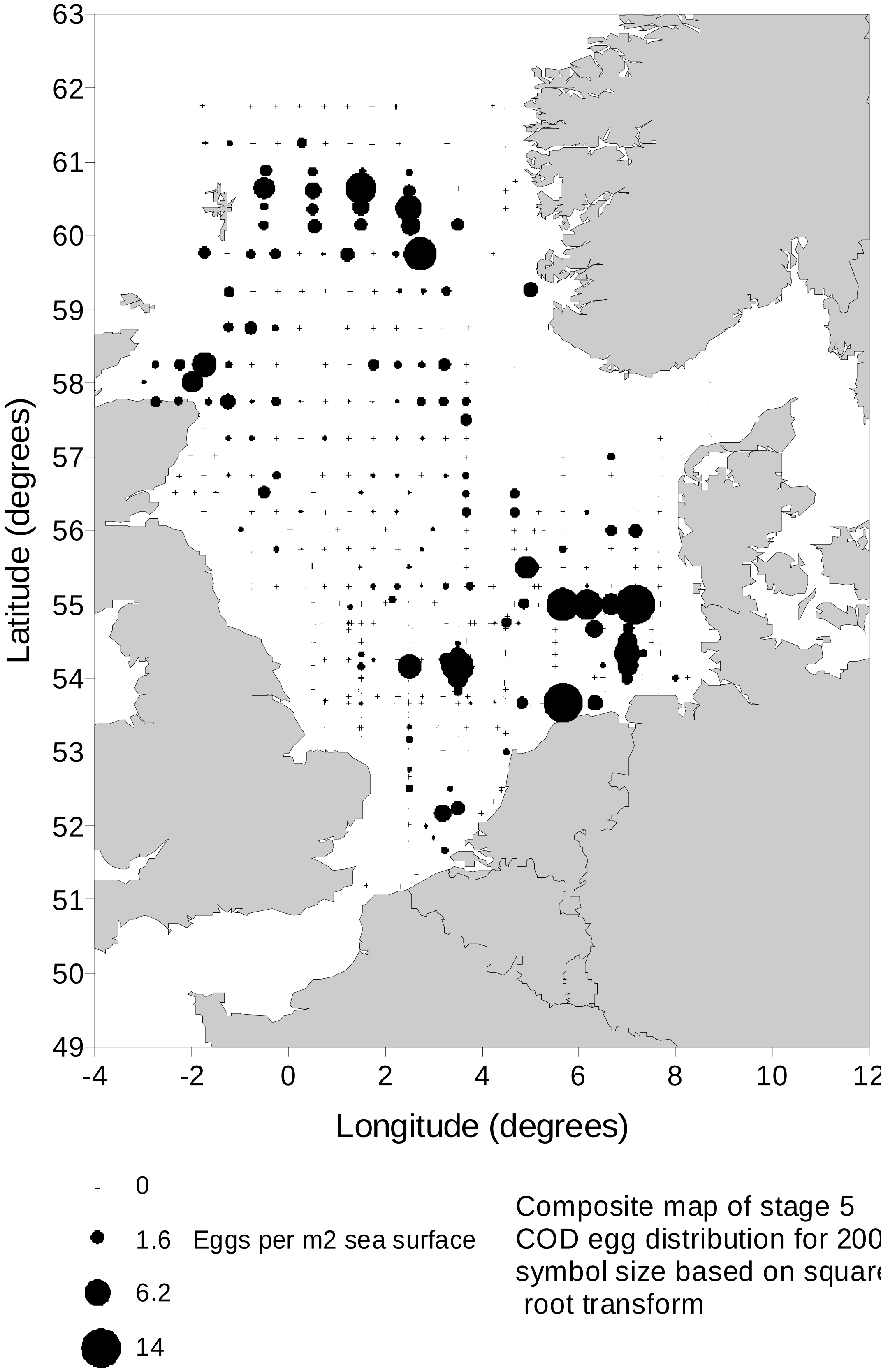


Composite map of stage 3 COD egg distribution for 2004, symbol size based on square root transform

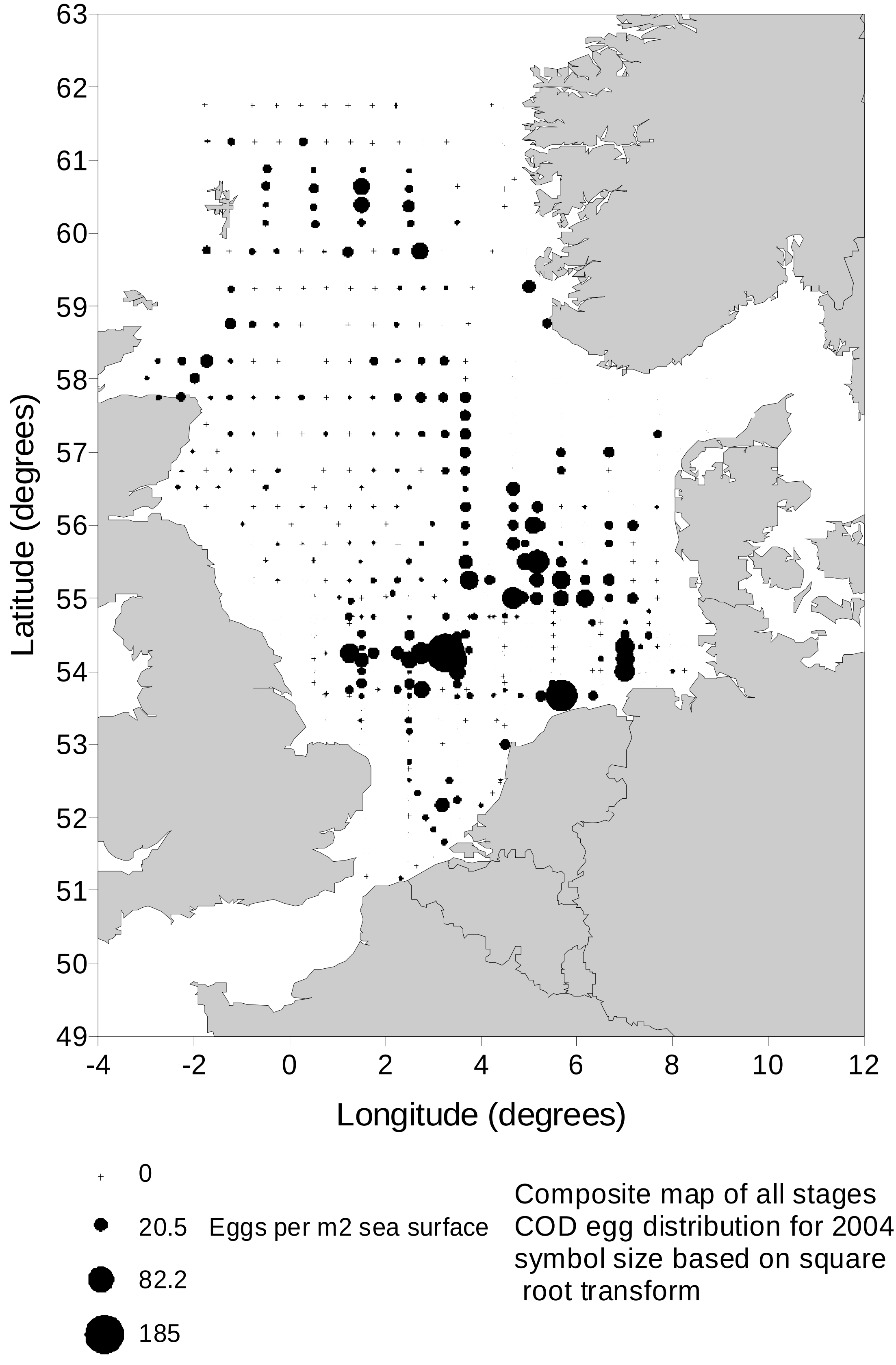


0
9.7 Eggs per m2 sea surface
38.7
87

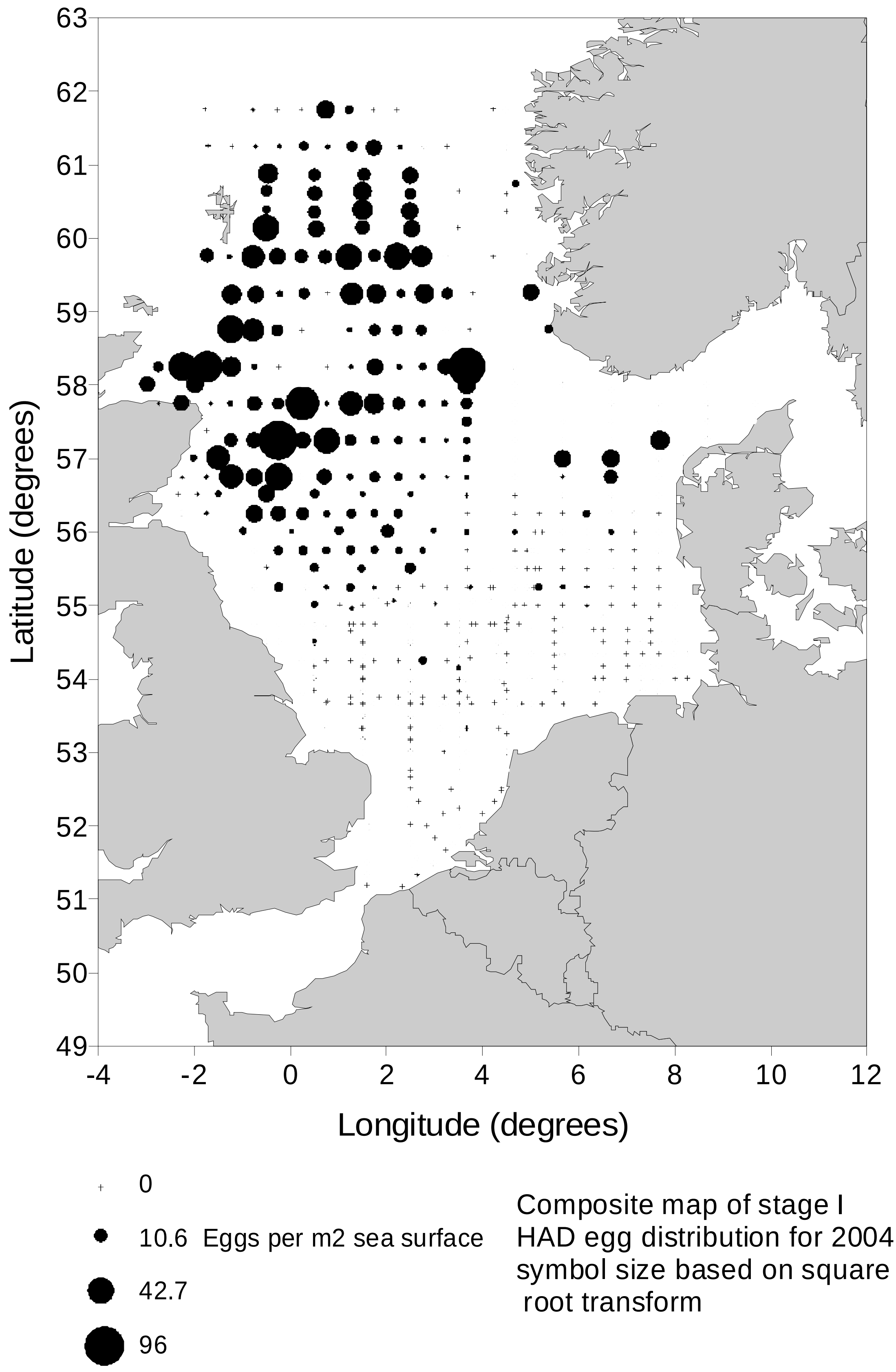
Composite map of stage 4 COD egg distribution for 2004, symbol size based on square root transform



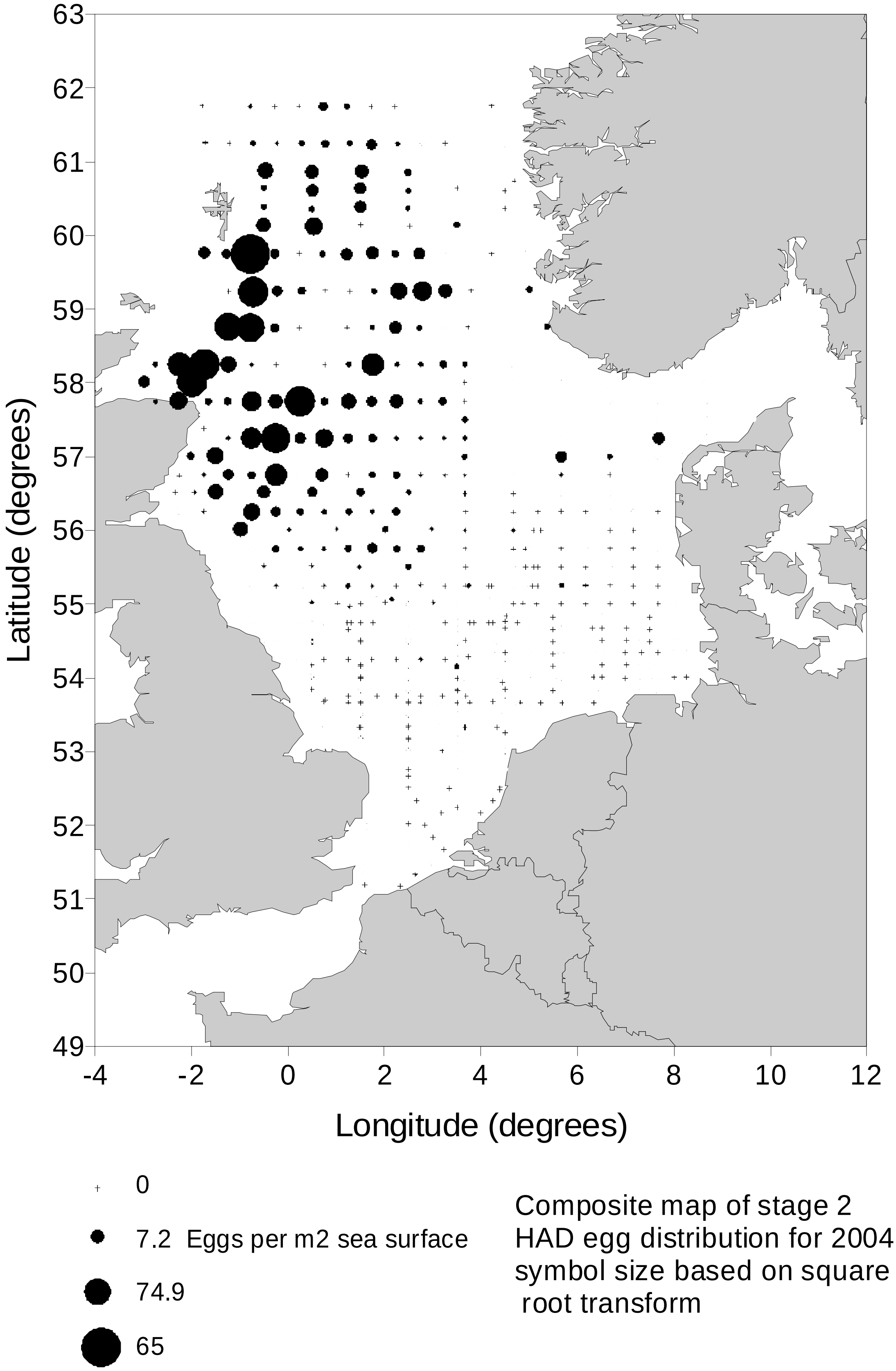
Composite map of stage 5 COD egg distribution for 2004, symbol size based on square root transform



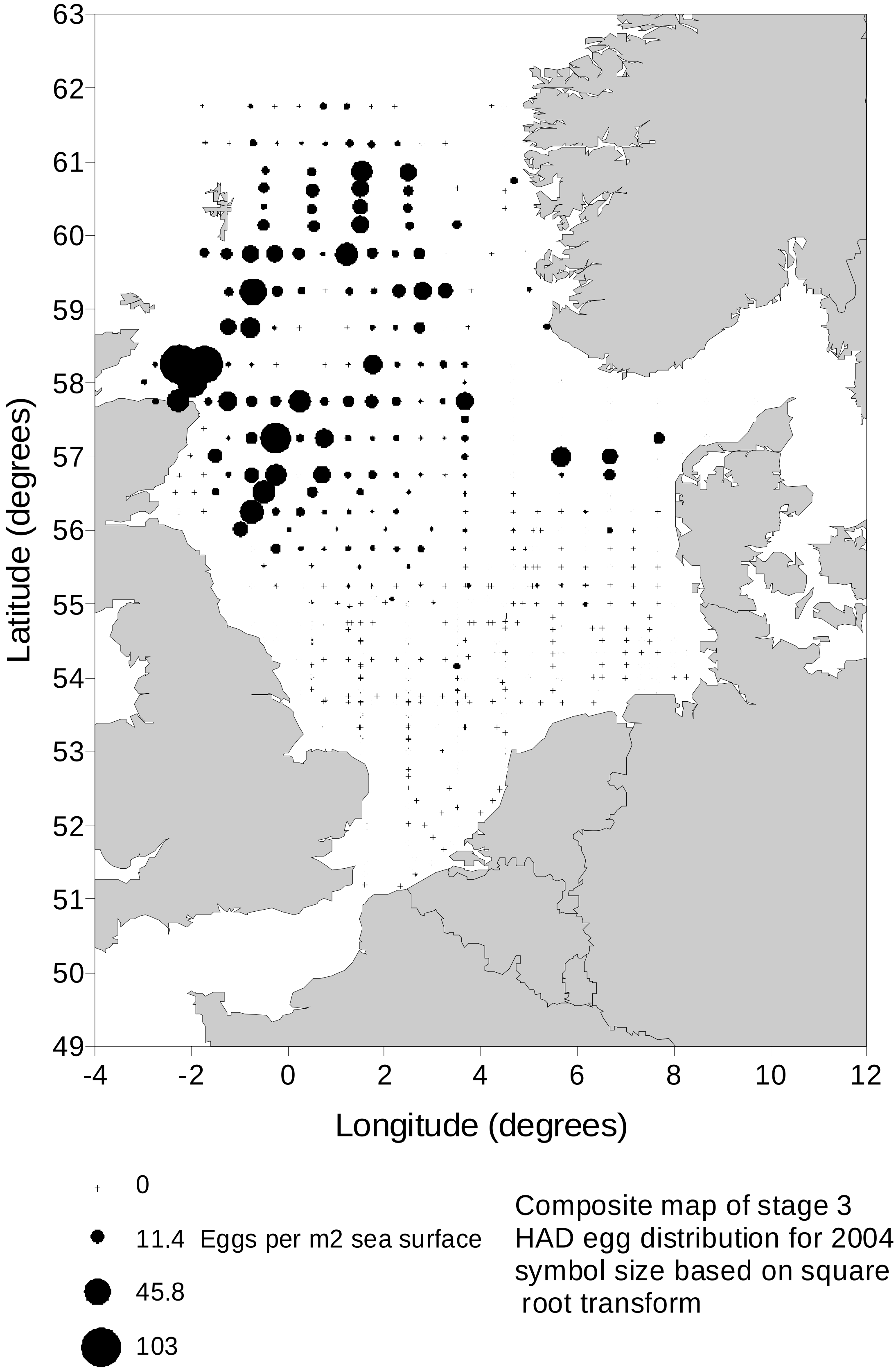
Composite map of all stages COD egg distribution for 2004, symbol size based on square root transform



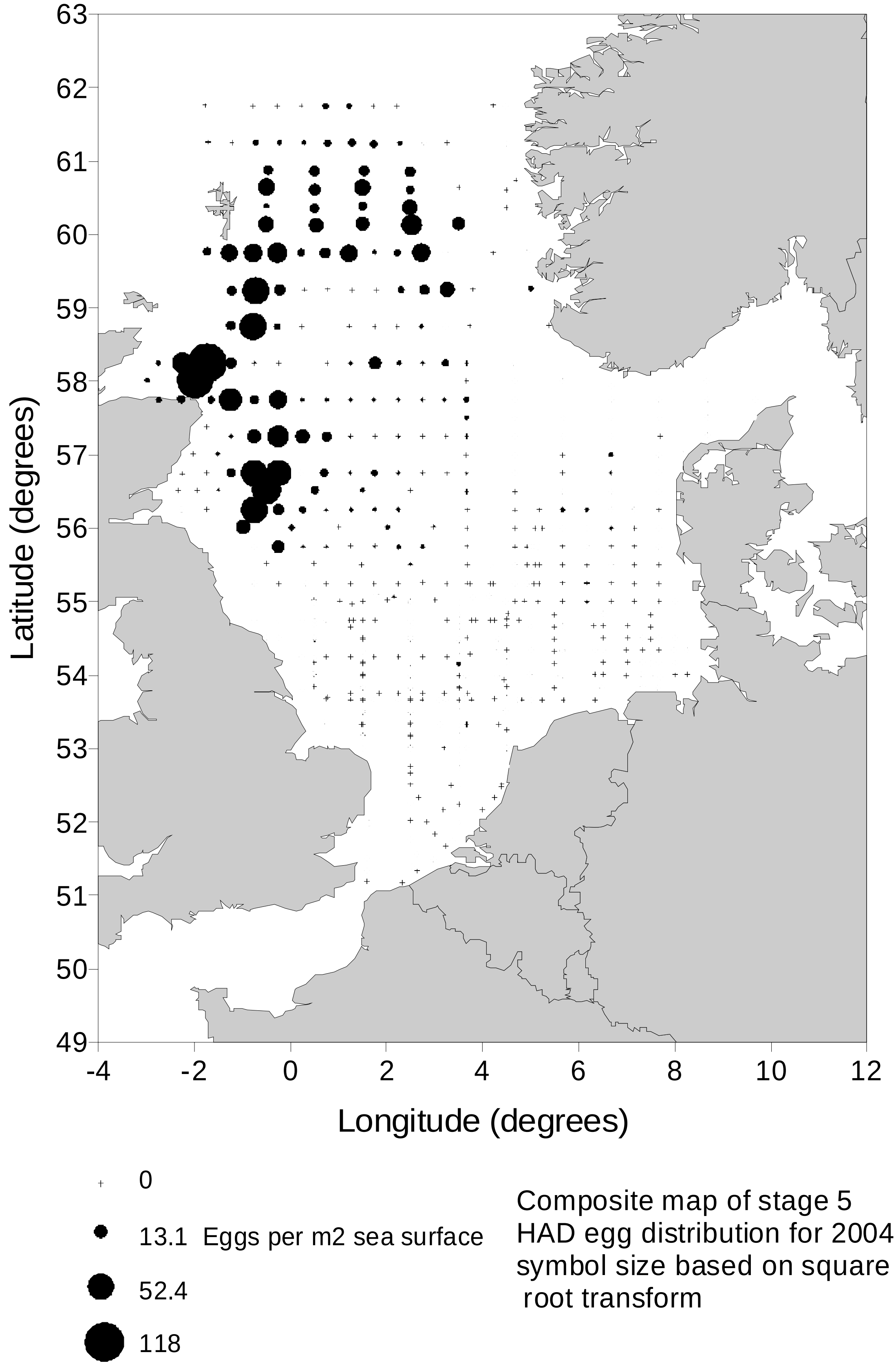
Composite map of stage I HAD egg distribution for 2004, symbol size based on square root transform



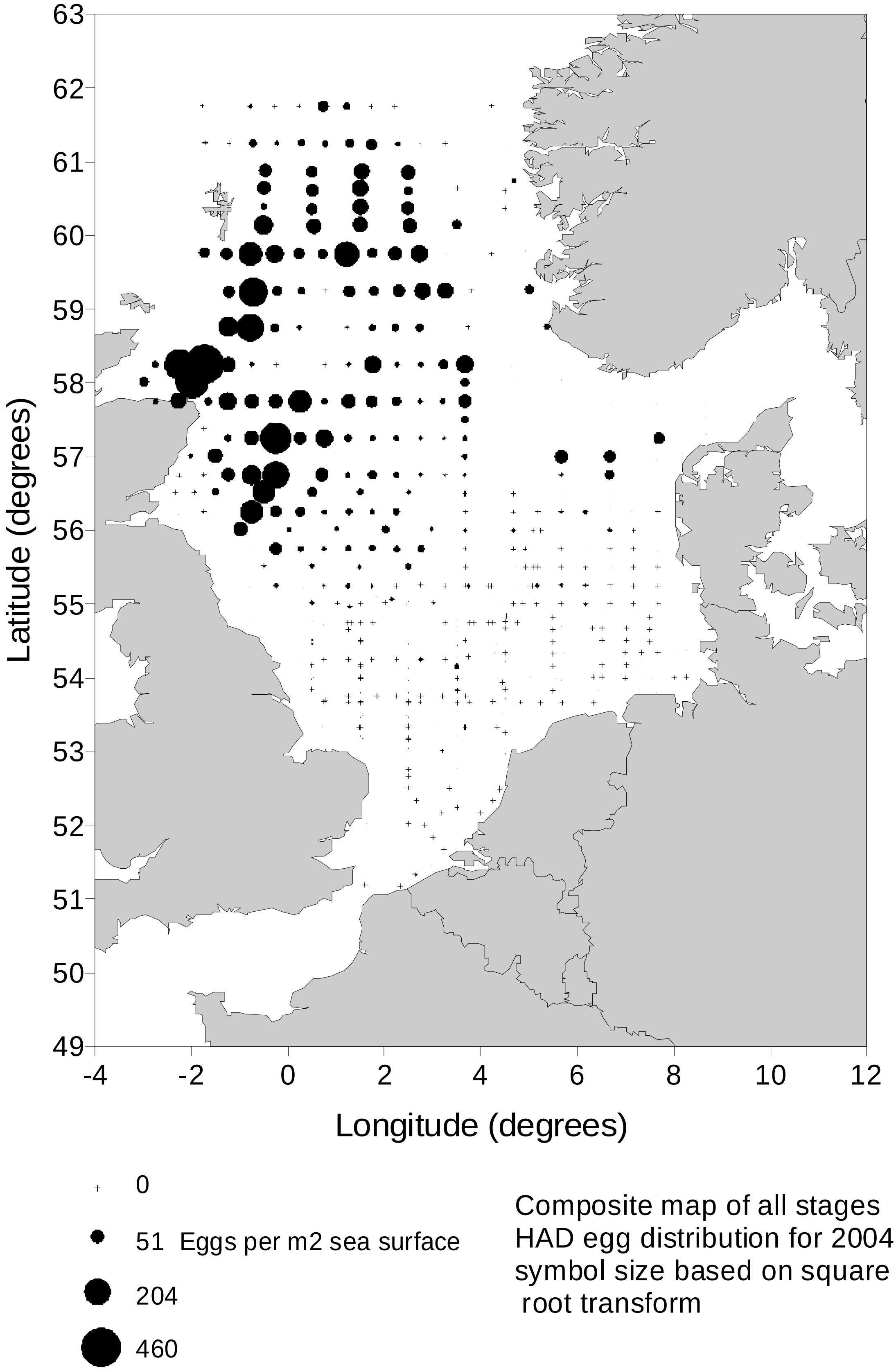
Composite map of stage 2 HAD egg distribution for 2004, symbol size based on square root transform



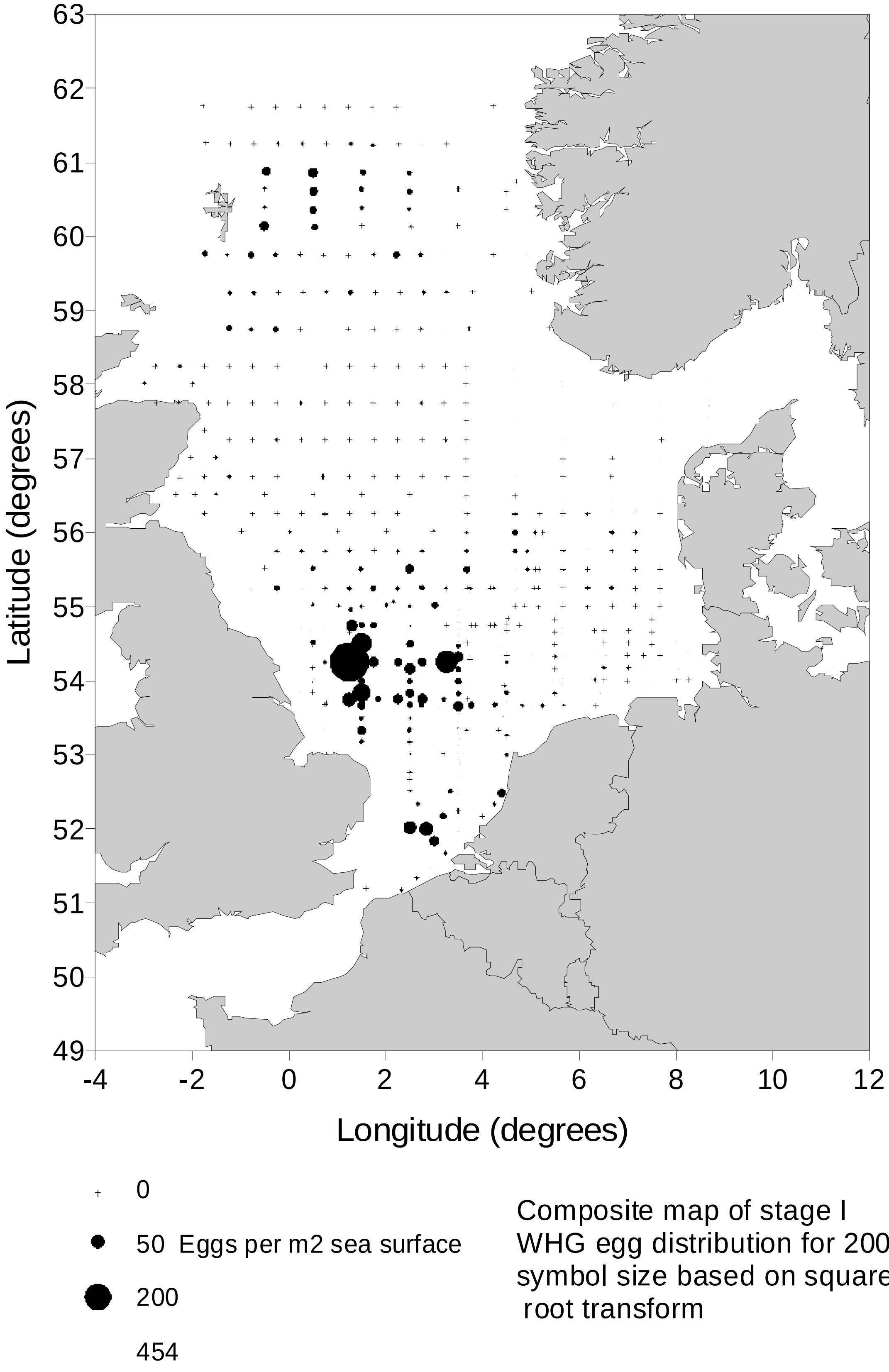
Composite map of stage 3 HAD egg distribution for 2004, symbol size based on square root transform



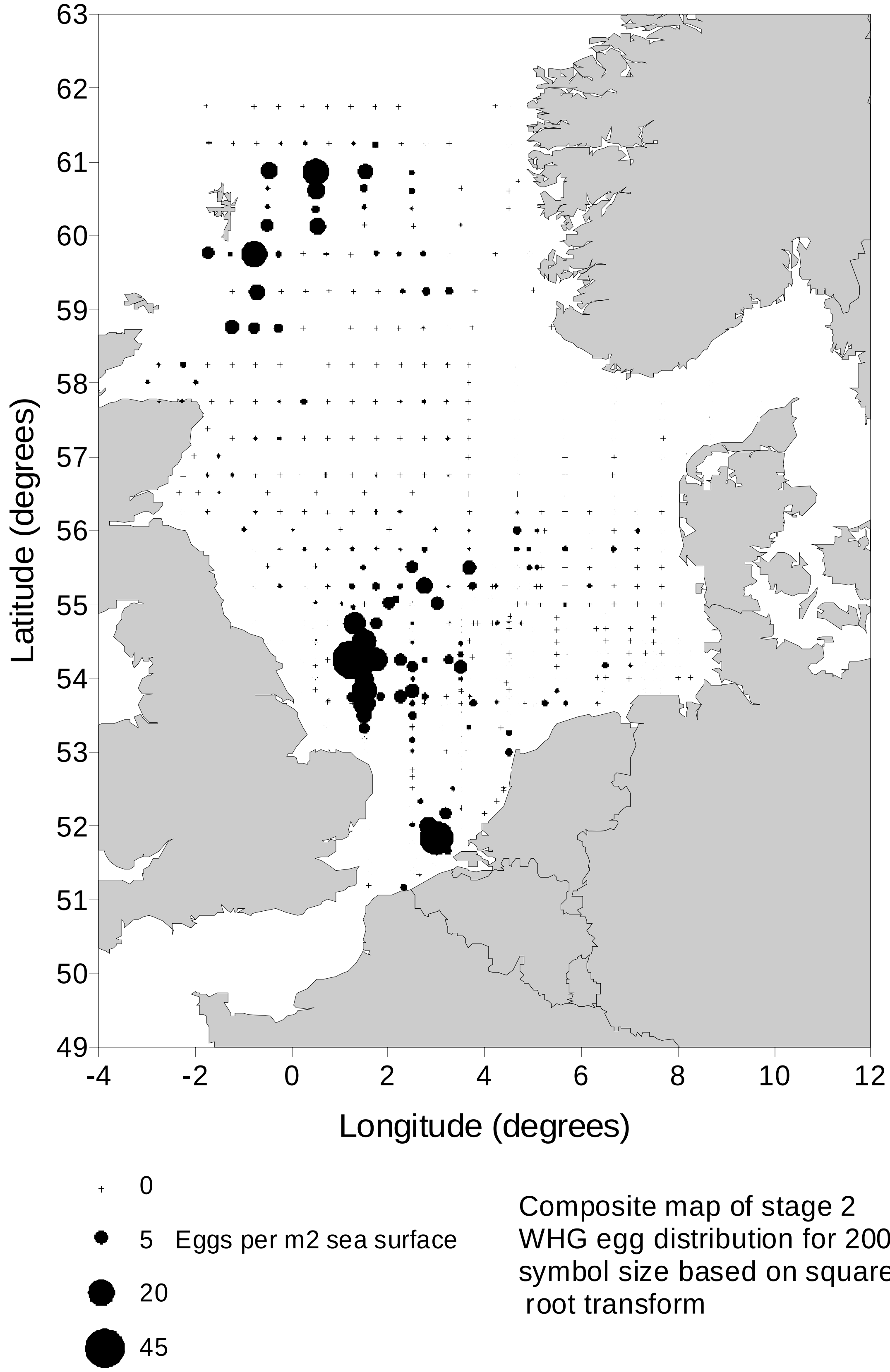
Composite map of stage 5 HAD egg distribution for 2004, symbol size based on square root transform



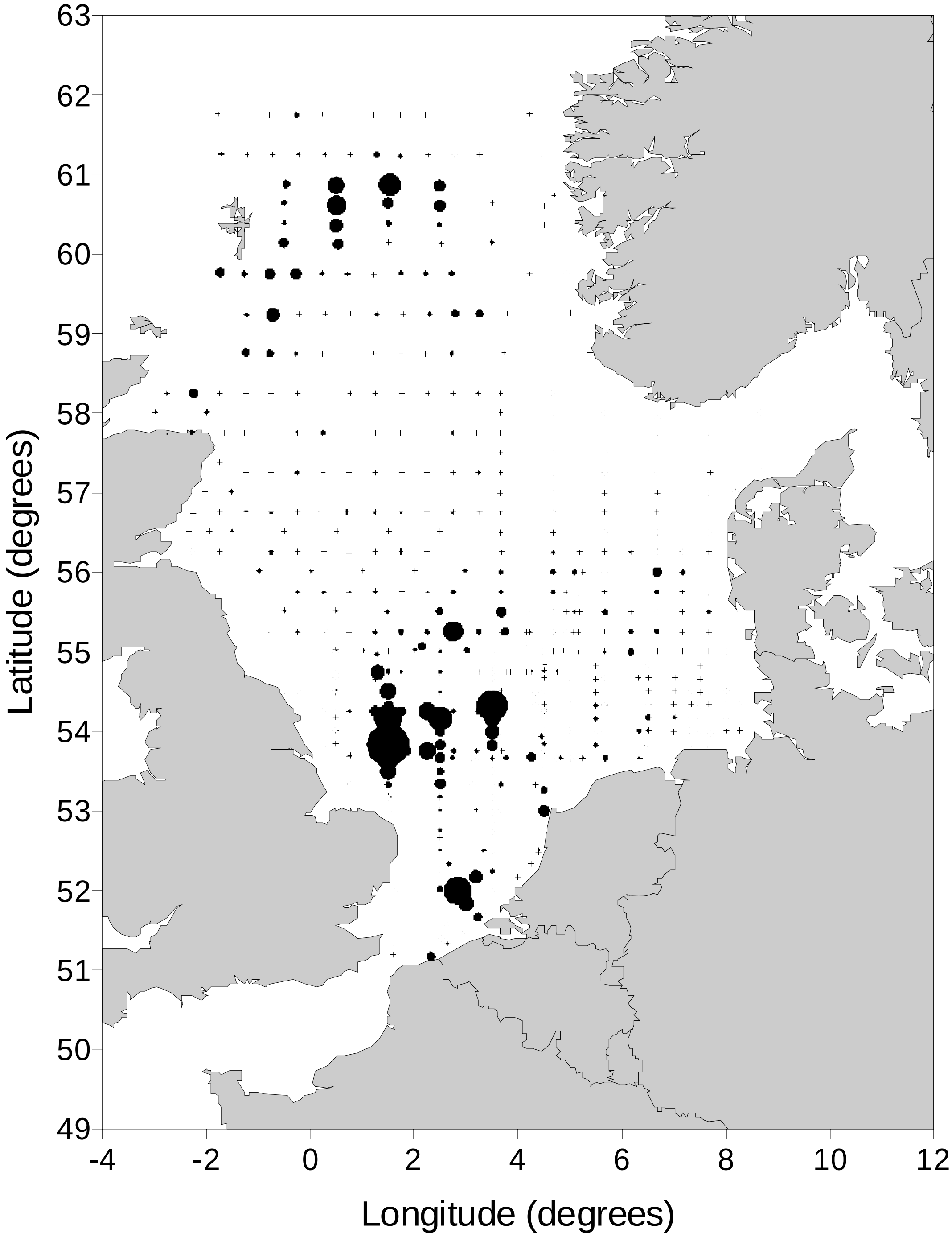
Composite map of all stages HAD egg distribution for 2004, symbol size based on square root transform

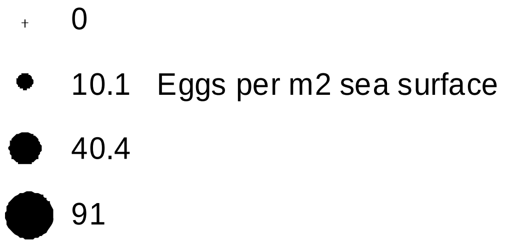


Composite map of stage I WHG egg distribution for 2004, symbol size based on square root transform

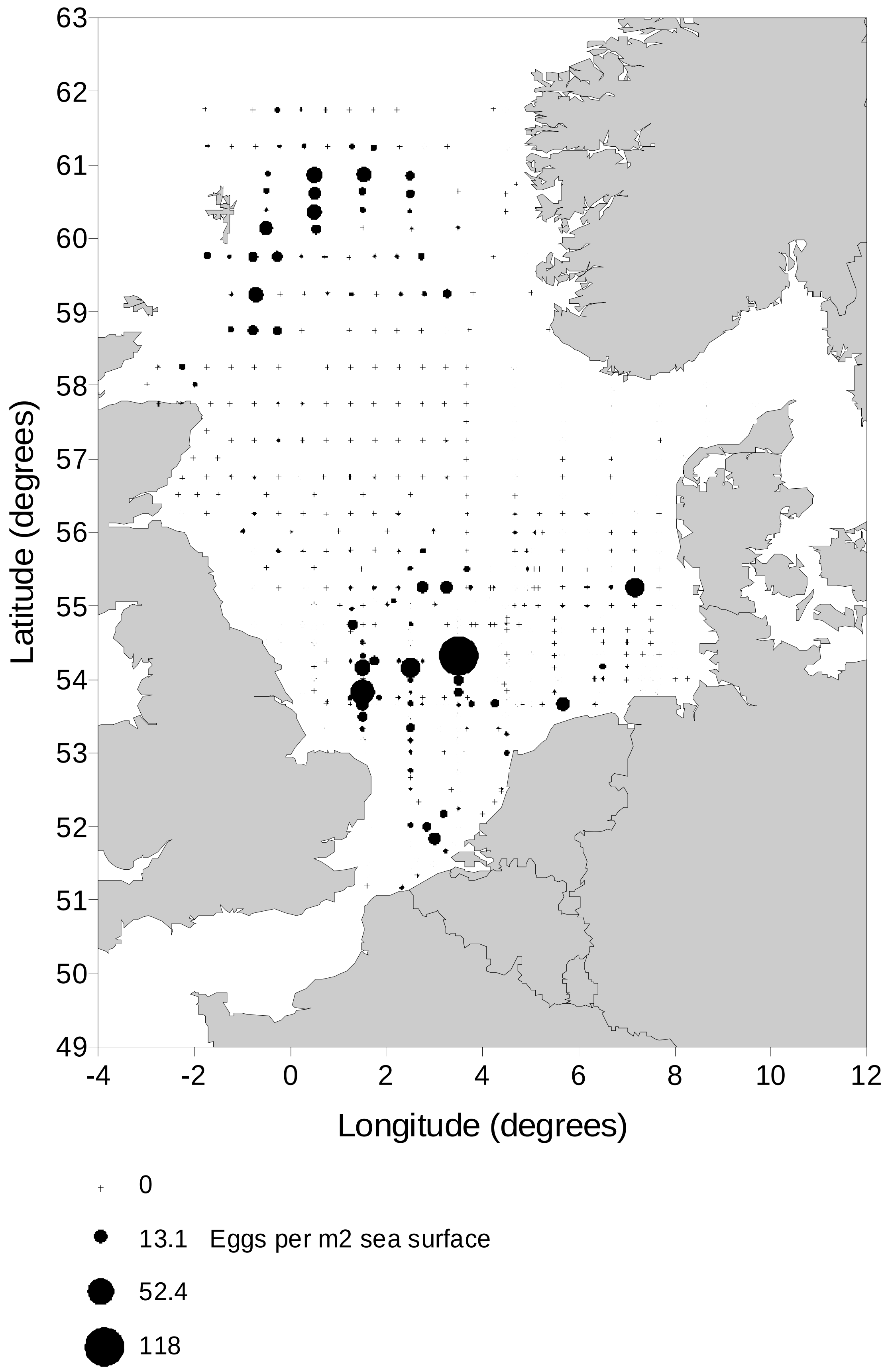


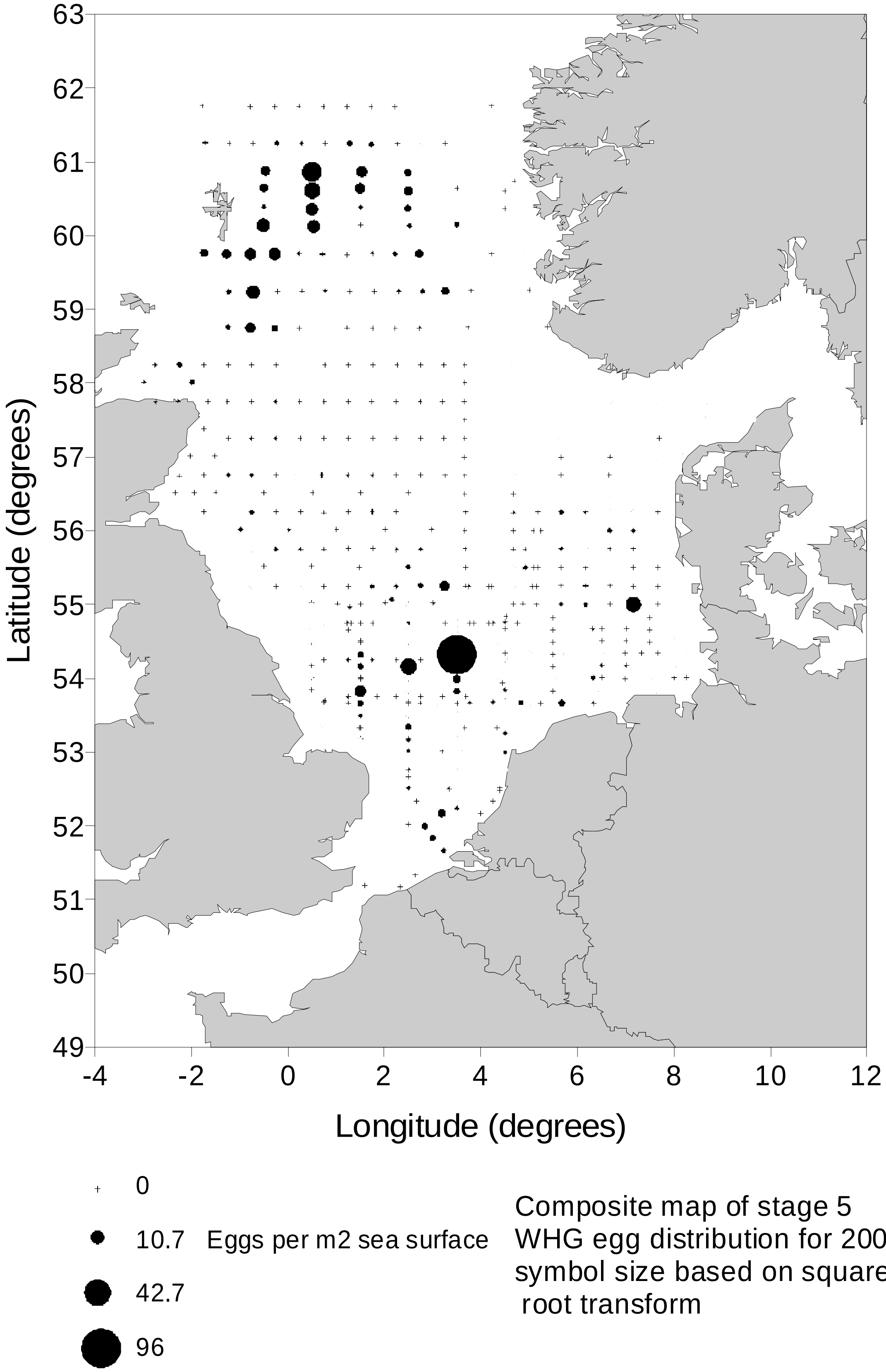
Composite map of stage 2 WHG egg distribution for 2004, symbol size based on square root transform



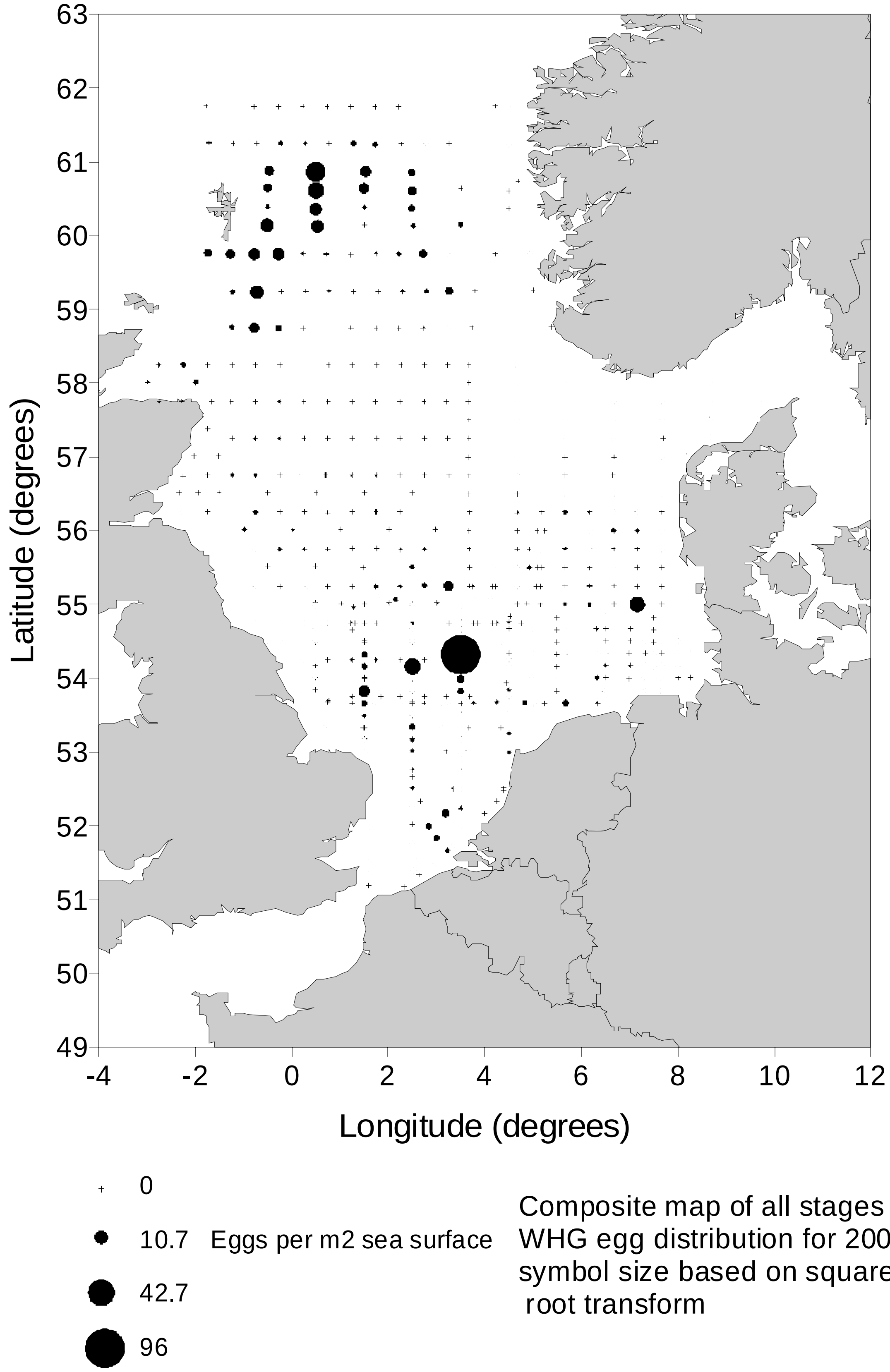


Composite map of stage 3 WHG egg distribution for 2004, symbol size based on square root transform

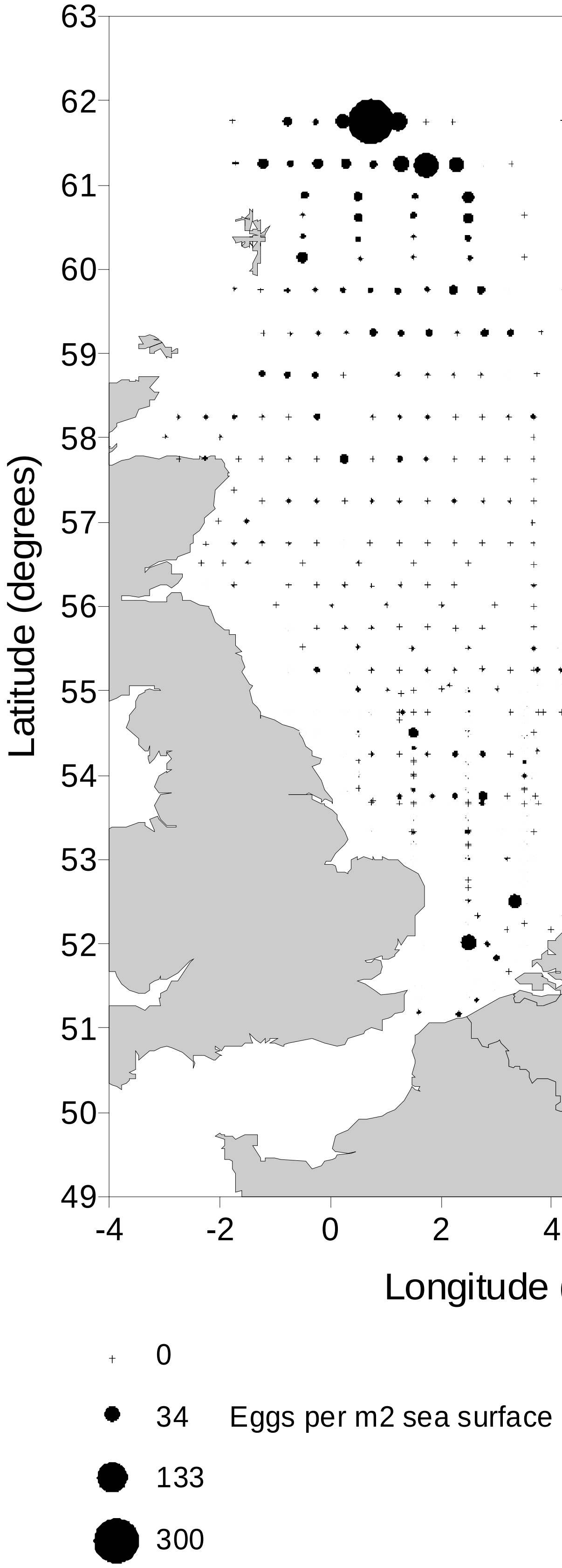

63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
-4
-2
0
2
4
6
8
10
12
Latitude (degrees)
Longitude (degrees)
0
13.1 Eggs per m2 sea surface
52.4
118



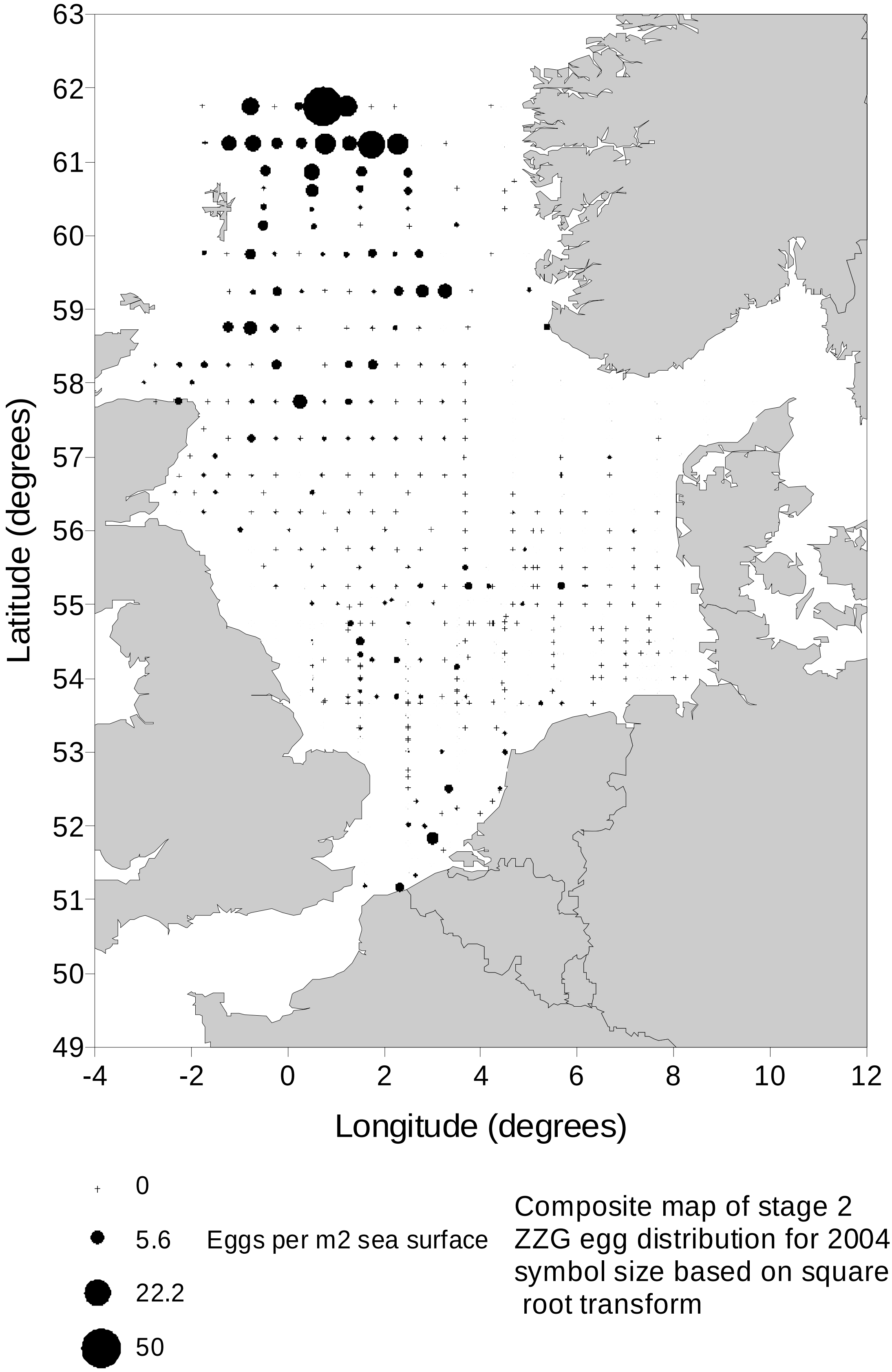
Composite map of stage 5 WHG egg distribution for 2004, symbol size based on square root transform



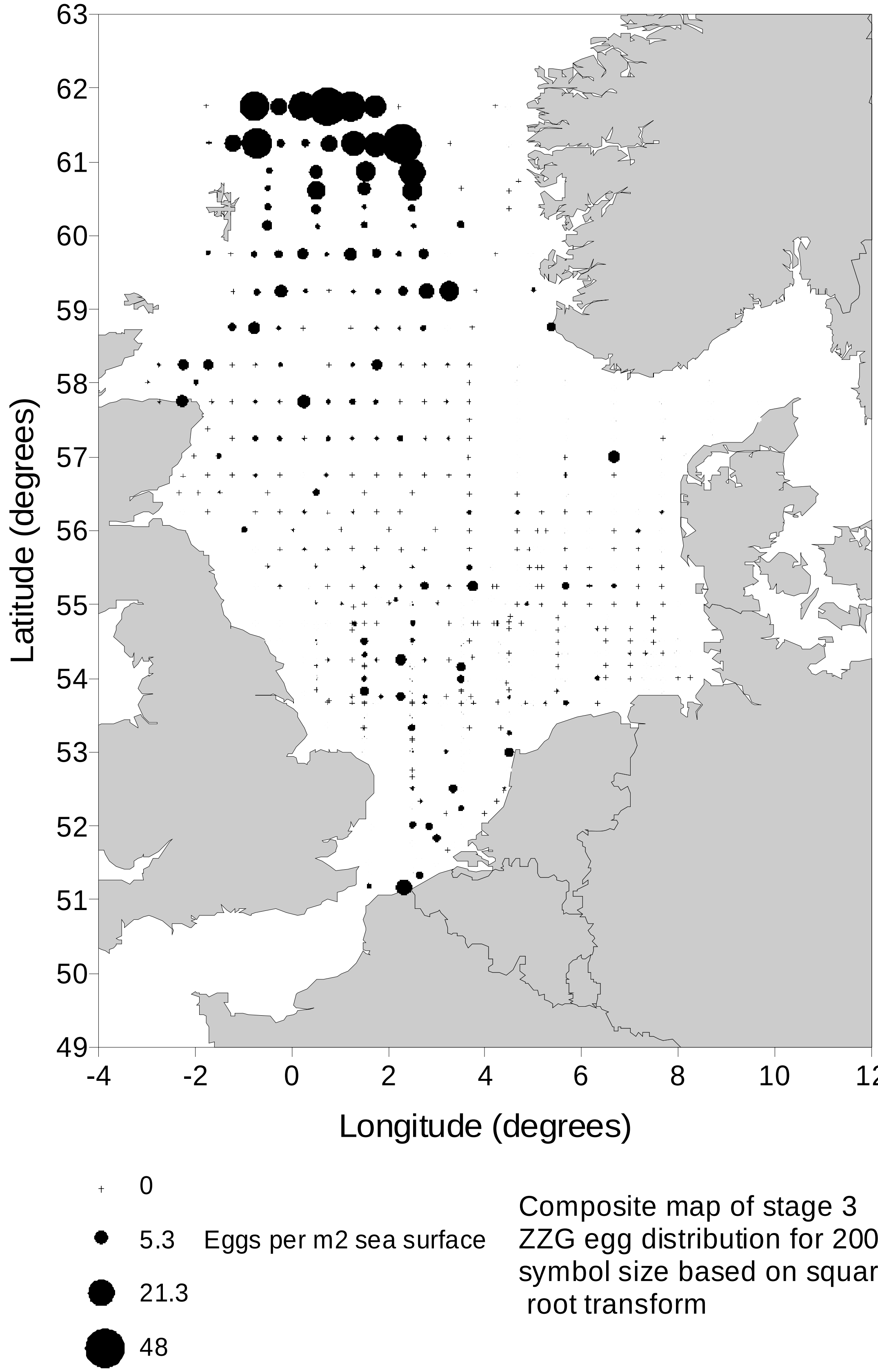
Composite map of all stages WHG egg distribution for 2004, symbol size based on square root transform



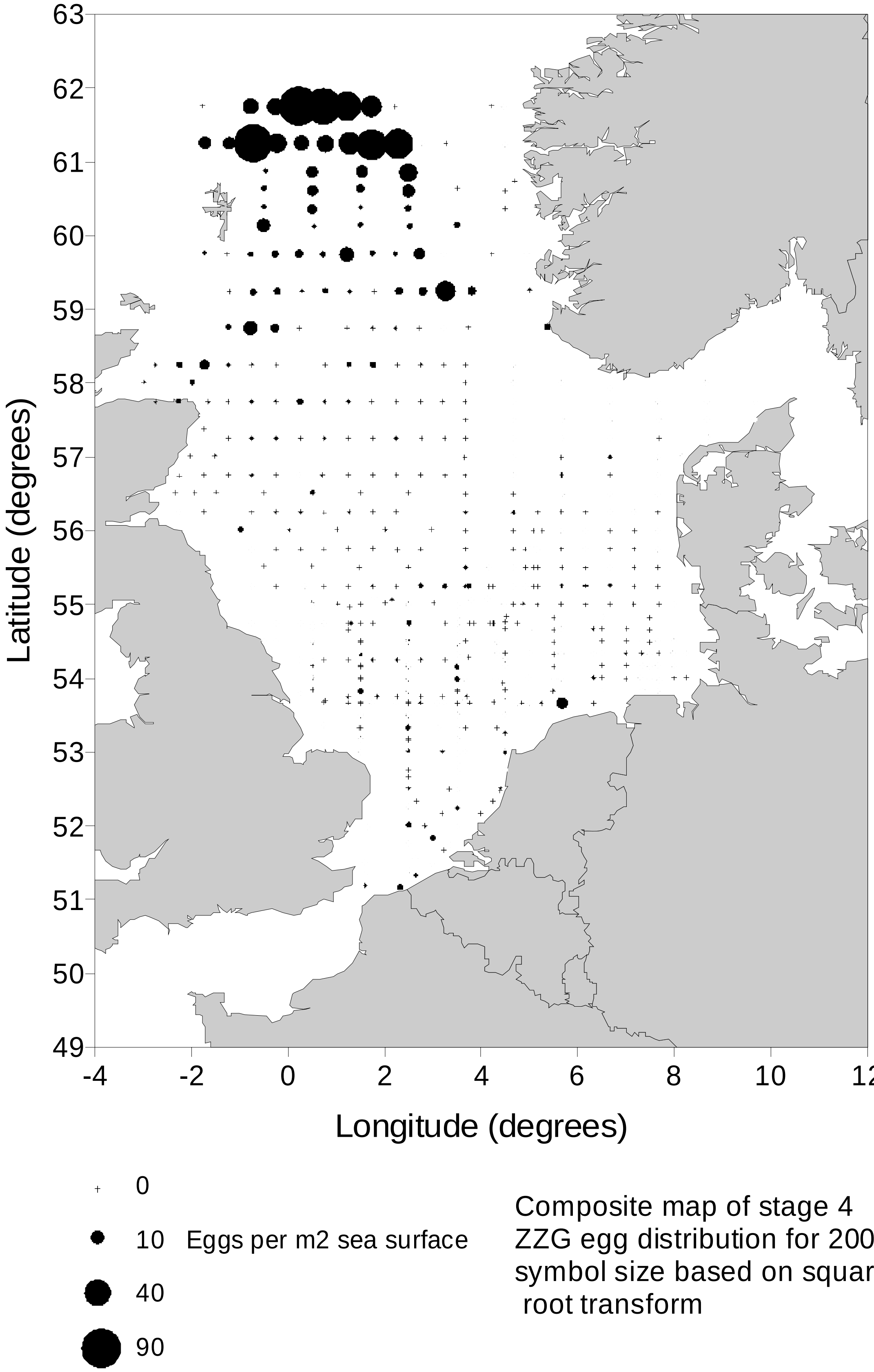
Composite map of stage I ZZG egg distribution for 2004, symbol size based on square root transform



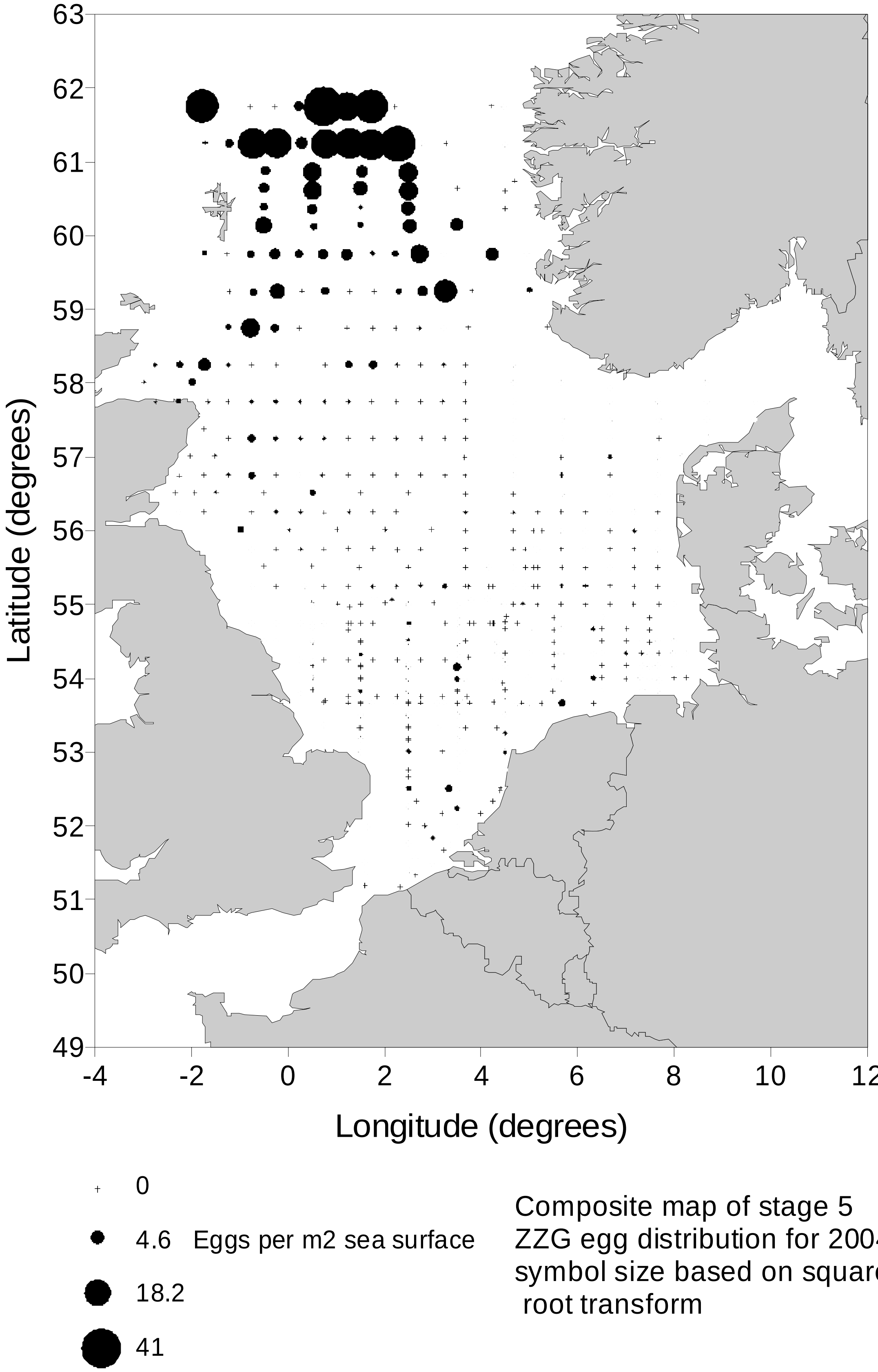
Composite map of stage 2 ZZG egg distribution for 2004, symbol size based on square root transform



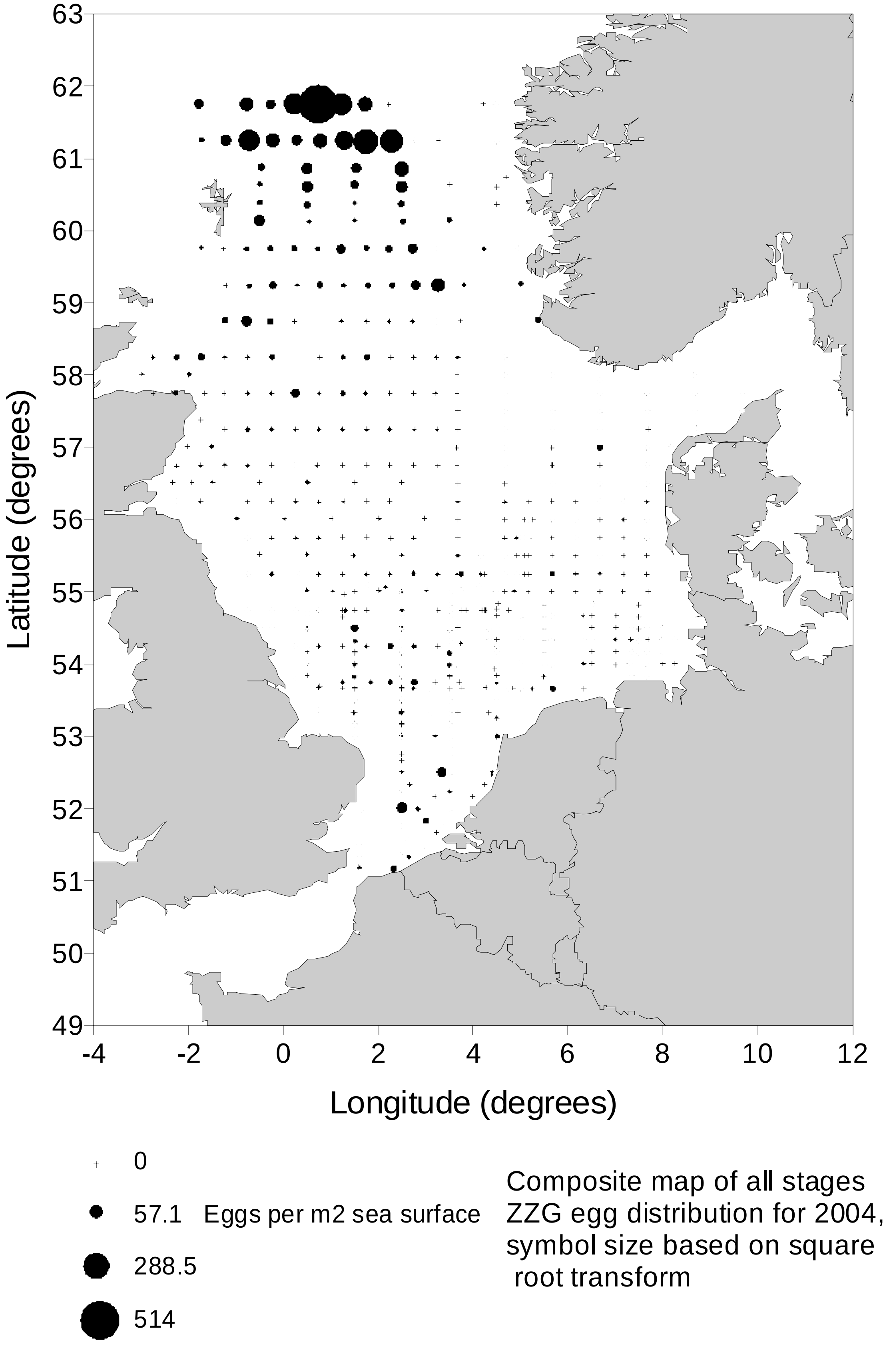
Composite map of stage 3 ZZG egg distribution for 2004, symbol size based on square root transform



Composite map of stage 4 ZZG egg distribution for 2004, symbol size based on square root transform



Composite map of stage 5 ZZG egg distribution for 2004, symbol size based on square root transform


63
62
61
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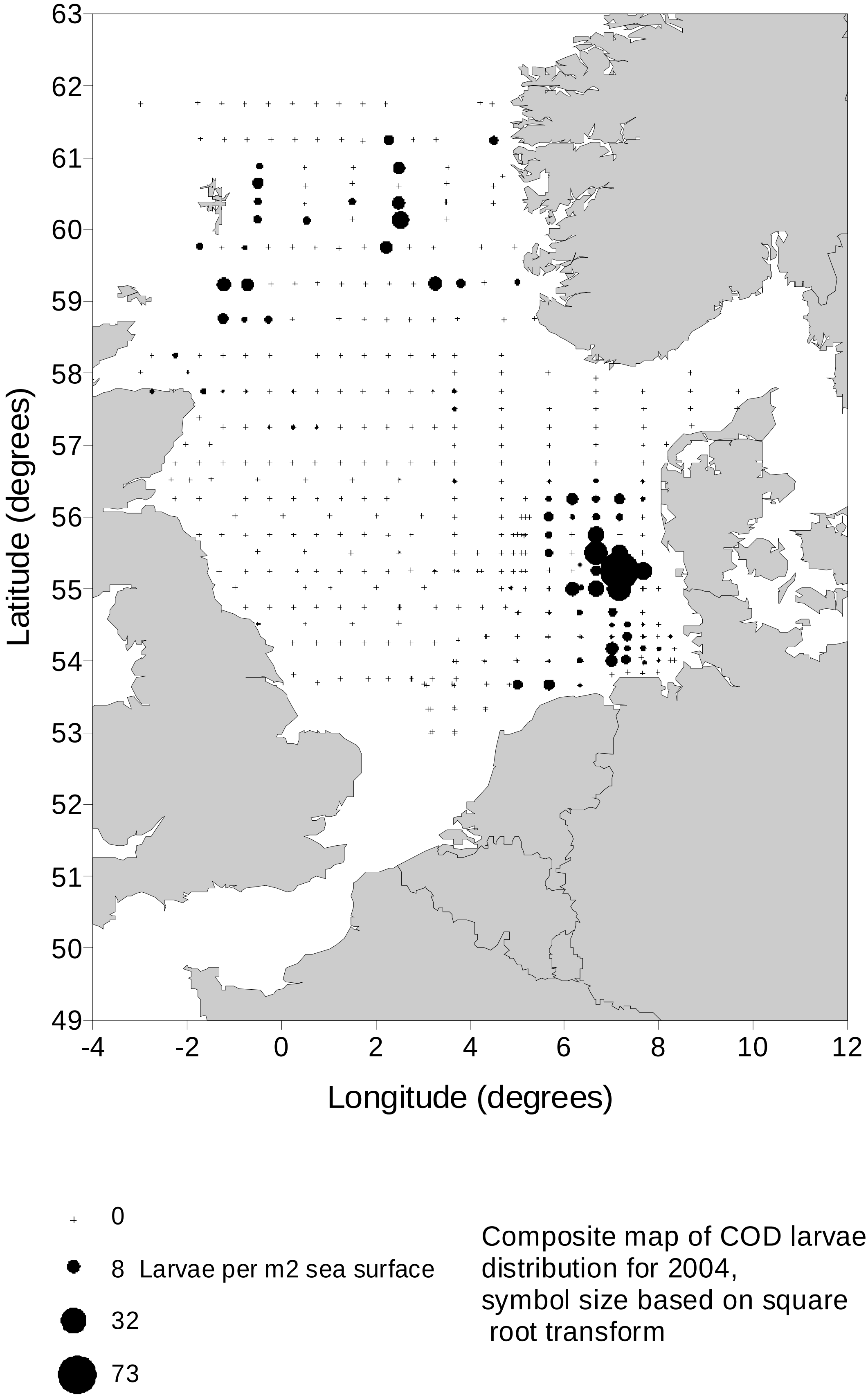
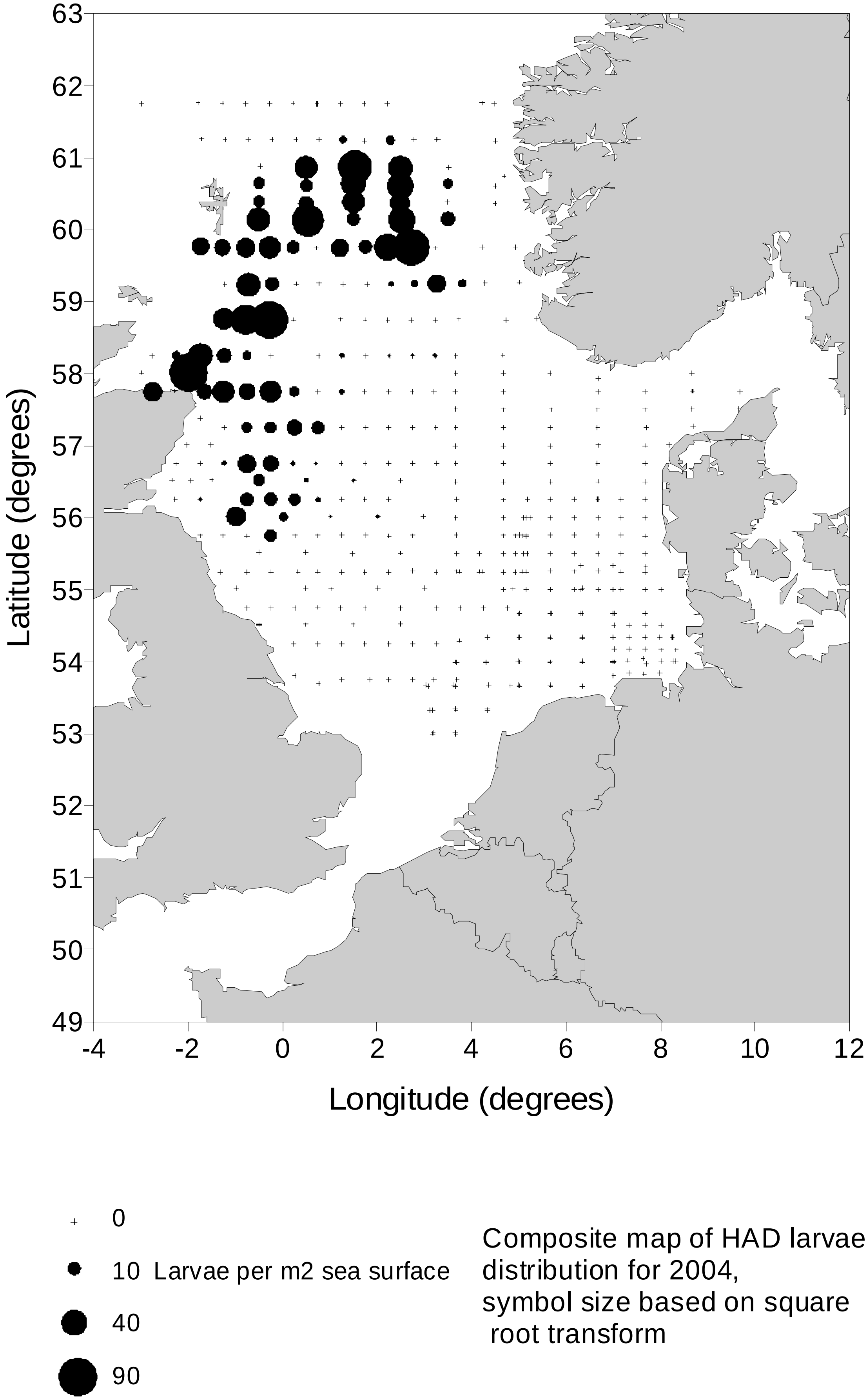
59
58
57
56
55
54
53
52
51
50
49
Latitude (degrees)
-4
-2
0
2
4
6
8
10
12
Longitude (degrees)
0
57.1 Eggs per m2 sea surface
288.5
514
Composite map of all stages
ZZG egg distribution for 2004,
symbol size based on square
root transform

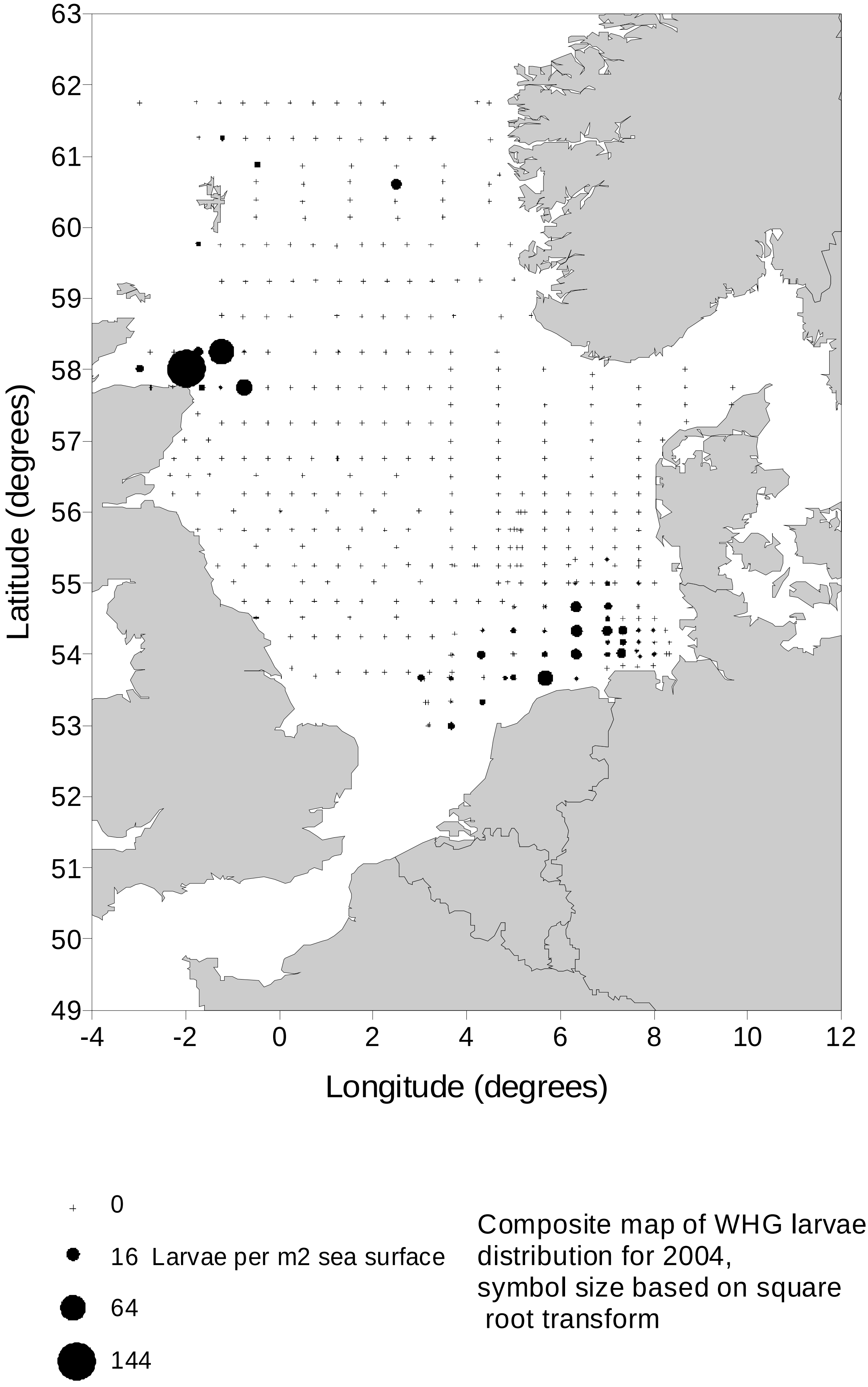
Maps of larval distributions



Composite map of COD larvae distribution for 2004, symbol size based on square root transform



Composite map of HAD larvae distribution for 2004, symbol size based on square root transform



Composite map of WHG larvae distribution for 2004, symbol size based on square root transform