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

13-14 May 2008

Lowestoft, UK

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The Planning Group on Egg Surveys was originally set up to address the fact that there had never been a complete ichthyoplankton survey of the North Sea. In particular, the need to monitor commercial fish spawning areas was identified by the Expert Panel (1) that followed the 2002 Bergen Ministerial North Sea Conference. Although spawning grounds can be monitored by trawl surveys, ichthyoplankton surveys have a number of potential advantages. Since individual fish spawn thousands of eggs it is often more reliable to sample the eggs rather than the adult fish and surveying spawning grounds of species producing planktonic eggs is also not restricted by bottom-type so a more complete spatial coverage can be achieved. Against that is the amount of additional sea-time required to undertake egg surveys and the additional laboratory analysis time needed to work up the samples.

Because of the current poor state of the cod stock and concern at the time about the trajectory for plaice, it was decided to focus on those species. Given the scale of the proposed ichthyoplankton survey it was hardly surprising that it took several years to organize but in 2004 the fieldwork was undertaken. This work has resulted not only in the most complete maps of cod and haddock spawning areas in the North Sea ever produced (2, 3) but also distribution maps of several other species of interest (3), in an egg-production estimate for plaice in the southern North Sea and new insights into the relationship between oceanography and fish egg and larval distributions (submitted manuscripts). The data generated by the 2004 survey are now being used to support fisheries conservation and wider marine conservation e.g. consideration of management plans for Natura 2000 sites.

Clearly a single survey, even of the scale undertaken is of limited value because we need to build up a picture of changes over time. This is especially relevant in the context of environmental changes that may exacerbate the conservation challenges of dealing with low stock sizes for valuable species such as cod. This was recognized in the original Expert Committee wording which calls for 'monitoring of the spawning grounds'. It was therefore planned to repeat and extend the 2004 survey in 2009, i.e. after a period of five years, to begin the process of examining changes in the spawning grounds over time.

A new survey should:

a) Use comparable methods to 2004 to enable examination of changes in spawning activity between the two surveys.

b) In addition, collect data on the reproductive state of adult fish to validate egg survey timing, this is principally an issue for cod. This information would enable improved interpretation of egg production levels in different areas of the North Sea. The lack of these data in 2004 has reduced the degree of confidence that can be placed in interpretation of the 2004 results, particularly for the northeastern North Sea.

During the workshop available resources for 2009 were reviewed. It became clear that member countries had committed inadequate resources to enable a repeat and extension of the 2004 survey design. Given committed resources, the group concluded that planning should be taken forward for a coordinated comparison of cod egg production and reproductive state in two areas of the North Sea (southern and northern), as opposed to covering the whole North Sea. Plaice egg production in

the southern North Sea remains of interest, particularly for the Netherlands. The proposed 2009 surveys may be able to provide an additional egg production index for plaice if some additional support from IMARES is obtained. Information will also be collected on egg and larval production for other species such as haddock and sandeels.

To facilitate, the workshop concluded that PGEGGS should continue, but a replacement chair could not be identified during the meeting (the current chair has served the maximum term allowed under ICES rules). This issue is referred back to the parent committee.

1) Scientific Expert Committee for the Fifth International Conference on the Protection of the North Sea (2002) Priority scientific issues for North Sea ecosystem management, Bergen, p 2

2) Fox, C. J., Taylor, M., Dickey-Collas, M., Fossum, P., Kraus, G., Rohlf, N., Munk, P., van Damme, C. J. G., Bolle, L. J., Maxwell, D. L., and Wright, P. J., 2008. Mapping the spawning grounds of North Sea cod (Gadus morhua) by direct and indirect means. Proceedings of the Royal Society London Series B, doi:10.1098/rspb.2008.0201

3) Taylor, N., Fox, C. J., Bolle, L., Dickey-Collas, M., Fossum, P., Kraus, G., Munk, P., Rolf, N., van Damme, C., and Vorbach, M., 2007. Results of the spring 2004 North Sea ichthyoplankton surveys. Cooperative Research Report, 285, International Council for the Exploration of the Sea, Copenhagen.

1 Opening of the meeting

PGEGGS met 13–14 May 2008 at CEFAS, Lowestoft, UK.

2 Adoption of the agenda

The **Planning Group on North Sea Cod and Plaice Egg Surveys** [PGEGGS] (Chair: C. Fox, Scotland) will convene a meeting in late 2007 or early 2008 (venue and dates to be decided) to:

a) take forward planning for a North Sea wide ichthyoplankton survey in 2009.

b) to consider the advice from WGZE on the value of collecting additional zooplankton samples during future North Sea ichthyoplankton surveys in 2009.

c) prepare an action plan to ensure archiving of data collected in 2004.

PGEGGS will report by June 15 2008 for the attention of the Living Resources and the Resource Management Committees.

For the workshop the following tasks were considered:

a) Brief review of outputs from 2004 survey.

b) Presentations on relevant research (Scotland, France, England).

c) Planning for a North Sea wide ichthyoplankton survey in 2009.

d) Consider the advice from WGZE on the value of collecting additional zooplankton samples during future North Sea ichthyoplankton surveys in 2009.

e) Prepare an action plan to ensure archiving of the data collected in 2004.

f) Election of new chair for the group.

3 Participants

A list of participants is given at Annex 1 of this report.

4 Progress with publications of results from the 2004 survey (workshop aim a)

ICES Co-operative Research Report: The ICES Co-operative Report was published in 2007 as Taylor, N., Fox, C. J., Bolle, L., Dickey-Collas, M., Fossum, P., Kraus, G., Munk, P., Rolf, N., van Damme, C., and Vorbach, M. 2007. Results of the spring 2004 North Sea ichthyoplankton surveys. International Council for the Exploration of the Sea, Cooperative Research Report, 285, 59 pp. This report contains results for all species except those identified using gene-probes which have previously been published as Fox, C., Taylor, M., Dickey-Collas, M., van Damme, C. J. G., Bolle, L., Daan, N., Rohlf, N., Kraus, G., Munk, P., Fossum, P., and Bailey, N. 2005. Initial results from the 2004 ichthyoplankton survey of the North Sea. International Council for the Exploration of the Seas, ICES CM 2005/AA:04, 40 pp.

Proceedings Royal Society: The main results on the cod spawning grounds have recently been published in the high-impact journal Proceedings of the Royal Society as Fox C.J., Taylor, M., Dickey-Collas, M., Fossum, P., Kraus, G., Rohlf, N., Munk, P.,

van Damme, C.J.G., Bolle, L.J., Maxwell, D.L., Wright, P.J., 2008 Mapping the spawning grounds North Sea cod *(Gadus morhua)* by direct and indirect means. Proc. Royal Society Series B, doi:10.1098/rspb.2008.0201.

Fisheries Oceanography: Based on the information obtained from the 2004 surveys we investigated the distributional patterns of eggs and larvae and their correspondence to oceanographic characteristics. We used the information from the oblique hauls for eggs and larvae and hydrographical description from the vertical profiles of temperature and salinity. Salinity-based fronts were seen in coastal areas and off the Dogger and Fisher Banks, and egg and larval abundances of several fish species including cod, haddock, plaice and long rough dab peaked in frontal areas. Hence our findings support the hypothesis that the spawning locations for many fish are linked to recurrent hydrographic features such as salinity fronts. This linkage would provide complementary survival advantages since fronts are thought to provide increased abundance of prey organisms for larvae. Furthermore, the related physical processes confine egg and larval dispersal and constrain transport towards suitable nursery habitats. These findings have been submitted for publication in the Journal "Fisheries Oceanography".

Plaice AEPM: A re-analysis of North Sea plaice spawning-stock biomass from 1948 to 2004 using the annual egg production method. Using the results from the 2004 PLACES survey and fecundity data collected prior to the egg surveys, the annual egg production method was used to estimate the spawning-stock biomass of North Sea plaice in the southern North Sea. A new estimation of by AEP was considered necessary for two reasons. First, the catch-at-age based stock assessment of North Sea plaice has changed greatly in recent years. Large retrospective changes in the estimated absolute levels of plaice SSB in the North Sea have been observed. The stock assessment (XSA) has also been revised to include estimates of discards. This revised method has not been sensitivity tested and further fisheries independent validation would be valuable.

The daily egg productions were calculated for the different survey areas using egg development rates from the literature. Egg mortality rates were either treated as fixed values or were related to seawater temperature. Annual egg production and female only and total SSB were estimated for each survey area and for the whole southern North Sea. The published data from earlier plaice egg surveys, 1948–1950 and 1987–1988 was re-calculated using the same method as applied to the 2004 data, but using fecundity data from those periods. The female only and total SSB for the 2004 and older surveys were then compared to the XSA estimates.

Comparing the years 1987, 1988 and 2004, the production of plaice eggs does not appear to have changed greatly. However, when the 2004 data and 1980s data are compared to that around 1950, differences do become apparent. Egg production in the Southern Bight appears to have been much higher around 1950. It also appears that spawning on or south of the Dogger Bank occurred earlier in the year around 1950.

The current AEP estimate of North Sea plaice SSB is in broad agreement with the current ICES standard XSA stock assessment. The decline in SSB from 1988 to 2004 was approximately 60% as estimated by XSA, and was 50% as estimated by AEP. The AEP method supports the current ICES XSA stock assessment both in terms of the relative trend in SSB and the current absolute biomass. The AEP also suggests that most of this decline has occurred in the Dogger Bank area and the German Bight. The

manuscript will be send to a journal in the summer of 2008 and results will also be presented at the Flatfish symposium, November 2008.

5 Presentations on relevant research – Scotland, France and England (workshop aim b)

Scotland: FRS Marine Laboratory has national funding (MF760) for an investigation of population structuring in cod, with a focus on the major fishing grounds in the northern North Sea. A 15 day cruise timed to coincide with the average peak in cod spawning in the northern North Sea (20 February–6 March) has been agreed for 2009. The survey will undertake trawling to sample adult cod from previously identified spawning areas and plankton gears to sample ichthyoplankton. This project will be able to provide staff time for plankton analysis, cod reproductive development and genetic identification of eggs.

France: The current poor status of many exploited fish stocks has led to calls for marine fish spawning grounds to be regarded as "sensitive habitats". Monitoring and study of these spawning grounds can be seen as an essential component of an ecosystem-based approach to marine resource management. The present study aims to identify and characterize the location and habitats of spawning grounds in the Eastern English Channel and the southern North Sea and to specify the importance of environmental factors on their location.

The continuous fish egg pumping device CUFES (Continuous Underway Fish Egg Sampler) has been added to IFREMER's "Ressources Halieutiques" (RH) "International Bottom Trawl Survey" (IBTS) on "Thalassa".

As a result, a standardized sampling of the southern North Sea and the Eastern English Channel has been carried out in February 2006, 2007 and 2008. This action is planned to be repeated in February 2009. During the survey CUFES was operated continuously and a sample taken every 30 minutes. The eggs collected were preserved in formalin. CUFES efficiency in sampling pelagic eggs in the North Sea has been tested by comparing vertical sampling of the whole water column, using a traditional plankton net in 2006 and 2007 and using an oblique Bongo net in 2008 with CUFES results.

The creation of an identification key that includes all the characteristics of the main fish species encountered in the sampled areas will allow a fast and accurate identification of eggs. In each sample, fish eggs are identified to species as far as practical, sorted by developmental stage, and then counted (eggs per m^3).

The ZooScan imaging system has also been developed to automatically count and identify pelagic fish eggs collected with CUFES device. The ZooScan is relatively recent laboratory imaging system capable of taking good resolution images (2,400 dpi – 16 bits) of zooplanktonic samples, at high sampling rates. These images are then analysed using object classification allowing automated taxonomic identification for species with characteristic visual features (egg size, shape and appearance). This approach was applied on fish egg samples collected by the CUFES during the 2006 French IBTS, in order to investigate the applicability of this approach to future French IBTS in the North Sea, and hence potentially speeding-up the taxonomic identification and counting of egg samples.

Fish egg taxonomic identification based on visual criteria cannot always be carried out effectively. This is particularly the case for a number of Gadiforme species. In these cases recent developments in molecular identification may help. Two separate

studies were carried out with molecular tools. Nine species were considered: *G. morhua, M. merlangus, M. aeglefinus, T. luscus, T. minutus, Trisopterus esmarkii* (no eggs sampled during the 2006 French IBTS), *C. mustela, E. cimbrius* and *C. septentrionalis*.

The difference in abundance and in the number of species identified was compared with samples taken over the whole water column (using a plankton net, in vertical hauling). The effects of vessel and windspeed on these comparisons have also been explored. Annual distribution maps are being produced and interannual variation will be explored

The habitats of the spawning areas are being modelled using available explanatory variables (chlorophyll, surface temperature and salinity), depth, seabed stress and sediment type using linear (glm) regression. The results of the models are being applied to the entire study area in order to define the potential spawning areas in non-sampled zones.

The first results of this approach for 2006 are available. Funding has come from the Interreg 3A micro-project "ISADO" (Identification of the Spawning Areas in the Dover Strait and adjacent marine areas). Online maps and downloadable scientific report are available on the project website: http://charm.canterbury.ac.uk/isado.

England: Applying gene probes to identify cod haddock and whiting eggs fixed in formalin. In the 2004 survey it was necessary to extract cod-sized eggs from plankton samples before formaldehyde fixation and to place the eggs in ethanol in order to preserve good quality DNA for application of TaqMan gene probes to identify the proportions of cod, haddock and whiting. This presorting requires additional staff on the cruise to extract the eggs, and excludes working in bad weather when the vessel was unstable. Recent work has shown that TaqMan gene probes can be successfully applied to formaldehyde fixed eggs produced from captive females under defined conditions providing; 1) the duration in fixative does not exceed 15 days and 2) the fixed eggs were subsequently stored in a non formaldehyde based preservative for up to 90 days[1]. Results on trials to show whether this method can be applied to plankton samples collected at sea are expected by August 2008 and will be made available to subsequent PGEGGS planning meetings. If the trials confirm that the protocol is successful it will be possible to delay sample sorting until after the cruise and target DNA analysis more effectively thereby reducing costs whilst improving precision.

1) Goodsir, F., Armstrong, M. Milligan, S., Shaw, M., Goddard, M., Creach, V, Witthames, P.R. 2007 Can formaldehyde gadoid eggs be identified by gene probes ICES theme session Q23.

6 Planning for a North Sea ichthyoplankton survey in 2009 (workshop aim c)

Rationale for a modification in survey protocols applied in 2004.

One of the issues arising from the 2004 ichthyoplankton results for cod was the apparent disparity between the low egg densities estimated for the northern North Sea and that predicted from the distribution of mature cod in research surveys and fishery landings. It is feasible that the egg survey in the northern area was conducted either too early or late in the spawning season to accurately reflect egg production in this region, although the timing was as far as known around the expected peak. In a future survey it would be desirable to estimate the stage of the spawning season for different regions of the North Sea in order to remove this uncertainty. This should be possible from sampling running female fish, as the size frequency distribution of

vitellogenic oocytes in Atlantic cod changes in a predictable manner as spawning progresses. Spawning state can be estimated using the relationship between the portion of the total number of eggs spawned per season to the number of vitellogenic oocytes per gramme of the ovary. As spawn time tends to peak earlier in the southern than in the northern North Sea, samples of running females would be required from different regions of the North Sea (Annex 5).

The meeting reviewed the committed and potential resources available in spring 2009.

Table 6.1. Resources committed for 2009.

	Committed resources				Potential resources
Country	PGEGGS	Egg deformation	Additional IBTS sampling	NSAS Herring larvae survey	
Netherlands				End Dec–Jan: Gulf VII	Possible cruise or staff time
France			Feb: CUFES, Bongo		
Germany		27 Feb–18 Mar Egg deformation Survey, Nackthai GULF III or Bongo	Jan–Feb GULF III or Bongo	Jan 1–15 Gulf III	
Denmark			Feb extra days Bongo		Might be willing to come in with extra seatime if 2009 survey became North Sea wide
Scotland	20 Feb–6 March: 15 days Gulf VII, Bongo		Jan–Feb		
Norway					Request for 15 days Gulf III/VII
England					

At present only one country has committed ship resources specifically for plankton sampling under PGEGGS (above) whilst other countries can make additional sampling under other programmes. The German contribution will be part of their survey on egg malformations, Germany and Netherlands can make egg samples collected during the herring larval surveys available, France will be undertaking CUFES (continuous underway fish egg sampling, along with occasional Bongo net samples during their IBTS in the southern North Sea. Germany, Denmark and Scotland may also commit to undertaking some additional sampling of eggs (e.g. using Bongo nets) during their portions of the first quarter IBTS. England cannot at present make any commitment.

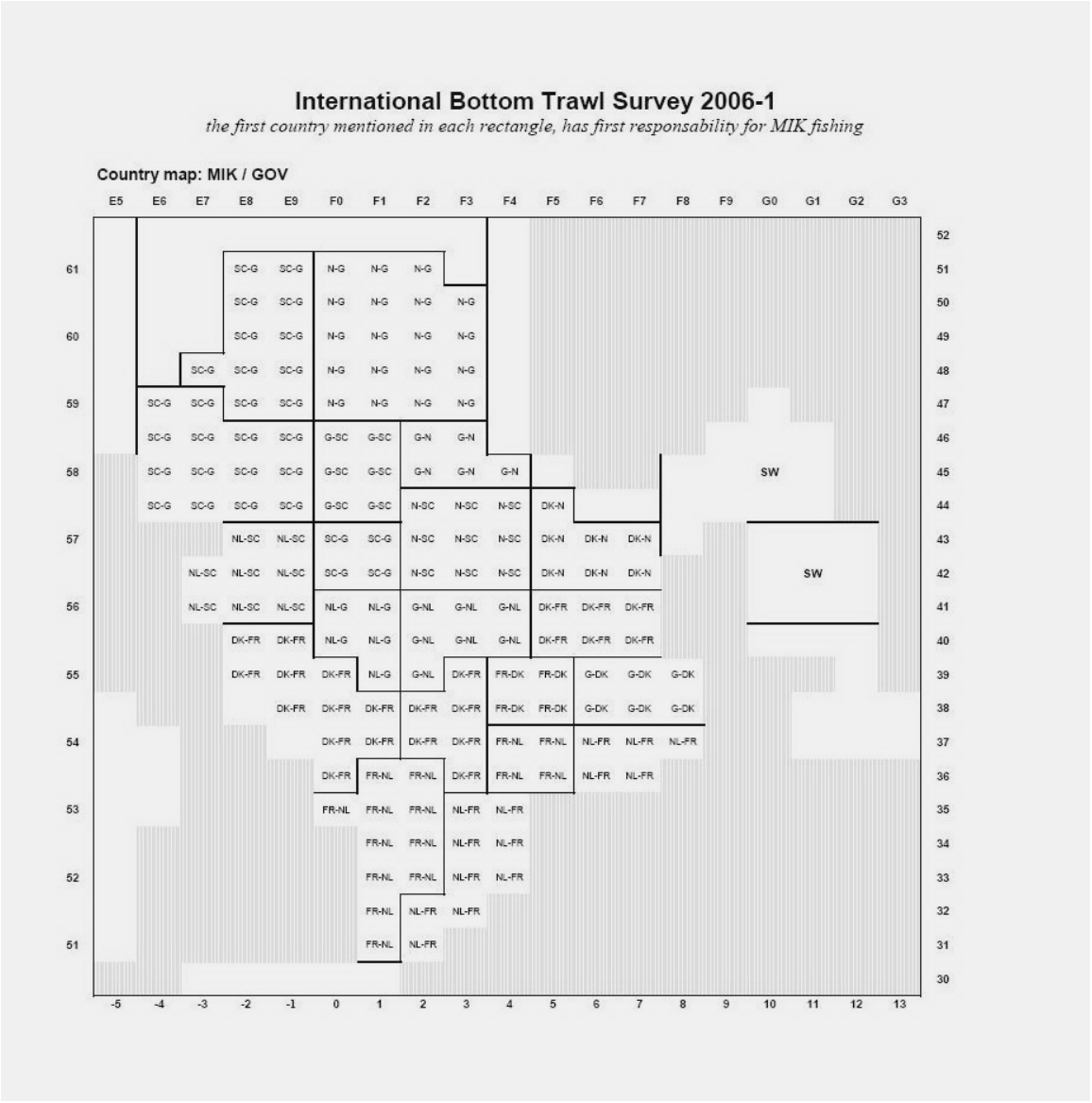

Figure 6.1: IBTS sampling locations.

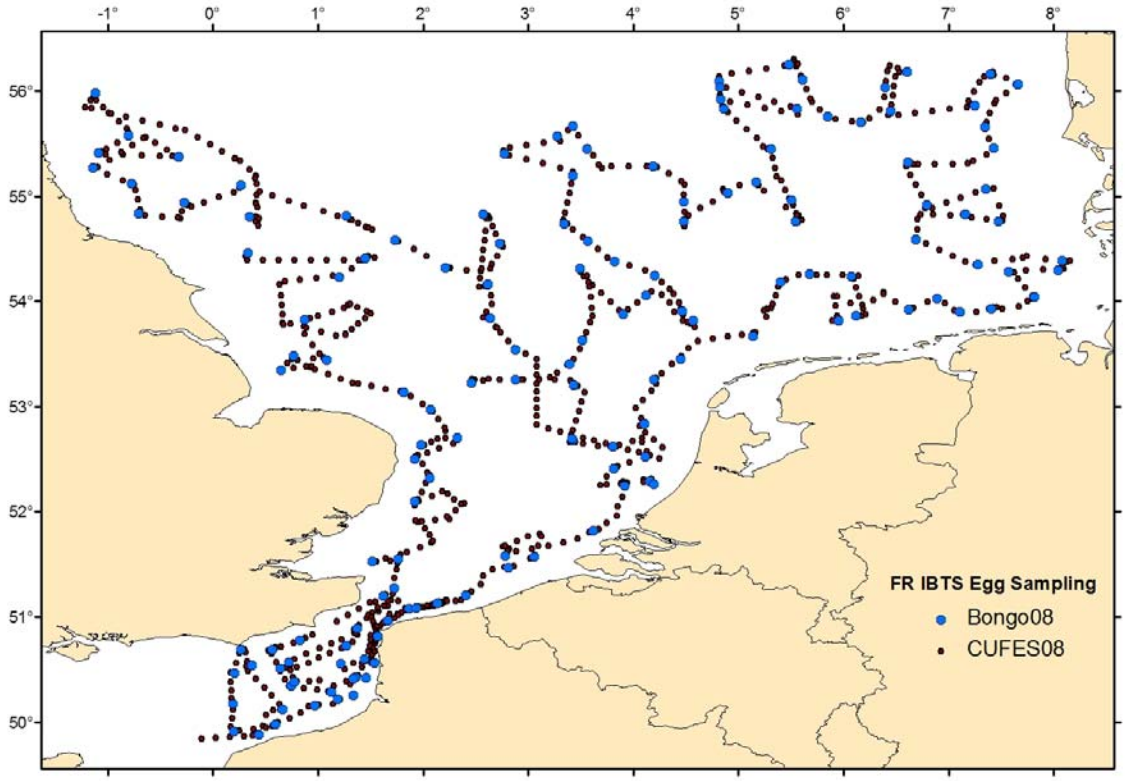

Figure 6.2: French IBTS/CUFES route.

The workshop concluded that **at present there is insufficient time allocated by each participant to provide full coverage of the spawning grounds of cod and plaice in the North Sea as undertaken in 2004**. With available commitments, the northeastern sector of the North Sea is still an area that cannot easily be covered. As such there is a request that **Norway participates to make the area coverage complete**. Norway was a participant in the 2004 survey and has a proven track record in this type of sampling.

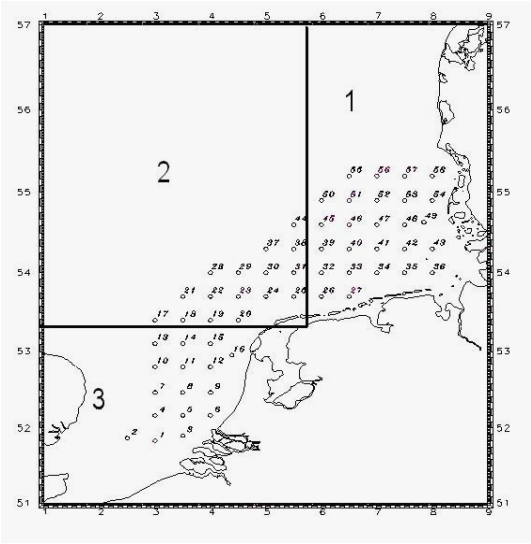

Figure 6.3: German malformation cruise stations.

If Norway is able to participate it would be possible to undertake an ichthyoplankton survey of ICES Area IVa in collaboration with the Scottish survey. The French survey would be able to provide an extensive coverage of ICES Area IVc and part of IVb using IBTS during the spawning season of cod (see figure above). Denmark would be able to supplement the French sampling particularly the central North Sea. Germany would be able to collect fish eggs during the malformation survey in the German Bight and southern North Sea. Adult sampling undertaken in conjunction with these surveys would provide an estimate of the phase of the spawning season and the proportion of spawning cod. This additional information would be important to ensure that the perception of regional spawning intensity from egg surveys is not biased by sampling too early or late in the spawning season. All countries agreed to collect ovary tissue samples during surveys for this purpose. FRS offered to undertake the analysis of the ovary samples from all participants.

This level of coverage would allow a comparison of egg production between the northern and southern North Sea, a key aim of the planned 2009 actions.

Requests for collection of additional samples on IBTS will be made through national PGEGGS delegates.

The methods to be used for plankton sampling were briefly reviewed and a draft protocol was written (Annex 6).

7 Consider the advice from WGZE on the value of collecting additional zooplankton samples during future North Sea ichthyoplankton surveys in 2009 (workshop aim d).

No advice has been received by PGEGGS from WGZE. Given the problems identified with resources for 2009 surveys, PGEGGS felt that additional zooplankton sampling should not be considered at this stage.

8 Prepare an action plan to ensure archiving of the data collected in 2004 (workshop aim e)

Substantial progress on this issue has not yet been achieved. Clive Fox contacted the ICES data management team to discuss this issue. The response was that if data could be got into a form suitable for inclusion in DATRAS then it could be imported relatively easily. Unfortunately the data from these surveys are complex due to the subsampling, use of genetic probes and other issues such as species coding. The ideal approach to this problem would be for Clive Fox to spend a week at ICES to work with the database team to translate the data into a suitable format. However, funding this is an issue because Clive Fox is no longer employed in a government fisheries laboratory. Action should be initiated by the new PGEGGS chair to ensure long-term archiving of the data from 2004 and future surveys. At present the data are held at the national laboratories and backup copies held by Clive Fox at SAMS.

9 Election of new chair for the group (workshop aim f)

No resolution could be attained on a new chair to replace Clive Fox i.e. no-one at the meeting was willing to volunteer for the post. If the parent body wish PGEGGS to continue, they will need to appoint a new chair.

Annex 1: List of participants

Name	Address	Phone	Email
Clive Fox *(Chair)*	Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban, Argyll, PA37 1QA	+44 1631 559423	clive.fox@sams.ac.uk
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Annex 2: Letter requesting support for 2009 surveys (Norway)

Tore Nepstad
Director
Institute of Marine Research
PO Box 1870 Nordnes
5817 Bergen, Norway

Dear Sir:

Re: Norwegian participation in an ICES coordinated survey of cod spawning locations in the North Sea in 2009

In January to March 2009 it is planned to undertake sampling of pelagic eggs to determine relative egg production in the northern and southern North Sea. At present we have commitments from Germany, France and Denmark to cover the southern North Sea and a commitment from Scotland to undertake part of the Northern North Sea. The participation of Norway is essential to give adequate sample coverage of the Northern North Sea and as such PGEGGS request that you consider making ship and personnel time available. Without participation by Norway the coordinated survey is unlikely to go ahead.

The surveys are designed to provide indices of the relative spatial importance i.e. northern and southern spawning components of North Sea cod. By periodically checking the proportions every four to five years ICES will have input to the spatial aspect of the Ecosystem Approach to Fisheries Management. This information is also needed for marine conservation management plans e.g. designation of Natura 2000 sites.

Yours faithfully

Chair of ICES Expert Group PGEGGS

Annex 3: Letter requesting support for 2009 surveys (Netherlands)

Frans van Beek
Head of Centre of Fisheries Research
PO Box 68,
1970 AB IJmuiden, Netherlands

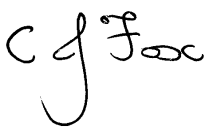
Dear Sir:

Re: Dutch participation in an ICES coordinated survey of cod and plaice spawning locations in the North Sea

In January to March 2009 it is intended to undertake sampling of pelagic eggs to determine relative egg production in the northern and southern North Sea. At present we have commitments from Germany, France and Denmark to cover the southern North Sea and a commitment from Scotland to undertake part of the Northern North Sea. The participation of the Netherlands in the Southern North Sea, by working up the herring larvae survey samples for eggs, would give the possibility of calculating an AEP for plaice and as such PGEGGS request that you would make personnel time available.

The surveys are designed to provide indices of the relative spatial importance i.e. northern and southern spawning components of North Sea cod and a fisheries independent estimate of the status of the southern North Sea plaice stock. By periodically checking the proportions every four to five years ICES will have input to the spatial aspect of the Ecosystem Approach to Fisheries Management and give an extra opportunity for tuning the XSA for plaice. This information is also needed for marine conservation management plans e.g. designation of Natura 2000 sites.

Yours faithfully



Chair of ICES Expert Group PGEGGS

Annex 4: PGEGGS Terms of Reference for the next meeting

The **Working Group on Planning Egg Surveys** [PGEGGS] (Chair: To be appointed) will meet during November 2008 to:

a) Confirm planning for 2009 North Sea ichthyoplankton surveys.

b) PGEGGS will also arrange for archiving of data collected in 2004 in the North Sea ichthyoplankton survey.

Supporting Information

PRIORITY:	The planned 2009 surveys are important in that they will confirm findings from 2004 in relation to locations of cod spawning and further investigate whether cod in the northern North Sea are actively spawning. These results are important in relation to ongoing management issues with these two key commercial stocks. Consequently, these activities are considered to have a high priority.
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 coordinated international North Sea ichthyoplankton surveys was presented in the report of PGEGGS which met in IJmuiden from 24-26 June 2003 and endorsed by the LRC. A successfull survey was planned and undertaken in 2004 under the direction of PGEGGS. The results confirmed reduced egg production for plaice compared with earlier surveys and raised important scientific questions regarding effective cod spawning areas. Particularly for cod the 2004 results need to be confirmed and in particular the apparent low egg production of northerly areas investigated. The situation should be monitored by regular surveys. Because of the cost of undertaking such surveys, PGEGGS has recommended that they be undertaken every 5 years. 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.
RESOURCE REQUIREMENTS:	ICES secretariat support for PGEGGS reports only, some advice from the ICES Data Centre is required to facilitate preparation of data collected in 2004 for archival
PARTICIPANTS:	See Annex 1
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.
LINKAGES TO OTHER COMMITTEES OR GROUPS:	No formal linkages
LINKAGES TO OTHER ORGANIZATIONS:	No formal linkages
SECRETARIAT MARGINAL COST SHARE:	

Annex 5: Recommendations

RECOMMENDATION	ACTION
1. PGEGGS to send letters to request support for sampling in 2009 to Norway, Netherlands, England (Annex 2 and 3)	PGEGGS Chair to send out request letter
2. Need to appoint a replacement chair for PGEGGS	Living Resources Committee
3. A meeting of PGEGGS be convened for November 2008 to confirm planning of the 2009 surveys	Replacement chair of PGEGGS to action
4. PGEGGS to archive the 2004 North Sea ichthyoplankton survey data with ICES Data centre	Replacement chair of PGEGGS to action

Annex 6: Methodology for additional maturity sampling

In order to assess the phase of the spawning in different North Sea regions samples of mature cod (IBTS stages MI, MA and SP) are required. A maximum of 200 cod per cruise with a maximum of 50 female cod for a given station are required for the analysis. Information on total length to the nearest cm and ovary and whole body weight to the nearest g should be recorded for each fish sampled. A piece of ovary (approx 100 mg) should be taken with a Wiretroll pipette from the mid-section of the ovary stages MI and MA and fixed in 3.6% formaldehyde buffered to pH 7.0 in labelled Eppendorf-type tubes. FRS will provide sampling kits for this purpose. The resulting samples will be examined using image analysis to determine the size composition of oocytes. This information will be used to assess spawning state based on published methodology.

Annex 7: Methodology for fish egg sampling

The following procedures are tentative; these will be further discussed and agreed upon at the following meeting.

Samplers

We recommend the use of GULF VII or Bongo nets. The BONGO net is 60 cm in diameter and can be equipped with nets of different mesh sizes (330 and 500 μm). Two samples are taken at each hauls in parallel. Both nets can be set up with flowmeters that should be placed in the net-opening. The Gulf high-speed plankton sampler has a 76cm diameter body fitted with a 40cm or 20 cm diameter aperture, conical nosecone. The standard net of this gear will be made of 270μm aperture mesh.

Deployment of samplers

The plankton samplers should be deployed on a double oblique tow, from the surface to within 2 metres of the bottom (or as near as bottom topography will allow) and return to the surface. In certain cases (French supplemental sampling, vertical hauls are specified). Speed when hauling should be between 2-3 knots or would be carried out from a non-moving ship in the case of vertical hauls. At shallow stations, multiple double-oblique dives may be necessary to enable a sufficient volume of water to be filtered. At deep stations the sampler should be deployed down to 100 m. A minimum sampler deployment time of 15 minutes is recommended.

The standard procedure for recovery of the plankton sample will be as follows:

Remove the end bag used on the station and place in a jug before washing down the net.

Attach a clean end bag and **gently** wash down the net playing the deck hose over the **outer** surface of the net from both ends of the sampler, taking care to wash any accumulated material on the lower surface of the net just in front of the end bucket.

Remove the end bag and place in the jug for transfer into the wet lab on the ship. This jug **must** be kept free from formaldehyde so should be clearly labelled.

Make sure the net is clean, using more than one end bag and repeating the first 3 steps if necessary.

Check the plankton net for tears, replace if necessary.

Make sure that a clean end bag is left on the sampler ready for the next station.

Move the jug containing th end-bags and plankton samples into the ship's laboratory and proceed with the presorting of cod-sized eggs.

Fixing plankton samples

Once collected zooplankton must be fixed and preserved to await identification and count. The identification may require an important time and is facilitated by species characteristic chromatophores visible on live specimen. The very fast photochemical oxidation of these chromatophores is a cause for slower and inaccurate identification.

We propose to modify the fixation fluid as follows following work by France:

- Ascorbic acid 2g
- disodic EDTA 20g
- BHA (buthylhydroxyanisol) 8g

- Monopropylene glycol 1l
- commercial formalin (36%) 2l
- distilled and deionised water to make up 5l
- buffer at pH 7 using sodium glycerophosphate (about 200g)

Dissolve BHA and 1/2l of propylene glycol. Dissolve separately EDTA in 1/2l of distilled water, add ascorbic acid and buffer at pH 7 using sodium glycerophosphate (about 90g). In a 5l recipient, pour the formol and while mixing bring at pH 7 using sodium glycerophosphate. Add the BHA solution, the remaining propylene glycol and make up to 5l with the distilled water. Mix 1/2 hour.

Finally, the samples are fixed in seawater using 6% of this solution. It is important to note that the resulting concentration of formalin in the sample is less than 1%.

Transfer of fixed material

It is recommended that the material is transferred to the 'observation fluid' (Steedman 1976) between 48 h and 3 weeks from sampling. This solution will act as a preservative on fixed material and enables the sample to be used for genetic analysis (dependant on results to be obtained from Cefas during the autumn of 2008)

Recipe for observation Fluid (30 litres)

To make 30 litres of observation fluid for use as medium for analysis and short-term storage of plankton samples in the laboratory:

1) Mix together 150cm3 Propylene phenoxetol and 1500cm3 Propane-1,2-diol. This must be done vigorously as the two chemicals are not very miscible.

2) Add deionised water to the mixture to make it up to 30 litres.

3) Mix thoroughly again.

Sub-sampling protocol

Where large numbers of eggs and larvae occur in plankton samples it becomes impractical to sort the total sample. The recommended method for subsampling is by using a folsom splitter. In this way, samples can be subdivided repeatedly to achieve the optimum sampling level. It is recommended that at least 100 eggs of the target species (cod and plaice) are present in the subsample. If more than 100 eggs of these species are sorted from the sample (or subsample) then only 100 need to be staged and the rest apportioned across the stages found in that particular sample. If 100 eggs of the target species are NOT found in the subsample the whole sample will have to be sorted.

In some samples there might be large numbers of fish eggs present but relatively few eggs of the target species. In these cases the smaller eggs can be subsampled and all the larger eggs should be sorted from the sample. It is useful to make a glass pipette of a known aperture (e.g. 1.1mm diameter) and then any eggs that will not go into the pipette should be sorted from the sample for identification under a microscope.

All cod and plaice larvae should be identified and all larvae should be identified if resources allow.

Identification of and staging of eggs in plankton samples

Eggs will be identified on the basis of the presence/absence of oil globules, size of the egg and in some cases the characteristic appearance as described in (Russell 1976).

The identification of cod, haddock and possibly some smaller diameter plaice eggs can be difficult if all three species are spawning in the same area. Plaice eggs are generally much larger than those of other species spawning in the North Sea. Russell (1976) gives an egg diameter of 1.66–2.17mm. In addition, plaice eggs have a thicker membrane than either cod or haddock. Based upon experience from sampling in the Irish Sea, plaice eggs will be classified as those above **1.75 mm** diameter.

The main identification problem will be to distinguish between cod and haddock eggs. The egg diameter range is given by Russell (1976) as 1.16–1.89mm for cod eggs and 1.2–1.7mm for haddock. Neither egg has any distinct morphological features, which would aid identification. In the later stages of egg development the embryos develop characteristic larval pigmentation that enables separation of the two species. There may also be some overlap between whiting eggs at the top of their range and the lower size of cod. Genetic methods will be employed to distinguish early stage cod and haddock eggs.

They will therefore be recorded as ZZY (un-identified) along with measurement of their diameter (in mm) and developmental stage (for eggs in size range 1.10–1.75 mm). Eggs smaller than 1.10 mm diameter without oil globules only require to be measured.

Cod-like eggs and those of plaice will be also classified into one of six developmental stages (IA, IB, II, III, IV, and V) following the development criteria described for cod (Thompson and Riley 1981) and plaice (Ryland and Nichols 1975).

Sorting of cod-like eggs for genetic analysis

Examine each egg and assemble eggs between **1.1 mm** and **1.75 mm** in diameter which do not contain oil globules.

Record the development stage of each 'cod-like' egg and its diameter on record sheets (these data will later be transferred using electronic recording described later on)

Transfer these eggs into a labelled eppendorf.

Add 1 ml of ethanol and store eppendorfs in closable eppendorf boxes.

Each eppendorf **must** be clearly labelled with the ship name, cruise number, station number and egg type

Ideally eppendorfs should be labelled with a chemical resistant pre-printed label (e.g. Brady PTL thermo-printer but this is expensive). Note that ethanol can remove many types of marker pen so test your labels for resistance to ethanol. A sticky label marked with pencil is preferable to using marker pens.

Data handling

Protocols will be agreed at the later meeting.