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

2–3 December 2008

IMARES, IJmuiden, the Netherlands

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CIEM Conseil International pour l'Exploration de la Mer

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1 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 organise but in 2004 the field-work 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 since 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 recognised 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 north-eastern North Sea.

During the meeting available resources for 2009 were reviewed. It became clear that member countries could not commit adequate resources to enable a repeat and extent the 2004 survey design. The committed resources will only allow for a one-time coverage of almost the whole North Sea. Thus no data on the whole spawning season of cod can be collected. The single coverage will allow for a coordinated comparison of cod spawning areas and reproductive state in two areas of the North Sea (southern and northern).

Plaice egg production in the southern North Sea remains of interest, particularly for the Netherlands. The proposed 2009 surveys will be able to provide an additional egg production index for plaice.

Information will also be collected on egg and larval production for other species such as haddock and sand-eels.

During the meeting the future of PGEGGS was discussed. An overview was produced of the available ichthyoplankton surveys in the ICES areas. It was recommended that a central group for ichthyoplankton surveys is constituted to discuss developments and problems in sampling, sampling equipment, protocols and data archiving and formats.

References

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.

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.

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.

2 Opening of the meeting

PGEGGS met from 2–3 December 2008 at IMARES, Ijmuiden, The Netherlands.

3 Adoption of the agenda

The **Planning Group on North Sea Cod and Plaice Egg Surveys in the North Sea** [PGEGGS] (Chair: Cindy van Damme*, the Netherlands) will meet in IJmuiden, the Netherlands, from 2-3 December 2008, and by correspondence from January 2009 to August 2009 to:

a) Confirm planning for 2009 North Sea ichthyoplankton surveys;

b) Arrange for archiving of data collected in 2004 in the North Sea ichthyoplankton survey.

PGEGGS will report by 1 January 2009 and by 15 August 2009 for the attention of SCICOM and TGISUR.

For the meeting the following tasks were considered:

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

b) Discuss future of PGEGGS and possibilities of joining with other groups to form an expert ichthyoplankton group.

c) Theme session on ichthyoplankton surveys at ASC 2010.

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

4 Participants

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

5 Planning for a North Sea wide ichthyoplankton survey in 2009 (workshop aim a)

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 gram of the ovary. As spawning 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 3). FRS offered to undertake the analysis of the ovary samples from all participants.

Only one country has committed ship resources specifically for plankton sampling under PGEGGS (Table 4.1) whilst other countries can make additional sampling un-

der other programs. 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 WP2 samples during their IBTS in the southern North Sea. Denmark, Norway and Scotland have also committed to undertaking some additional sampling of eggs using Bongo and Gulf sampler nets during their portions of the 1st quarter IBTS.

Table 4.1. Resources committed for 2009.

	Committed resources			
Country	PGEGGS	Egg malformation	Additional IBTS sampling	IHLS Herring larvae survey
Netherlands				End Dec-Jan: Gulf VII
France			Mid-January to Mid-February CUFES, WP2	
Germany		27 Feb–18 March Embryo malformation Survey, Nackthai		1–15 Jan Nackthai
Denmark			Feb Bongo	
Scotland	20 Feb–6 March: 15 days Gulf VII, Bongo		Jan–Feb	
Norway			5–28 February Gulf III or VII	

There is still **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 whole North Sea can be covered once.

Netherlands and Germany will cover the Southeastern North Sea and English Channel 3 times during December and January (Figure 4.1) during the herring larvae survey. Samples will be taken in oblique hauls with high speed plankton samplers with CTD mounted on top. Samples will be sorted for fish eggs and larvae.

Data on >1.1mm eggs will be provided on agreed format. It is not possible for the Netherlands to do DNA analyses on cod-like eggs. Cod-like eggs will be counted and kept separated from the remaining eggs for further analysis if funding is available.

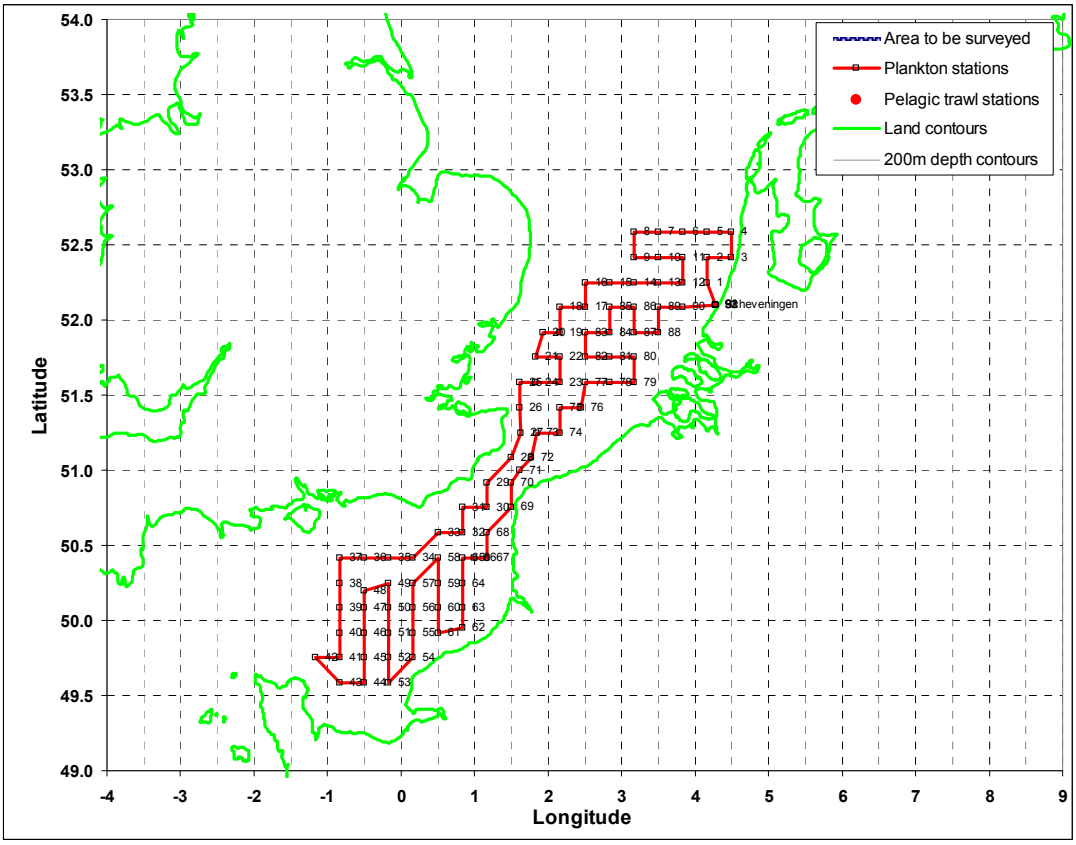


Figure 4.1. Proposed sampling grid during the International Herring Larvae surveys.

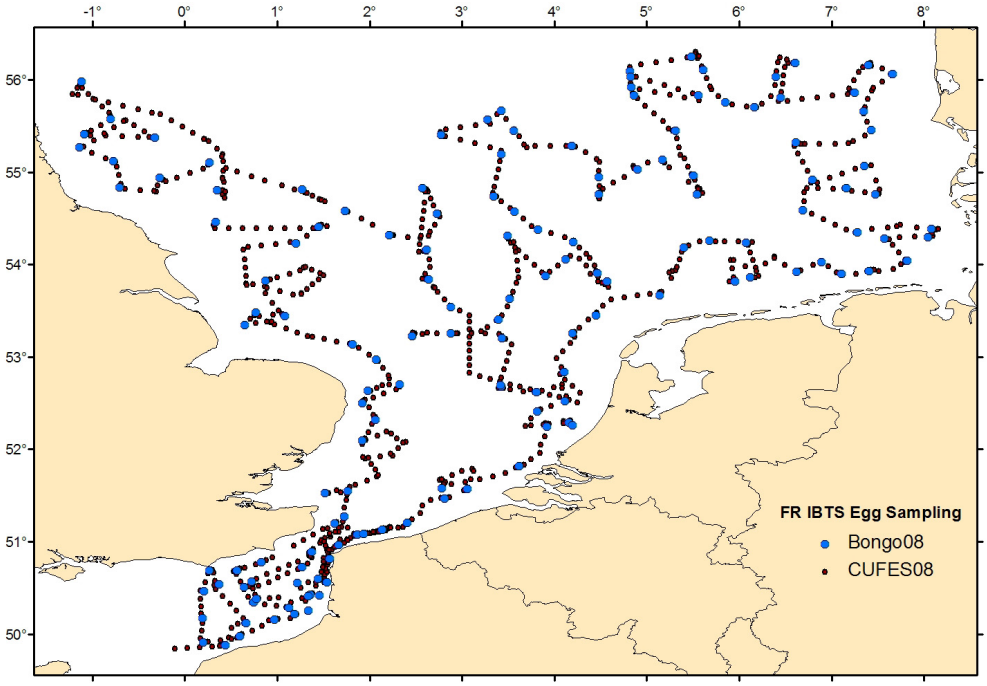
The French survey will be able to provide an extensive coverage of ICES area VIId, IVc and part of IVb. France will sample the North-Sea during the IBTS survey from 15 January to 15 February 2009 covering Eastern English Channel and Southern North Sea (Figure 4.2).

Two types of sampling gear will be used:

- WP2 (plankton net): Double WP2 with 500µm (ichthyoplankton) and 200 µm mesh (zooplankton) used with off-centered weight to allow for oblique vertical sampling in station (nets filtering both on their way down and back). Off-centered flow meter at both net mouths to measure filtered volume. About 100–150 stations will be sampled throughout the whole survey duration (day and night).
- CUFES: Continuous Underway Fish Egg Sampler fitted with 500µm mesh concentrator and collectors. Each sample is accumulated for every 30 minute during the entire survey. Pump flow is measured automatically every 30 seconds and averaged over each 30 minute sampling duration.

Eggs in samples will be measured, counted and identified semi-automatically by automated image recognition (ZooScan). Visual re-classification of image objects by an expert will further refine identification and staging of cod-like and plaice. Please note that full sample will be sorted "virtually". Individual species and stage will not be physically sorted into different flasks or ependorf tubes at that stage.

Depending on egg diameter distribution and known overlap range where confusion of species may be possible (cod-like eggs), samples will be identified and sorted for genetic identifications. Genetic identification of French samples will be obtained with alternative methods than those developed at CEFAS. DNA extraction with magnetic beads, PCR amplification and Restriction Fragment Length Polymorphism methods will be used on Cyt b mitochondrial DNA.



Data on >1.1mm eggs will be provided in an agreed format. Results from DNA analyses on cod-like eggs will be made available to group.

Figure 4.2. French IBTS/CUFES proposed sampling grid.

Germany will be able to collect fish eggs during the malformation survey in the German Bight and southern North Sea (Figure 4.3). This level of coverage will allow a comparison of egg production between the northern and southern North Sea, a key aim of the planned 2009 actions.

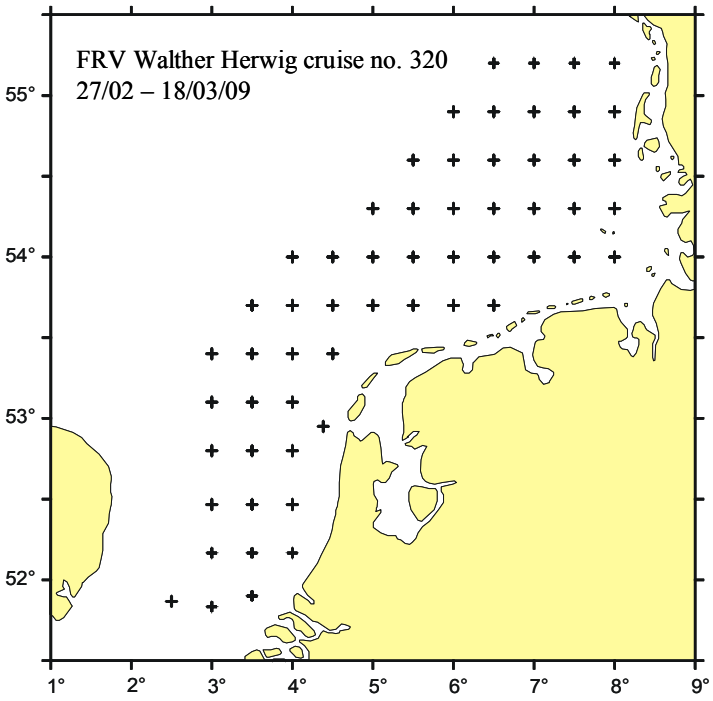


Figure 4.3: German egg malformation cruise stations.

Denmark would be able to supplement the French sampling particularly the central North Sea (Figure 4.4).

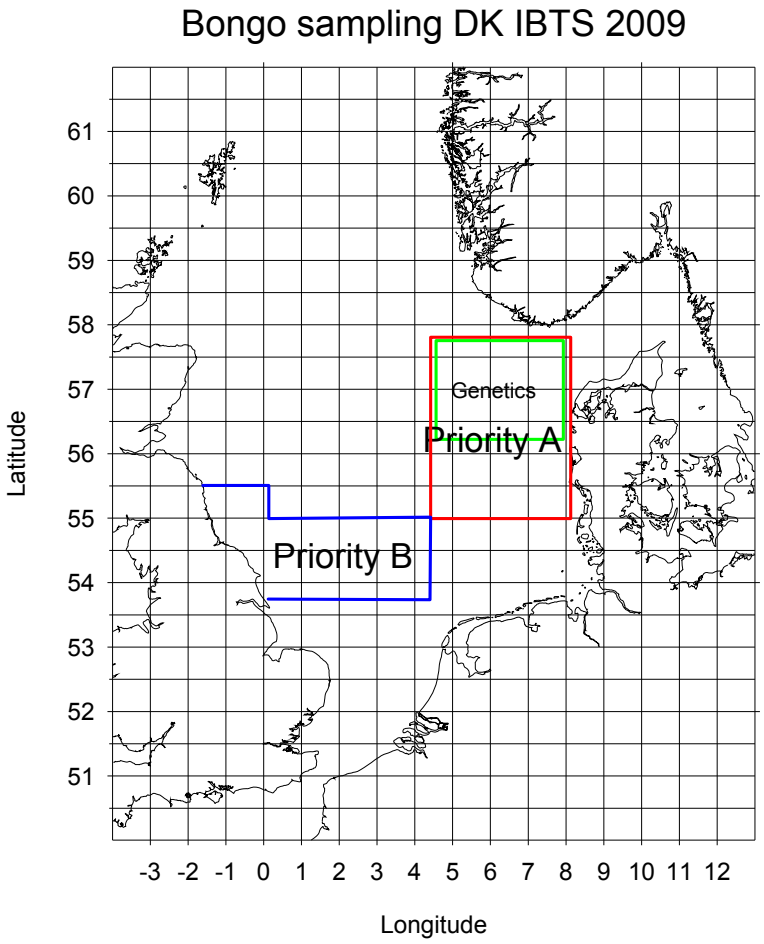


Figure 4.4 Proposed Danish sampling areas.

The sampling by Denmark will take place during the IBTS, planned for 30/1–17/2 2009. Sampling will be carried out by double oblique Bongo hauls, 1–2 hauls in allocated rectangles west of 4E, and 2–3 hauls in rectangles east of 4E. Highest priority is given to the area east of 4E. In addition to this sampling Denmark will investigate the applicability of attaching an egg-sampler on the MIK gear which can be used during all IBTS cruises. All samples will be preserved in formalin, and later sorted for eggs and larvae. In the area east of 4E and north of 56 20′ Denmark will preserve single eggs in alcohol from selected stations for later molecular identification.

Both Norway and Scotland and will cover the entire ICES area IVa. The FRS Marine Laboratory have a 15 day cruise timed to coincide with the average peak in cod spawning in the northern North Sea, between 20 February and 6 March in 2009 (Figure 4.5). The survey will undertake trawling to sample adult cod from previously identified spawning areas and deploy plankton gears to sample ichthyoplankton. The area to be sampled will extend from 55 30′ – 61° 30′ N and 5° W to 1° E, although priority will be given to stations north of 57 ° N. A chart showing the main 51 stations to be sampled and the 11 additional stations is given in Figure 4.5.

Trawling will take place at five or more areas previously identified to contain high densities spawning cod over the survey area. A maximum of 200 mature female cod will be taken for analysis. Information on total length to the nearest cm as well as ovary and whole body weight to the nearest g will be recorded for each fish sampled. A piece of ovary will be taken from the mid-section of the ovary stages MI and MA either with a Wiretroll pipette or using a scalpel to remove a section ~ 3 mm thick and 10 * 10 mm wide. The ovary section will be fixed in 3.6% formaldehyde buffered to pH 7.0 in labelled Eppendorf-type tubes. The resulting samples will be examined using image analysis to estimate the number of vitellogenic oocytes.

Plankton sampling will involve either oblique tows with a 40 cm Bongo net or 20cm diameter Gulf VII. Volume filtered will be determined using off-centered flow meter at net mouths. Stations will be sampled during both day and night. Eggs will be separated from zooplankton and then eggs will be sorted. Cod like eggs within the range 1.1-1.6 mm will be sorted whilst fresh and a sub-sample of 50 will be fixed in 100% ethanol for later molecular identification using the approach of Taylor *et al*. (2002). Remaining eggs in samples will be fixed in formalin and transferred to observation fluid as agreed in earlier meeting. Egg will be counted, measured and identified possibly semi-automatically by automated image recognition (ZooScan). Visual reclassification of image objects by an expert will further refine identification and staging of cod-like eggs. Data on >1.1mm eggs will be provided to IMARES on agreed format. Results from DNA analyses on cod-like eggs will also be made available to group. All fish larvae will also be extracted for analysis by partners in the ICES PGEGGS.

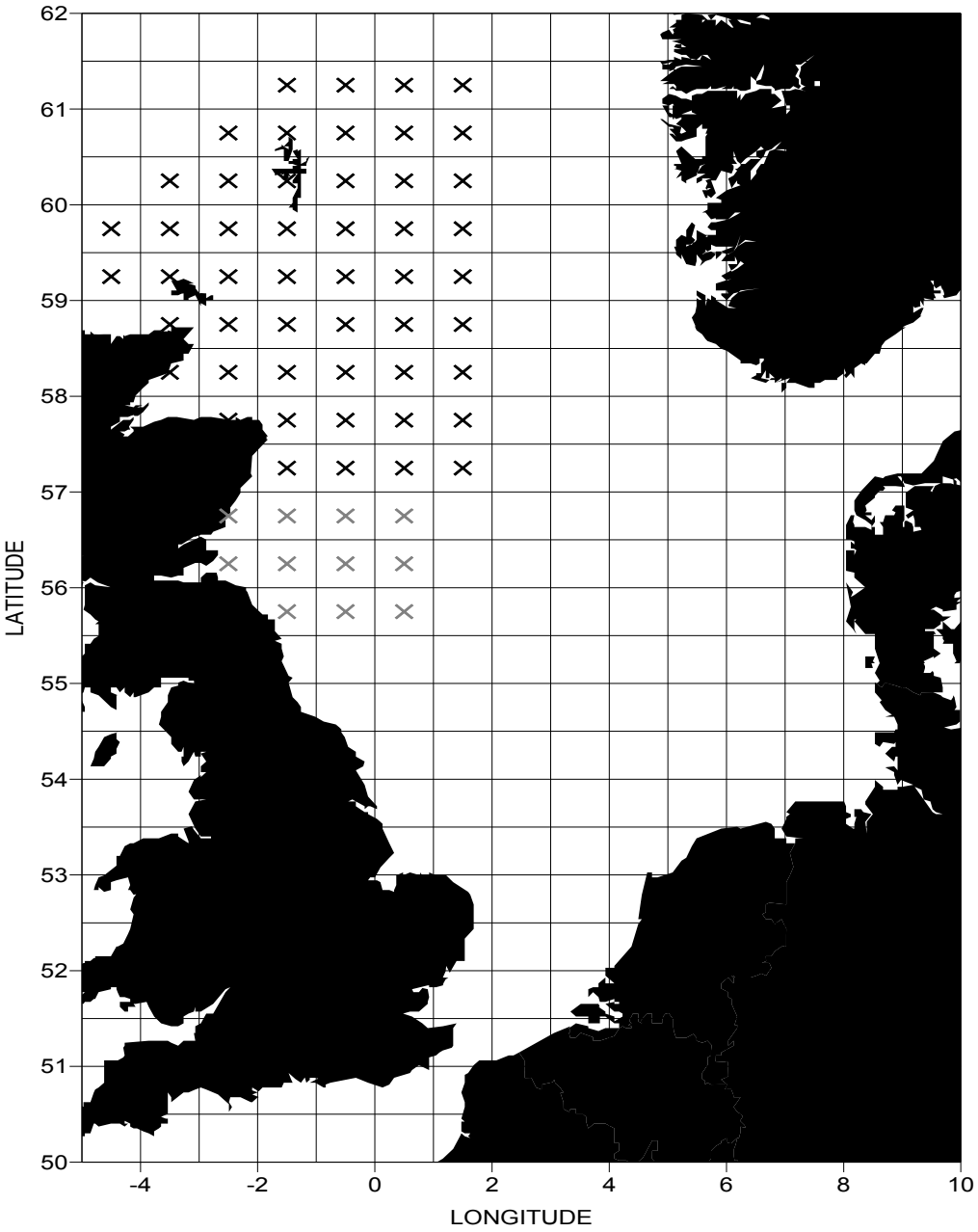


Figure 4.5. Proposed Scottish Ichthyoplankton stations. Black and grey crosses refer to main and additional stations, respectively.

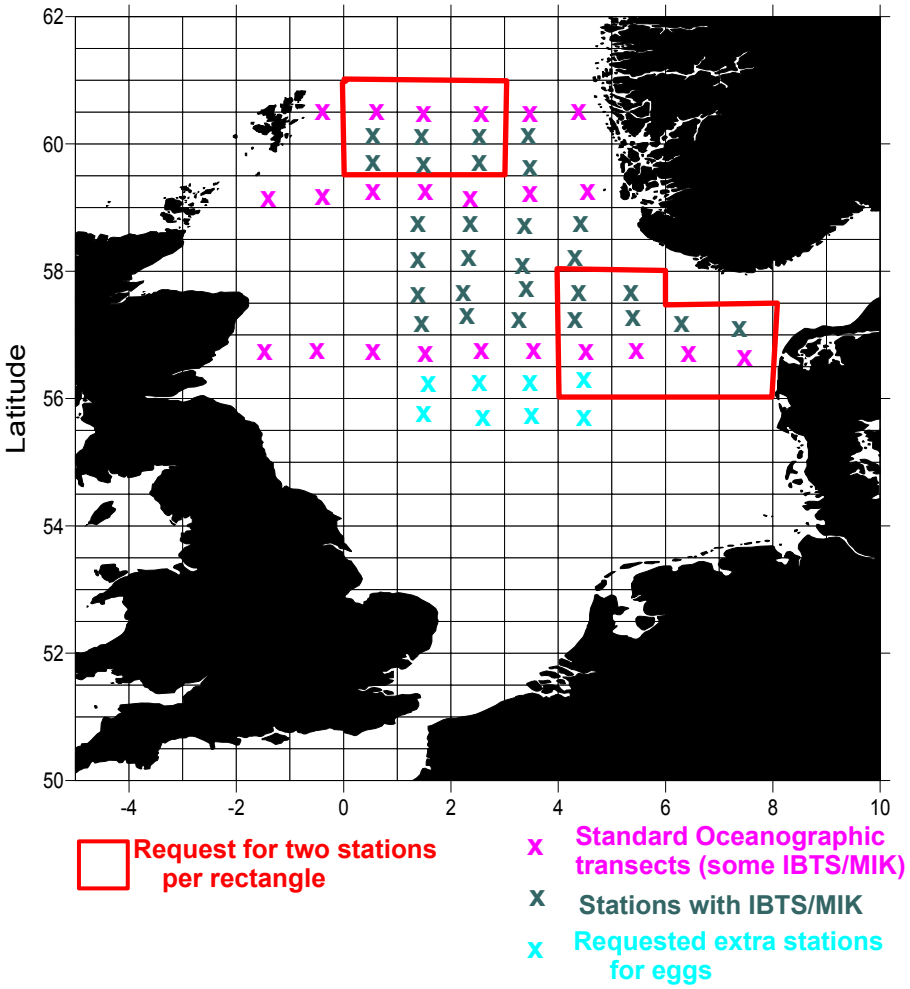


Figure 4.6. Proposed Norwegian Ichthyoplankton stations. See legend below figure for an explanation of the symbols.

The Institute for Marine Research, Bergen will undertake the ichthyoplankton survey in conjunction with their IBTS/MIK survey between the 5–28 February 2009 (Figure 4.6). The survey will take extra samples of adult cod from previously identified spawning areas (see text above). Since the surveys will also entail three of the regular IMR oceanographic transects, ichthyoplankton sampling will be extended east and west of the standard IBTS area (see Figure 4.6).

The methods to be used for plankton sampling, identifying and staging of eggs and identifying of larvae were reviewed and the protocol is given in Annex 4.

Measurements of temperature and salinity

It is suggested that each haul for fish eggs are accompanied by measurements of temperature and salinity. The priorities for these measurements are, in subsequent order: 1) Separate casts for vertical profiling between surface and bottom, 2) Measurements from an instrument mounted on sampler, 3) measurements of surface and bottom values, solely. Data should be stored either in standard format of the gear used, or in columns of a spread sheet. Data from all surveys will be collated by the Danish participant, and made available to the entire group.

6 Discuss future of PGEGGS and possibilities of joining with other groups to form an expert ichthyoplankton group (workshop aim b)

There are a number of ichthyoplankton surveys currently being conducted in the ICES area, primarily for providing information that can be used in stock assessments (see Table 5.1). The surveys are targeted at a number of species such as plaice, cod, herring, anchovy, sardine, mackerel and horse mackerel. The surveys are targeted at sampling either eggs or larvae and use a variety of types of sampling equipment with a range of sampling protocols. In addition the surveys are either national programmes or consist of a variety of levels of international cooperation.

The surveys have a variety of goals, and with them come a variety of levels of complexity in the sampling programmes. There are a number of herring larvae surveys where the abundance of larvae is used as an index of the Spawning Stock Biomass (SSB) i.e. North Sea Autumn Spawning, Irish Sea Autumn Spawning and Norwegian Spring Spawning herring. In one instance (Rügen herring in IIIa) the production of young herring larvae is used as an index of herring recruitment. In this case assumptions need to be made on larvae growth and mortality rates.

The abundance of later larvae is often used as an index of recruitment such as for North Sea Autumn Spawned herring and Irish Sea gadoids. In all of these cases the sampling equipment has been standardized to Methot Isaac Kidd (MIK) trawls.

There are also a number of egg surveys that are used to provide indices of SSB. The surveys currently undertaken involve anchovy, sardine, mackerel, horse mackerel, cod and plaice in areas such as the western margin of the northeastern Atlantic, the Bay of Biscay to off Cadiz, North and Irish Seas. In these surveys the egg abundances are used to estimate egg productions and through estimates of fecundity back to the SSB. These techniques involve a greater level of data as they require not only pelagic egg data but also information on the reproductive potential of individuals in the stock. These analyses often come under the heading of Annual Egg Production (AEP) or Daily Egg Production (DEP) Methods. As with larvae production methodology these techniques also require information or assumptions on egg development rates and egg mortalities.

Table 5.1. Summary of current ichthyoplankton surveys undertaken in the ICES area, primarily for use in stock assessments. Note this list is not exhaustive and does not include surveys being undertaken over limited time periods (years) solely for process studies.

Survey	Quarter	Area	Target species	Years/ frequency	Types of equipment	Purpose	Notes
Plaice and cod eggs (PGEGGS)	1	North Sea	Cod, Plaice	2004	Gulf, Bongo	Egg distributions (spawning locations), plaice EP	International
Demersal egg surveys	1	Irish Sea	Plaice and Cod	1995, 2000– / Annual	Gulf	Eggs to EP to SSB	International, now England and Northern Ireland

Survey	Quarter	Area	Target species	Years/ frequency	Types of equipment	Purpose	Notes
International Herring Larvae Survey (PGIPS)	4 & 1	North Sea	Herring	1973– / Annual	Gulf	Larvae as SSB index	International
Northern Irish	4	Irish Sea	Herring	1993– / Annual	Gulf	Larvae as SSB index	Only Northern Ireland
Mackerel Egg (WGMEGS)	2	North Sea (North/ Central)	Mackerel	Every 3 years	Gulf	Eggs to EP to SSB	International
Rügen Herring	1	IIIa, Baltic	Herring	1977 / Annual	Bongo	Larvae to recruitment	Only German
MIK	1	North Sea	Herring	1976-/ Annual	MIK	Recruitment index	International
MIK	2	Irish Sea	Gadoids	1993– / Annual	MIK	Recruitment Index	Only Northern Ireland
North Sea CUFES	1	Southern North Sea/ English Channel	Eggs	2006– / Annual	CUFES, Vertical WP2	?	Only France, done in conjunction with IBTS.
Baltic eggs and larvae		Baltic (Bornholm Basin)	Cod eggs and larvae	Annual	?	EP?	Only Denmark, Germany?
Malformed eggs	1	South-eastern North Sea	Plaice eggs	Annual since 1980s	?		Only Germany
Mackerel and Horse mackerel egg surveys	2	North-east Atlantic	Mackerel and Horse mackerel eggs	1992– / Every three years	Gulfs	Eggs to EP to SSB	International
Anchovy and Sardine egg and acoustic survey (WGACEGG)	2	Biscay to the Gulf of Cadiz	Anchovy and Sardine eggs and larvae	Annual since 1995	?, CUFES	EP	
NVG Sild surveys	1 or 2	Norwegian coastal zone	Norwegian Spring Spawned herring	Annual since 1982	Gulf and Vertical hauls	Larvae	Only Norway
Herring larvae	2	Stettin lagoon and Vistula lagoon	Herring larvae			Larvae	Poland
Russian surveys?		Barents Sea					

There is a need for the various groups working on ichthyoplankton surveys to communicate on a number of topics. These include discussions on developments and problems in sampling, sampling equipment, protocols and data archiving and formats. There is also a need to keep informed on new or novel techniques for e.g. species identification using e.g. genetic probes or automated procedures. A number of

these surveys are being undertaken at a regional level and as such there is also a need to bring people together so that surveys are not undertaken in isolation. Experience gained in one area can be transferred to others and there can be some semblance of standardization across all similar surveys.

We recommend that a central group for ichthyoplankton surveys is constituted which may not necessarily take the form of a Working Group. A suggested name for this group would be 'Standards in Ichthyoplankton Surveys (SIPS). The group will be required to provide coordination that ensures that every three years the group (encompassing all regional and species based ichthyoplankton survey planning, working and study groups) either meets or a special session is requested for the ASC. The final product for this group will be the cross fertilisation of ideas and standards for ichthyoplankton surveys in the ICES area.

7 Theme session on ichthyoplankton surveys at ASC 2009 (workshop aim c)

Theme Session Ichthyoplankton Surveys – value added beyond assessment

Conveners: Cindy van Damme & Matthias Kloppmann, third convenor?

Within ICES co-ordinated work a number of ichthyoplankton surveys have been carried out for many years. Many of them already constitute a long time series that would allow for data analysis beyond estimates of annual indices of recruitment or annual egg production. Some of those surveys also may have undergone changes in methodology or have adopted new techniques. This session invites contributions that analysed ichthyoplankton survey data with respect to changes in distribution, size and stage composition of species in relation to the changing physical and biological environment. Descriptions and analysis of change of methodology and the adoption of new techniques, and how they impacted the survey results are also expected. Since most of those surveys are carried out under the supervision of different ICES working groups it is expected that this theme session will also promote positive influences between different ichthyoplankton groups.

Contributions addressing the following topics are encouraged:

- The impact of changes in methodology on survey results
- Adoption of new techniques and their benefits for improvement survey results
- Changes in abundance and distribution patterns in relation to changing ecosystem
- Changes in size and/or stage composition
- Species composition, parasitisation of eggs and other issues of interest.

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

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 sub-sampling, use of genetic probes and other issues such as species coding.

For the 2009 survey the data will initially be gathered and stored in the IMARES database, until it can be included in DATRAS. IMARES will try to get funding to send a database manager to ICES to address the problem of importing the PGEGGS 2004 and 2009 data into DATRAS. It will require at least one member of PGEGGS to join them with background knowledge of the PGEGGS data.

Annex 1: List of participants

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Annex 2: Recommendations

RECOMMENDATION	ACTION
1. PGEGGS recommends that a central group for ichthyoplankton surveys is constituted	Science Commitee
2. PGEGGS recommends to plan a theme session on added values of ichthyplankton surveys at the ICES Annual Science Conference	Science Commitee
3. PGEGGS should meet by correspondence in 2009 to review where and when sampling was carried out	All participants to send cruise reports
4. PGEGGS should meet in 2010 to discuss results of the 2009 survey	All PGEGGS participants
4. PGEGGS should compare the results from the 2004 and 2009 surveys in order to assess whether there has been a change in spawning distribution of target species	All PGEGGS participants
5. PGEGGS to archive the 2004 and 2009 North Sea ichthyoplankton survey data with ICES Data centre	IMARES

Annex 3: 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. Participants taking PGEGGS samples during the IBTS, France, Denmark, Norway and Scotland, are requested to collect cod ovary samples,. 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.

FRS also requests cod otoliths for shape analysis and microchemistry analysis of cod caught during the IBTS. If institutes could send 1 otolith of 100 cod from the 2007 year class per cruise. FRS will send around a request with instructions to all the institutes involved.

Annex 4: Methodology for fish egg sampling and identifying and staging of the eggs and larvae

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 VII high-speed plankton sampler has a 50 cm diameter body fitted with a 40 cm or 20 cm diameter aperture, conical nosecone. The standard net of this gear will be made of 280 μm aperture mesh.

At sea – Deployment of samplers

The plankton samplers should be deployed on a double oblique tow, from the surface to within 2 meters 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 5 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 10 minutes is recommended.

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

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 the end-bags and plankton samples into the ship's laboratory and proceed with the pre-sorting of cod-sized eggs.

Fixing plankton samples

If genetic analysis requires the eggs to be preserved in ethanol (analysis by FRS, Scotland), a sub-sample of 50 cod-like eggs will be sorted from the fresh sample and fixed in 100% ethanol for later molecular identification. The remainder of the plankton will be fixed and preserved.

If genetic analysis will be carried out by France or Germany or no genetic analysis is possible, the 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 photo-chemical oxidation of these chromatophores is a cause for slower and inaccurate identification.

We propose to modify the fixation fluid as followed 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)

Disolve 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 sea water using 6% of this solution. It is important to note that the resulting concentration of formalin in the sample is less than 1%.

At sea or laboratory – 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.

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.

Mix together 150cm^3 Propylene phenoxetol and 1500cm^3 Propane-1,2-diol. This must be done vigorously as the two chemicals are not very miscible.

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

Mix thoroughly again.

Sort the whole sample for eggs larvae and keep these on observation fluid.

Laboratory

Put the eggs over a 1 mm mesh sieve. The smaller fraction of the sample is kept on observation fluid for 2 years. The larger fraction is identified, measured and staged.

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 sub-sampling is by using a plankton splitter. In this way, samples can be sub-divided repeatedly to achieve the optimum sampling level. It is recommended that at least 100 eggs of the target species are present in the sub-sample. If more than 100 eggs of these species are sorted from the sample (or sub-sample) 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 sub-sample the whole sample needs 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 sub-sampled 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 other larvae should be identified if resources allow. All larvae should be sent to Peter Munk for further analysis.

Identification 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; Munk and Nielsen, 2005).

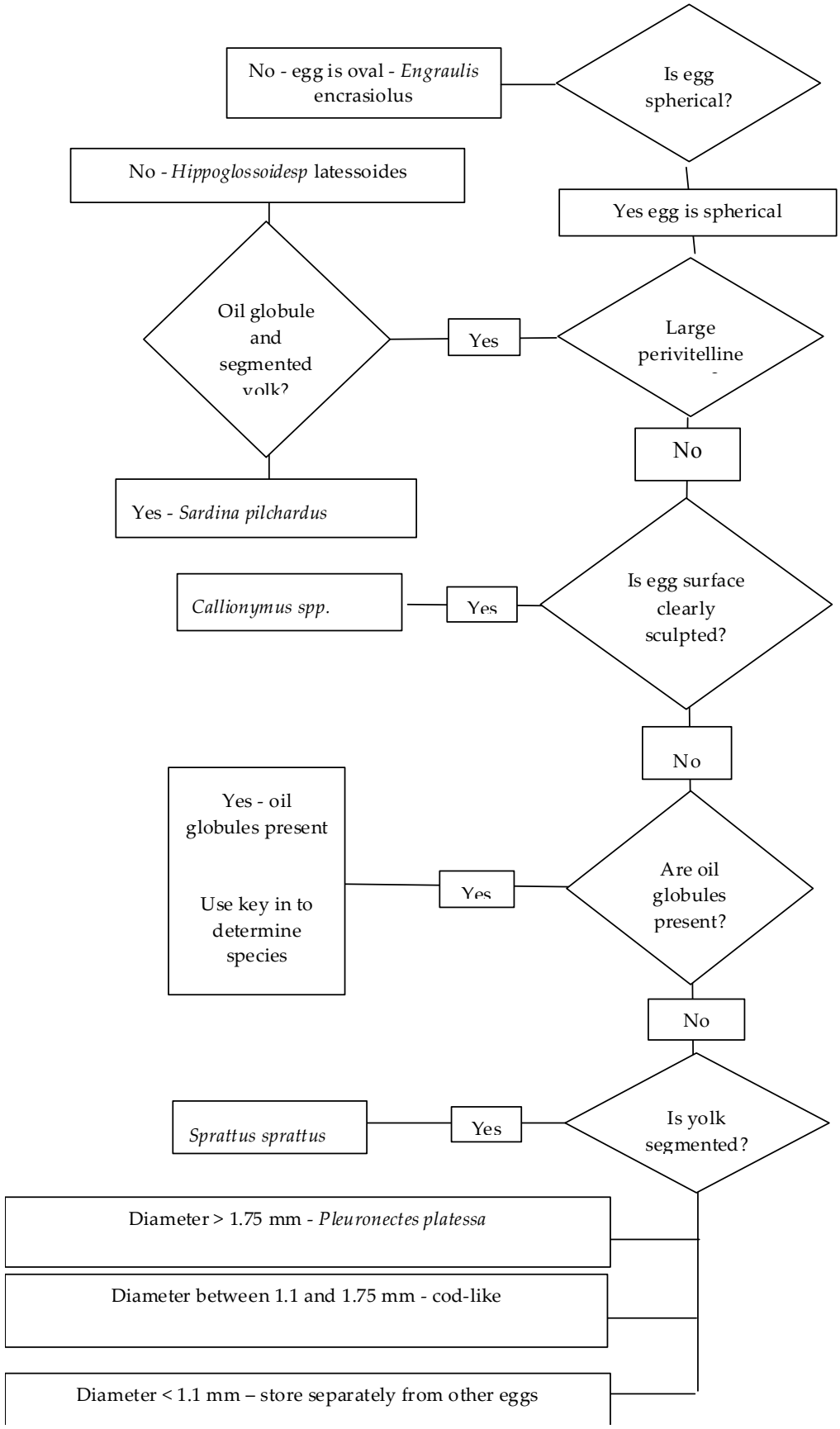
The identification of cod, haddock and possibly some smaller diameter plaice eggs can be difficult if all three species are spawning in the same area. Plaice eggs are generally much larger than those of other species spawning in the North Sea. Russell (1976) gives an egg diameter of 1.66–2.17 mm. In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

The main identification problem will be to distinguish between cod and haddock eggs. The egg diameter range is given by Russell (1976) as 1.16–1.89 mm for cod eggs and 1.2–1.7 mm 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, haddock and whiting eggs.

They will therefore be recorded as 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 are kept for 2 years after the survey.

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).

Flow chart for egg identification



Key to identification of pelagic eggs (size diameter)

PELAGIC SPHERICAL EGGS		
	Egg diameter (mm)	
Large eggs with large perivitelline spaces		
Sardina pilchardus	1.30–1.09	With oil globule and segmented yolk
Hippoglossoides platessoides	1.38–2.64	No oil globule and unsegmented yolk
Small eggs with sculptured membrane		
Callionymus spp.	0.7–1.0	No oil globule
Eggs with several oil globules and yolk with peripheral segmentation		
Solea solea	1.00–1.60	Oil globules small and clustered
Buglosidium luteum	0.64–0.94	12–15 oil globules scattered
Pegusa lascaris	1.28–1.38	50 or more scattered oil globules
Microchirus variegates	1.28–1.42	50 or more scattered oil globules
Eggs with several oil globules and unsegmented yolk		
Trachinus vipera	1.00–1.37	6–30 oil globules scattered
Eggs with one oil globule and segmented yolk		
Argentina sphyraena	1.70–1.85	Yolk wholly segmented
Trachurus trachurus	0.81–1.04	Yolk wholly segmented
Mullus surmuletus	0.81–0.91	Yolk with peripheral segmentation
Eggs with one oil globule and unsegmented yolk		
	Egg diameter (mm)	Oil globule diameter (mm)
Triglidae	1.10 –1.70	0.17 – 0.33
Zeus faber	1.96–2.00	0.36 – 0.40
Dicentrarchus labrax	1.20–1.51	0.36 – 0.46
Scophthalmus rhombus	1.24–1.50	0.16 – 0.25
Scomber scombrus	1.00–1.38	0.28 – 0.35
Lepidorhombus whiffiagonis	1.07–1.22	0.25 – 0.30
Scopthalmus maximus	0.91–1.20	0.15 – 0.22
Molva molva	0.97–1.13	0.28 – 0.31
Trachinus draco	0.96–1.11	0.19 – 0.23
Zeugopterus punctatus	0.92–1.07	0.17 – 0.20
Merluccius merluccius	0.94–1.03	0.25 – 0.28
Capros aper	0.90–1.01	0.15 – 0.17

Phrynorhombus regius	0.90–0.99	0.16 – 0.18
Serranus cabrilla	0.90 – 0.97	0.14–0.15
Phrynorhombus norvegicus	0.72–0.92	0.09–0.16
Raniceps raninus	0.75–0.91	0.14–0.19
Arnoglossus thori	0.72–0.74	0.12
Rocklings	0.66–0.98	0.14–0.19
Arnoglossus laterna	0.60–0.76	0.11–0.15
Eggs without oil globules		
With segmented yolk		
Sprattus sprattus	0.80–1.23	
With unsegmented yolk		
Pleuronected platessa	1.66–2.17	
Boreogadus saida	1.53–1.90	
Gadus morhua	1.16–1.89	
Melanogrammus aeglefinus	1.20–1.70	
Microstomus kitt	1.13–1.45	
Merlangius merlangus	0.97–1.32	
Micromesistius poutassou	1.04–1.28	
Glyptocephalus cynoglossus	1.07–1.25	
Pollachius pollachius	1.10–1.22	
Pollachius virens	1.03–1.22	
Trisopterus luscus	0.90–1.23	
Trisopterus esmarkii	1.00–1.19	
Platichthys flesus	0.80–1.13	
Trisopterus minutus	0.95–1.03	
Ctenolabrus rupestris	0.72–1.01	
Limanda limanda	0.66–0.92	
Pelagic oval eggs		
Engraulis encrasicolus	1.2–1.9 x 0.5–1.2	Segmented yolk

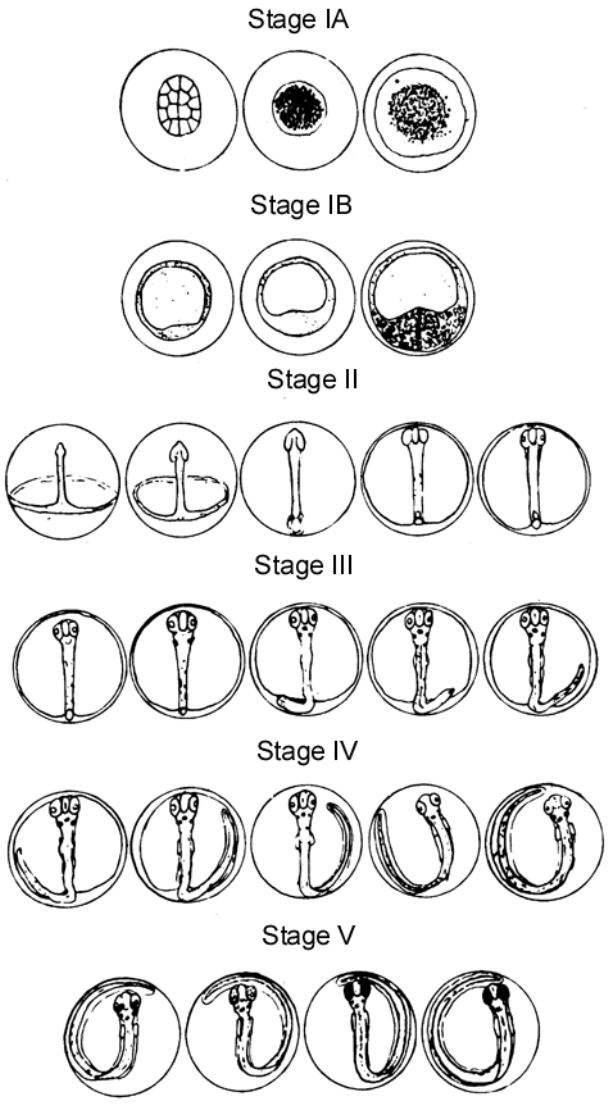
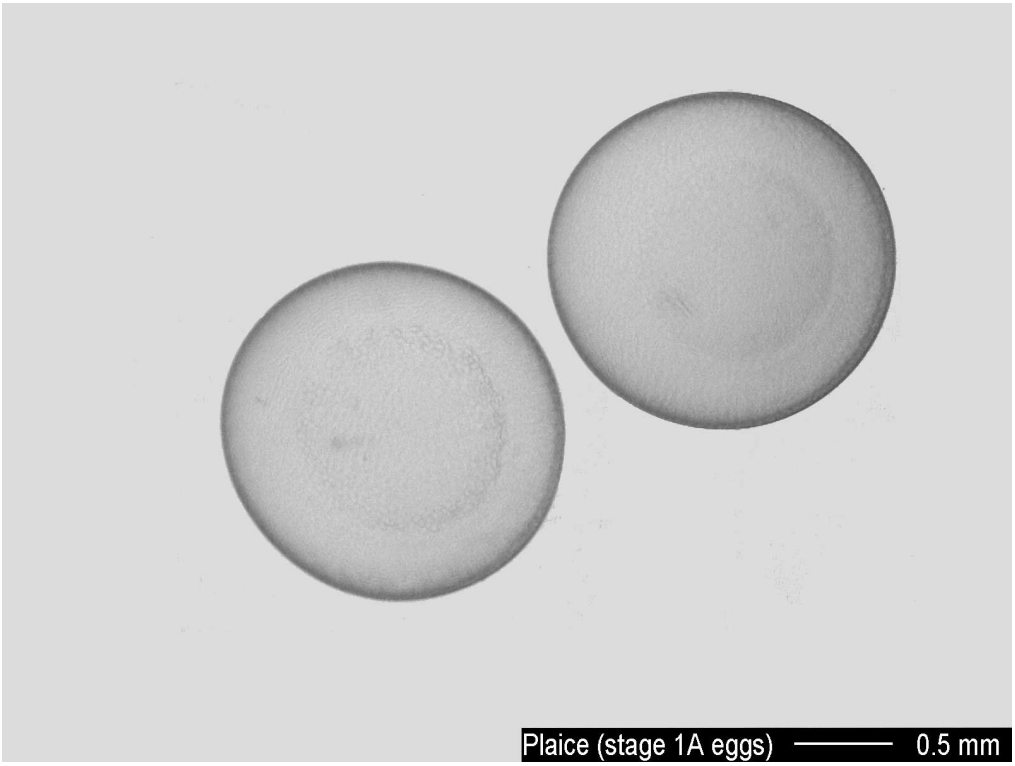


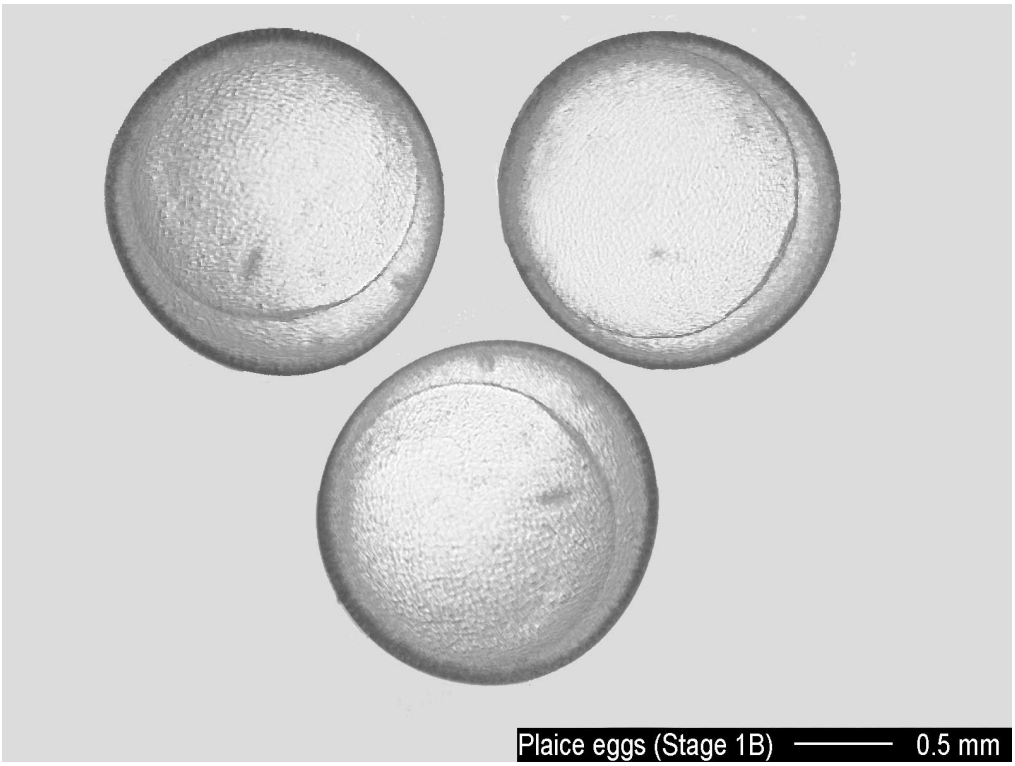
Diagram of egg development stages

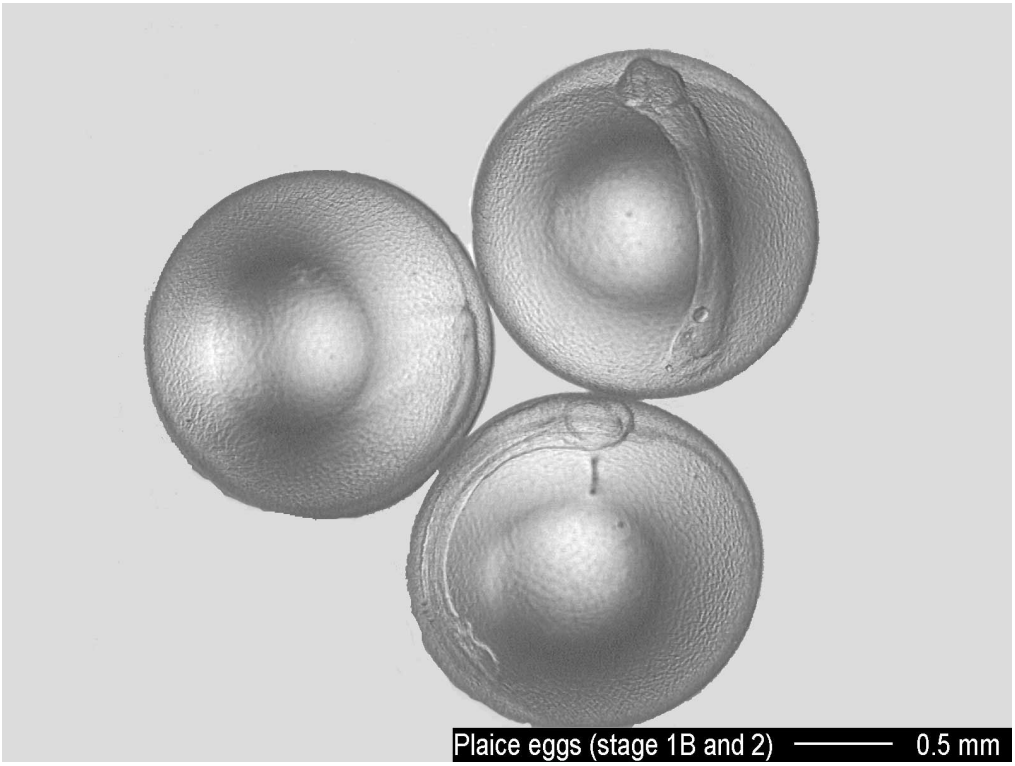
Criteria for egg staging following Thompson and Riley (1981) and Riley (1973)

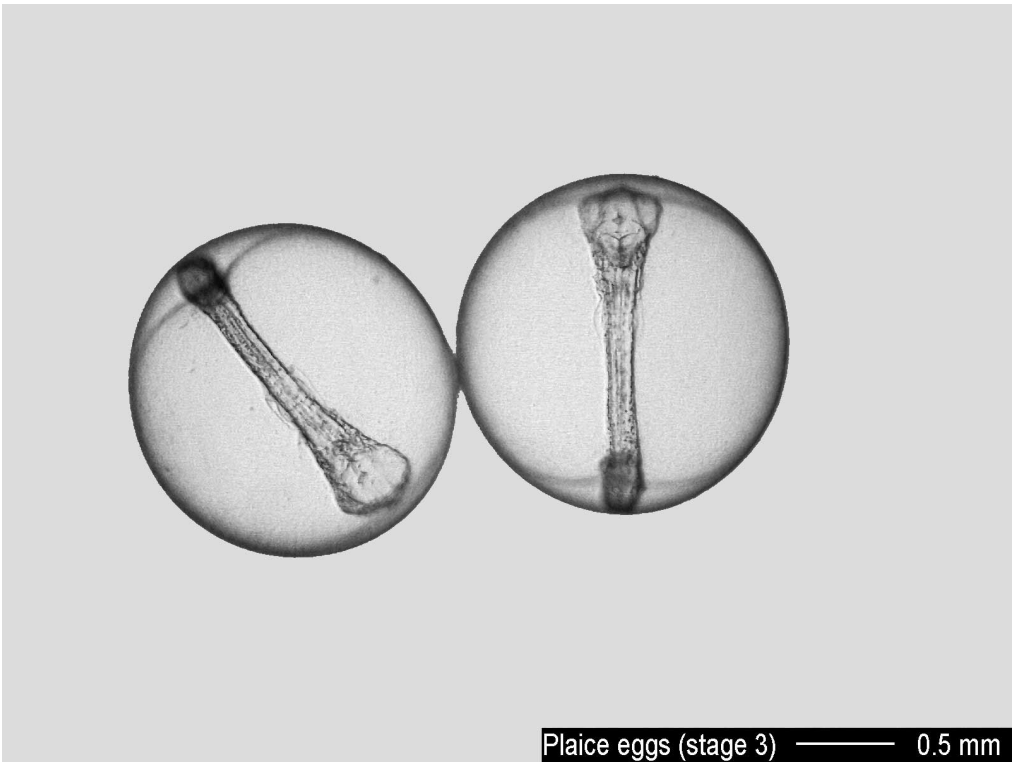
STAGE	PRIMARY CHARATERISTICS	SECONDARY CHARACTERISTICS
IA	Blastula stage lasting from fertilization until successive cleavages produce a cellular mass in which individual cells are not visible	There are no signs of a thickening of cells around the edge of the cell bundle. NB In preserved eggs the edge of the cell bundle can sometimes fold over giving the appearance of a 'signet ring' seen in a stage Ib.
IB	Continuing development of the blastodisc, which becomes visible as a signet ring, up to the first indication of the primitive streak	The cell bundle has thickened around the edge giving a distinct ring appearance. Cells in the centre of the ring form a progressively thinner layer and eventually disappear. NB At the end of this stage the ring can become very indistinct as it spreads towards the circumference of the egg.
II	Gastrulation stage lasting from the first sign of the primitive streak until the closure of the blastopore.	Early in this stage the primitive streak can be difficult to see, only appearing as a faint line in the surface of the yolk. Late in this stage the head is still narrow and the eyes are not well formed.
III	Growth of the tail occurs until the embryo spreads around three-quarters of the circumference of the egg. There is development of the eye structure and pigment spots.	Widening of the head and development of the eyes. Pigment spots develop on the embryo, usually close to the posterior end.
IV	Growth of the tail occurs until the embryo fills the whole egg with the tail touching the head.	Eyes continue to develop and the lenses become visible. Development of the marginal fin and the tail begins to separate from the yolk. Pigmentation of the body increases.
V	Growth of the tail past the head. Pigmentation of the eyes begins. At the end of this stage the larva hatches.	Pigmentation develops in the eye.

The preservation of eggs can cause shrinkage and distortion of the embryo. Therefore care should be taken when assessing the length of the embryo, as they do not always remain around the full circumference of the yolk. They may also become distorted giving a false impression of development stage.


Plaice (stage 1A eggs) 0.5 mm


Plaice eggs (Stage 1B) 0.5 mm


Plaice eggs (stage 1B and 2) 0.5 mm


Plaice eggs (stage 3) 0.5 mm


Plaice eggs (stage 4) 0.5 mm


Plaice eggs (stage 5) 0.5 mm

Identification of larvae in plankton samples

Since many more species are identifiable as larvae compared with the eggs, considerably more training and experience is necessary. It is not possible to present relatively simple keys for their identification as was done for the eggs. The standard text for the North Sea are Russell (1976) and Munk and Nielsen (2005).

Because many of the laboratories will be using inexperienced staff to sort samples, PGEGGS agreed the following protocol:

- All participants will sort, identify and measure larvae of the target species (cod and plaice).
- If resources and expertise are available the participants will also identify and measure (standard length) non-target species.
- In any case the participants will separate the non-target larvae from the sample and store them in separate vials.
- Larvae will be measured within 0.5 mm intervals, to the 0.5 mm below. If possible a computerised morphometric system should be used to allow measurements of curved larvae.
- Data will be entered using the standard input software and incorporated into the project database.

Description of cod larvae: When newly hatched the larvae are about 4 mm long and have a typical pigmentation pattern consisting of two postanal bars and one or two ventral caudal melanophores. At hatch the eyes are pigmented but the mouth closed. Yolk-absorption is completed when the larvae are around 4.5–5 mm long.

Description of plaice larvae: The larvae at hatching are considerably larger than dab or flounder, usually plaice larva at hatching are between 6 and 7.5 mm. The canary-yellow pigmentation is characteristic together with melanophores present in several longitudinal rows over the body. Yellow pigment cells predominate in the dorsal half of the body and melanophores predominate in the ventral half. These features may be somewhat obscured in preserved samples. The primordial fin is without pigmentation. The eyes are pigmented at hatching. Yolk-sac absorption is completed when larvae are 7–8 mm in length. Post-metamorphic larvae are unlikely to be caught in plankton samples.

Cod egg with late stage embryo and larvae

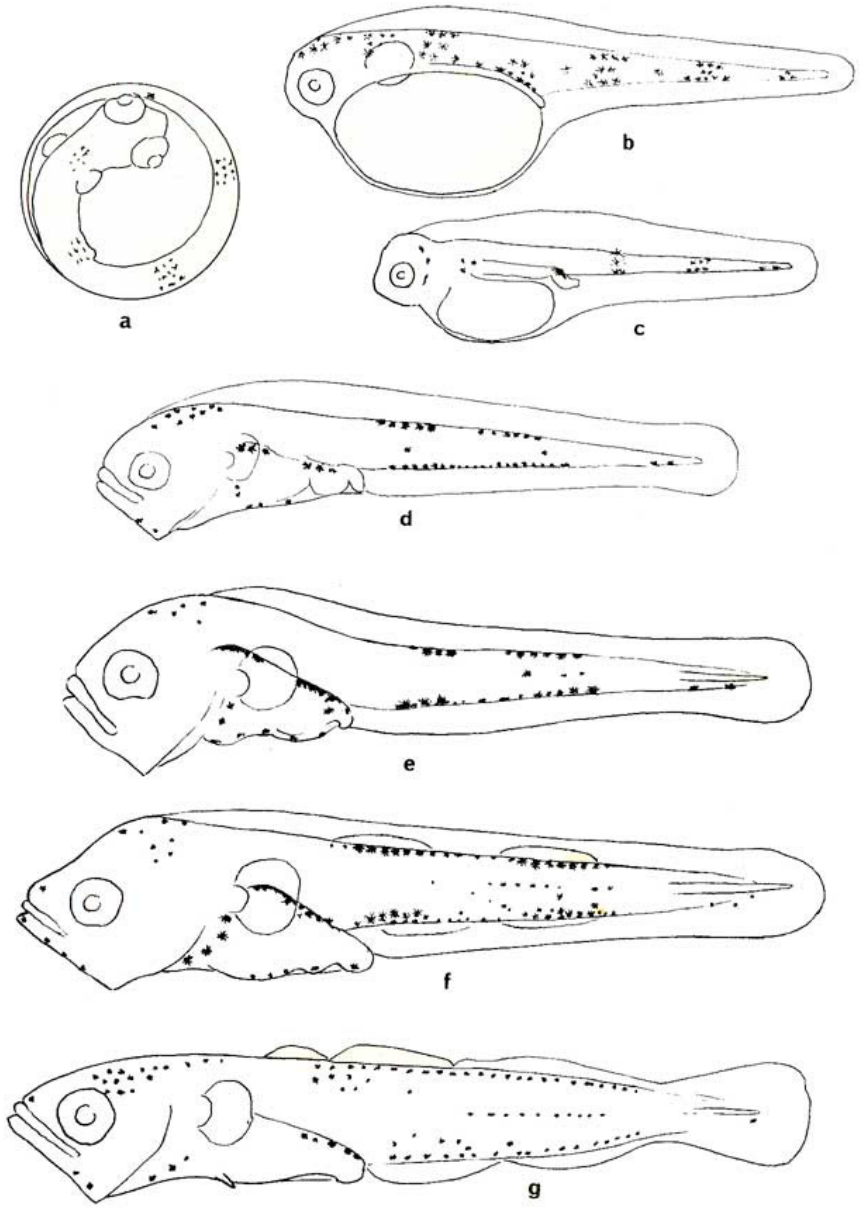


Fig. 19. *Gadus morhua*.

(a) Egg, 1·35 mm in diameter, after Heincke and Ehrenbaum, 1900, Text-fig. 13.
(b) Newly hatched larva, after M'Intosh and Masterman, Pl. IX, Fig. 1.
(c) Preserved larva from plankton, 3·0 mm long, eye pigmented, west coast of Scotland, 16.iv.74.
(d), (e), (f) and (g) Postlarva, 5·0 mm, 6·0 mm, 8·0 mm and 12·5 mm, west coast of Scotland, 30.iii.71, 31.iii.71, 16.iv.70 and 31.iii.71.

Plaice egg with late stage embryo and larvae

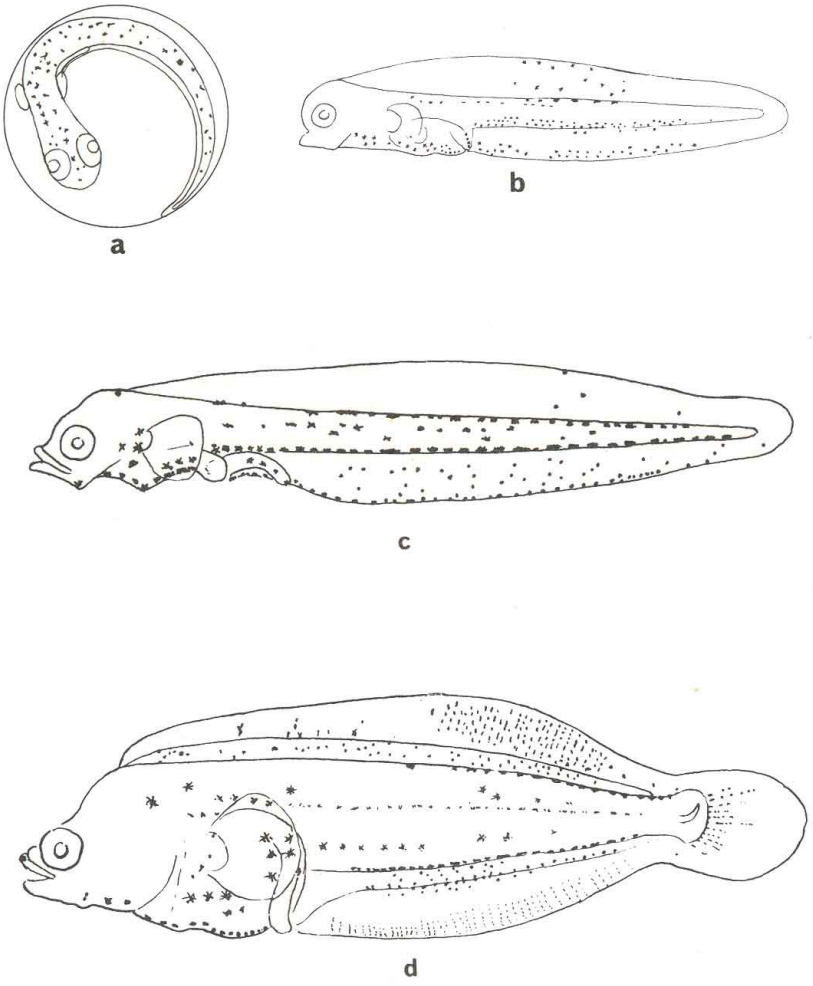


Fig. 121. *Pleuronectes platessa*

(a) Egg, 1·95 mm in diameter, artificial fertililization, after Heincke and Ehrenbaum 1900, Pl. IX, Fig. 5.

(b) Larva, *c.* 6·5 mm (5 days) artificial fertilization, after Cunningham, 1890a, Pl. XVIII, Fig. 4.

(c) and (d) Postlarva, 7·0 mm (12 days) and 9·8 mm (*c.* 7 weeks), reared at Dunstaffnage Marine Research Laboratory, May 1971.

Data handling

Excel worksheet template will made by IMARES and send round to all participants. The 2009 data will be stored in the IMARES database until the egg data can be transferred to the ICES DATRAS database.

References

Munk, P., and Nielsen, J. G. 2005. Eggs and larvae of North Sea fishes: Forlaget Samfundslitteratur.

Russell, F. S. 1976. The Eggs and Planktonic Stages of British Marine Fishes. London: Academic Press.

Ryland, J. S., and Nichols, J. H. 1975. Effect of temperature on the embryonic development of the plaice, *Pleuronectes platessa* L. (Teleostei). Journal of Experimental Marine Biology and Ecology, 18: 121–137.

Steedman, H. F. 1976. Miscellaneous preservation techniques. *In* Zooplankton fixation and preservation (Steedman, H. F., ed.), pp. 175-183. Paris: Unesco Press.

Thompson, B. M., and Riley, J. D. 1981. Egg and larval development studies in the North Sea cod (*Gadus morhua* L.). Rapports et Procès-verbaux des Réunions Conseils International pour l'Exploration de la Mer, 178 : 553–559.