Living Resources Committee
ICES CM 2004/G:03, Ref. D

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

11–12 November 2003
Kiel, Germany

International Council for the Exploration of the Sea

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1 INTRODUCTION

1.1 Background

The TOR of PGEGGS call 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 meeting 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 purpose of the third meeting held in Kiel was to review the plans for the surveys in 2004 in light of the responses to requests for ship time and staff and to produce agreed protocols for sampling, sample handling, data compilation, data exchange and statistical analysis.

1.2 Terms of reference

The **Planning Group on North Sea Cod and Plaice Egg Surveys** [PGEGGS] (Chair: C. Fox, UK) will meet in Kiel, Germany from 11–12 November 2003 to:

a) review existing plans for North Sea ichthyoplankton surveys for 2004 in light of funding decisions by member states;

b) agree protocols for sampling, sample analysis and data handling;

c) examine contingency planning for the surveys to deal with events such as poor weather;

d) plan for a subsequent workshop at which detailed spatio-temporal analyses of the data from the surveys will be analysed.

The third PGEGGS meeting will report for the attention of the Living Resources Committee and Resource Management Committee.

1.3 Participants

A complete list of participants is given in Section 10 of this report.

1.4 Status of PGEGGS in relation to Living Resource Committee Review at ICES Annual Science Conference

The report from the second PGEGGS meeting was delivered on time to the Living Resources Committee and reviewed at the Annual Science Conference. The report and work-plan were accepted and the request for the third PGEGGS meeting supported. Some concerns were expressed about the adequacy of the proposed survey coverage. In response we would point out that PGEGGS is attempting, as tasked, to coordinate a systematic survey of the entire North Sea, using the very limited resources that have been placed at its disposal. The plan presented is very much prescribed by these logistical constraints.

1.5 Paper on North Sea cod spawning grounds from FRS Aberdeen

PGEGGS noted that a paper reviewing data on North Sea cod spawning had been submitted to Working Group on North Sea and Skagerrak Demersal Stocks by FRS Aberdeen (Wright, Gibb, Gibb Heath and McLay, 2003). As well as reviewing published, historical data (also covered in the second PGEGGS report), the FRS document contains anecdotal evidence from a recent (2002) series of interviews with fishermen. This suggests that spawning is mainly in period Feb-March and that spawning may have become later in the year than occurred 10 years ago. In relation to spawning locations, fishermen report that cod in spawning condition occur dispersed over relatively large areas of the North Sea although some identified particular locations as being more important. Some areas that were historically important (e.g., Scottish north-east coast from Fraserburgh to Banff) do not appear to support spawning cod at present.

The paper draws several conclusions, two of which are pertinent to PGEGGS. Firstly, it is not possible to quantify long-term changes in the use of spawning grounds because of a lack of comprehensive survey data on eggs or spawning adults and the lack of suitable sampling within ICES bottom trawl surveys. Secondly, the limited data available do suggest a contraction in significant spawning areas since the 1980s.

Both of these conclusions lend support to the rationale for conducting coordinated surveys to ascertain the current distribution of cod egg production in the North Sea.

2 OVERALL AIMS

This PGEGGS report sets out six aims for the ichthyoplankton surveys planned for 2004. These are:

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 spawning based on distributional information of egg stages and larval sizes.
d) Correlate the distributional patterns of eggs and larvae to hydrographic features, and investigate potential physical/biological linkages
e) Assess the change in distribution of identified egg/larvae concentrations between separate surveys
f) Describe the distribution pattern of eggs/larvae of non-target species

During the Kiel meeting PGEGGS evaluated whether all six aims could be achieved with the resources now known to be available.

Aims (b) and (c) may be affected by the limited temporal coverage available, particularly in the northern North Sea. Given that the peak of spawning is thought to occur between Feb-March in this area, the proposed survey dates particularly for PLACES (PLAice and Cod Egg Surveys) sampling area (G) may be towards the tail end of spawning.

However, we are constrained by ship availability in not being able to undertake repeat sampling of this region. An offer has been received from FRS Aberdeen who will undertake some plankton sampling during the first quarter ground-fish survey in February. This will be a valuable addition to survey coverage but issues of staffing and sample analysis need to be addressed.

The possibilities for aim d) will be restricted by the relative spatial coarseness of the sampling grid. Hence, in parts of the sampling area, data will probably not be on a sufficiently fine-scale to identify meso-scale hydrographic structures such as fronts and then identify physical/biological linkages. However, in other regions, especially in the south-eastern areas, where additional sampling are planned, the resolution of hydrographic characteristics and spatial variation in egg/larval abundance should be sufficient for carrying out studies related to aim d).

Due to the limited number of repeat surveys in any given sampling area, it is unlikely that aim e) will be achieved for all areas.

Due to constraints in sample sorting support, aim (f) was now considered to be of secondary importance although still desirable, especially for the period when the entire North Sea is covered (weeks 9–11). It was agreed that participants would sort and identify non-target larvae where possible. Those participants who could not undertake this task (due to logistical constraints) would make larval samples available to other participants in PGEGGS as requested if funding becomes available to allow their analysis (see section on sample handling for further details).

The Netherlands have requested that we consider attempting to produce an egg production estimate, at least for the southern North Sea to compare with historical production estimates produced by (Daan 1981). This would require a minimum of three surveys in this region. PGEGGS considered this suggestion in regard to available sampling capacity and felt that it could be achieved within the current plan for the southern North Sea but not for other regions.

Considering the above, the aims of PLACES are modified to the following:

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 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.

3 RESPONSES TO REQUESTS FOR SUPPORT

All participants now have a firmer view on resources available for the surveys in 2004. These are listed below in the order ships, staffing of sea-time, post-cruise sorting of samples, data analysis and project coordination. PGEGGS then identified any shortfall in resources and suggestions are made as to how to deal with these.

3.1 Ship time

A summary is presented below of confirmed sea-time. More details can be found in Section 5 of this report.

Germany – Herring larval cruises – 12 days allocated to the herring larval surveys.

Germany – Dedicated PLACES cruises – In addition to the herring larval surveys, 4 extra days dedicated to PLACES work will be provided.

Germany - GLOBEC cruises – Four days within each GLOBEC cruise as laid out in the second PGEGGS report are confirmed. There is some flexibility in when within the GLOBEC cruise period sampling for PLACES should occur. The consensus was that weeks 8 and 15 were best.

Germany - Malformation cruises - The work program for these cruises is set and it is unclear how much additional sampling will be possible. This will be discussed internally between Gerd Kraus and Michael Vobach.

Scotland – First quarter North Sea ground-fish survey – Three weeks in January/early February, icthyoplankton sampling to be undertaken at night

England - Sea time as laid out in the second PGEGGS report is confirmed.

Norway - 14 days commencing in week 11 rather than week 10 are confirmed. This will likely coincide with the decline in cod spawning in sampling region (G) but because of other commitments it is not possible to make this cruise earlier.

Norway – 22 days available on the Norwegian spring spawning herring larval survey. Samples from the southern stations will be scanned for occurrence of fish eggs. This survey is confined to coastal waters.

Denmark - 11 days available but commencing in week 9 rather than week 10 (as stated in second PGEGGS report).

Netherlands – 10 days in addition to those available during the International Herring Larval Surveys are confirmed plus a probable 5 days using a charter vessel. The first 5 days will be in week 4 rather than week 3 as stated in the second PGEGGS report.

3.2 Staffing on cruises

PGEGGS feel that a minimum of 6 sea-going staff will be needed to undertake plankton sampling and egg sorting at sea (two watches of three). On longer cruises, 9 staff would be preferable (allowing three rotating watches).

Germany – Herring larval cruises – at least 6 staff will be available but the majority will likely be students. There is a requirement for training of supervisory staff in sorting techniques at sea.

Germany – Dedicated PLACES cruises - at least 6 staff will be available but the majority will likely be students. There is a requirement for training of supervisory staff in sorting techniques at sea.

Germany - GLOBEC cruises - at least 6 staff will be available but the majority will likely be students. There is a requirement for training of supervisory staff in sorting techniques at sea.

Germany - Malformation cruises – staffing to be agreed internally by participating German institutes.

England – cruise will be fully staffed with 9 participants. The majority of staff are experienced and extra training is not required.

Norway – 6 staff will be available for the cruise. There is good level of expertise and additional training is not required.

Denmark – 4 staff currently available. Training in sorting eggs at sea is required. At least 2 additional staff will be required for this cruise.

Netherlands – 4 staff are available. Some additional training in sorting samples at sea has been requested.

This section has identified some shortages of sea-going staff particularly for Denmark and Netherlands. Also identified is need for training in sorting fish eggs at sea. PGEGGS agreed that staffing issues would have to be dealt with by participating institutes. Clive Fox agreed to investigate whether an experienced member of staff from CEFAS would be able to participate in at least the first cruises for Germany and Netherlands to provide training in pre-sorting eggs at sea.

3.3 Sampling gear

3.3.1 Hydrographic measurements

Profiles of temperature and salinity will be made at all stations. Institutes may wish to record additional parameters such as fluorescence but these will not form part of the core hydrographic measurements for PLACES. Sampling protocols for hydrographic measurements are more fully described in Section 6. All participating institutes reported that they had suitable CTDs available, either mounted on plankton samplers or as separate units suitable for making vertical casts.

3.3.2 Plankton sampling gear

PGEGGS agreed that the recommended sampling gear should be the Gulf VII high-speed plankton sampler with a mouth opening of 40 cm diameter and fitted with 270 um mesh net (Nash *et al.* 1998). The sampler should carry an internal flow-meter mounted in the centre of the mouth opening and an external flow-meter mounted on the sampler body. A CTD package should also be carried. The end-bag should be partially made of waterproof material to reduce damage to eggs and larvae.

However, during PLACES it will be necessary to deploy a wider variety of plankton gear as some participants do not possess the Gulf VII sampler and there are no resources within PGEGGS to purchase additional samplers specifically for the PLACES survey. For International Herring Larval samples the gear is fixed as Gulf III since PLACES work is supplementary to the main purpose of these surveys. For the purposes of sampling fish eggs the volume of water filtered by the Gulf III is rather small (around 50 cubic meters on a typical deployment as opposed to between 100–200 cubic meters for the Gulf VII). Bongo nets filter larger water volumes compared to Gulf III, their main disadvantage being the lightness of the frame reducing the capacity to sample in rough weather.

The current availability of plankton sampling equipment for the proposed surveys is as follows.

Germany – International Herring Larval surveys – Plankton sampling gear will be Gulf III (20 cm diameter mouth opening) with on-board flow-meters and CTD. Because these surveys form part of a standard international series there is no chance to change the sampling gear.

Germany – Dedicated PLACES surveys and GLOBEC – Plankton sampling gear will be Bongo net. Again these surveys form part of another research program so the sampling gear cannot be changed.

England – Plankton sampling gear will be Gulf VII meeting the specifications laid out above.

Norway - Intend to use Gulf III sampler for plankton with separate CTD profiles being made. They will explore the possibility of borrowing the Gulf VII system from Aquatic Sciences Division, DARD, Belfast or Port Erin Marine Labs.

Denmark – Intend to use Bongo net for plankton with separate CTD profiles being made. They will explore the possibility of borrowing the Gulf VII system from Aquatic Sciences Division, DARD, Belfast or Port Erin Marine Labs.

Netherlands – Sampling gear will be Gulf VII sampler meeting the specifications laid out above.

PGEGGS Kiel recognised the lack of standardised sampling gear as a potential weakness in the program. Given the financial constraints on the project and the fact that several PLACES surveys form part of other research and monitoring programs, we will not be able to completely standardise sampling gear for 2004. A gradual move to standardised gear is recommended if surveys of this type are repeated in the future.

3.4 Resources for pre-sorting cod-like eggs at sea

Given the availability of sea-going staff, it may prove difficult to pre-sort cod-like eggs at sea from every station during some cruises.

Niels Daan questioned whether it was necessary to apply intensive genetic probing in the southern North Sea. This is not a region in which haddock normally spawn whilst spawning of whiting may commence later in the year. However, the group as a whole felt that given the desire to produce as scientifically solid a result as possible, as much genetic speciation should be undertaken as was practical. It was agreed that genetic analysis should be undertaken on every station in northern areas (sampling areas[1] C, D, E, F and G) but could be undertaken on every third station in the southern North Sea if necessary (sampling areas A and B).

3.5 Resources for post-cruise genetic identification of cod-like eggs

Clive Fox has applied to UK Defra for funding to support the genetic analysis of cod-like eggs pre-sorted at sea by the participating countries. A positive response has been received from Defra but it remains to be confirmed whether the funding will be made available.

3.6 Resources for post-cruise plankton sample analysis

The bulk of the plankton samples will be fixed in formaldehyde and returned to participating laboratories. Fish eggs will be sorted, identified and counted following protocols laid out in Section 6 of this report. Fish larvae of the target species (cod and plaice) will also be sorted and counted. Fish larvae of non-target species will be sorted, identified and counted where resources permit. There is therefore a significant requirement for staff to undertake post cruise sample sorting.

The current availability of plankton sorting staff for post-cruise work is detailed below.

Germany – Sorting staff will be available but the majority are students who will require training.

England – Experienced sorting staff available, funding is currently available for one person-year dedicated to PLACES samples.

Norway – Experienced sorting staff are available.

Denmark – Intend to send samples to the Polish sorting centre. Funding is available for this.

Netherlands – funding is available to support sorting staff but these are inexperienced and will require training.

3.7 Resources for database management and data analysis

Whether the resources required for data analysis and report writing within individual institutes have been allocated is currently unclear. Clive Fox reported to PGEGGS that he had been allocated some resources up to April 2004 (from Defra project MF0427) to support database management but that he had not yet been able to secure a commitment beyond this date.

3.8 Resources for project coordination

To ensure success of PLACES, PGEGGS IJmuiden (Section 9.9 of that report) clearly identified the need to appoint and properly resource a project coordinator. The resources required include funding of time and T&S for the coordinator. Clive Fox reported to PGEGGS that whilst he was happy to undertake this role and had been allocated some resources up to April 2004 (from Defra project MF0427) he had not yet been able to secure a commitment beyond this date.

3.9 Conclusions on resources currently allocated to PLACES

This section clearly identified the lack of trained, experienced plankton sorting staff in several of the participating laboratories. The PGEGGS meeting in Kiel high-lighted the provision of training in egg identification and staging as vital to the success of the surveys. Quality control of sorting is also vital given the inexperience of sorting staff available in several participating countries.

Clive Fox agreed to explore the possibility of holding a training workshop at Lowestoft in 2004 but pointed out this may be difficult since 2004 is also a triennial mackerel survey year and CEFAS plankton staff are all heavily committed. From the point of view of PGEGGS and mackerel survey activities, weeks 5–7 of 2004 may be suitable for training.

[1] Sampling areas are fully described in Section 5.1 of this report.

The role of project coordinator and support for database management are not currently resourced beyond April 2004.

The Netherlands pointed out that there is an urgent policy requirement for the data from PLACES to be provided (at least in preliminary form) by early autumn 2004. This places some pressure on training plankton sorting staff, on completion of sample analysis and on compiling the data. PGEGGS emphasise that we will collectively do our utmost to achieve this but given the resources available and other commitments in 2004 we cannot guarantee that this can be achieved.

4 UPDATE ON PROGRESS WITH ICHTHYOPLANKTON SURVEYS UNDERTAKEN IN THE IRISH SEA AND WITH DEVELOPMENT AND APPLICATION OF GENETIC PROBES FOR IDENTIFICATION OF COD-LIKE EGGS

As outlined in previous reports, the unambiguous identification of fish eggs of several species is problematic. Of particular relevance to PLACES are the eggs of cod, haddock and whiting. A biochemical method (iso-electric focussing) has been used to identify eggs in the past but has the disadvantages that eggs require to be stored frozen prior to analysis and that a large percentage of eggs often fail to yield positive reactions. A semi-automated genetic method has recently been developed involving robotic extraction of DNA from individual eggs stored in ethanol and subsequent analysis in an automated polymerase-chain-reaction (PCR) real-time detection system (Taylor *et al.* 2002). The system is capable of identifying whether eggs are those of cod, haddock or whiting with greater than 95% accuracy for all developmental stages.

In March 2003, an ichthyoplankton survey was undertaken in the Irish Sea in which cod-like eggs were pre-sorted at sea and individually preserved in ethanol. The samples have been genetically analyzed at the University of East Anglia, England using the TaqMan system (Applied Biosystems). In the previous report we stated that problems had been encountered with the TaqMan probe for whiting eggs. Clive Fox reported to PGEGGS that this has now been solved and was caused by problems in the synthesis of this particular probe by Applied Biosystems. The whiting probe has been re-synthesised and is working correctly. To date, two thousand eggs have been analysed and around 95% have given positive identifications as being cod, haddock or whiting. Since the majority of the remaining 5% amplify with universal primers, these represent other species for which we do not currently have probes developed rather than samples which have failed to react because of DNA degradation.

The original hope was that this system would prove reliable using eggs fixed in formalin. This would be an ideal solution since genetic analysis could then be bolted on the back of standard laboratory pre-sorting i.e., only those eggs identified as cod-like would need to be gene probed and pre-sorting of eggs at sea would not be necessary. Unfortunately we have not been able to get the TaqMan system to work reliably with formalin fixed material. It is thought that formalin degrades DNA so this result was not entirely unexpected although several workers have reported successful PCR from formalin fixed samples. We have investigated the effects of using other preservatives such as ethanol and di-methyl sulphoxide (DMSO) for fish eggs. Whilst these preserve high-quality DNA, they lead to shrinkage and cause the eggs to become opaque. These effects invalidate the use of standard size keys for identifying the eggs and would prevent egg-staging, a necessary step in estimating egg age. Work is continuing on the development of probes robust to formaldehyde under an EU project (MARINEGGS) but reliable results have yet to be demonstrated for the identification of cod, haddock and whiting eggs.

Because of the difficulties in applying genetic probes to formaldehyde fixed samples, Clive Fox recommended to PGEGGS that a sub-sample of cod-like eggs be pre-sorted and staged at sea and stored individually in ethanol for subsequent genetic analysis. This is more fully described in the sampling protocols contained in this report (Section 6 and Appendices).

5 UPDATE ON PROPOSED SURVEY DESIGN FOR NORTH SEA COORDINATED ICHTHYOPLANKTON SURVEYS

5.1 Spatial coverage

PLACES will cover the entire North Sea and the eastern English Channel. The survey area has been divided into 7 regions, based on the coverage of ongoing research programmes and on the expected North South gradient in spawning activity (Figure 5.1.1).

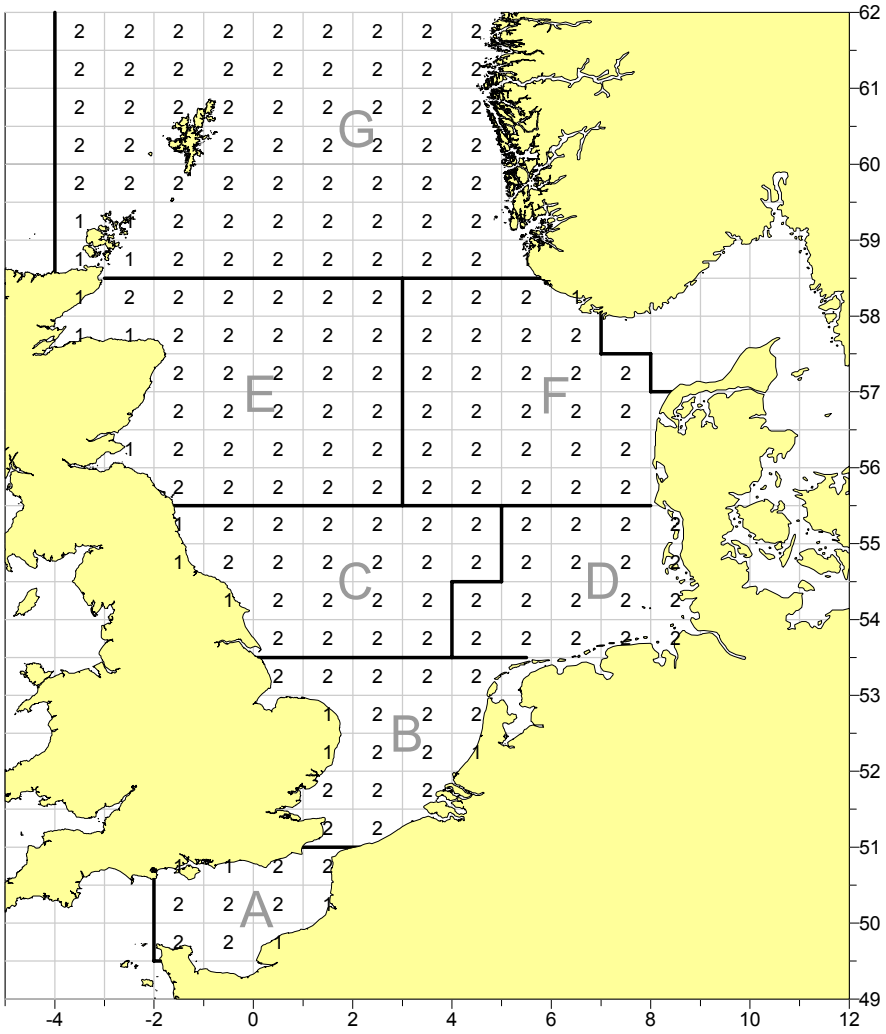


Figure 5.1.1. Map of the survey area indicating the regions (coded by letters) and the number of samples per ICES rectangle.

5.2 Sampling grid

In order to obtain a large spatial coverage the sampling grid is set at two samples per ICES rectangle and at one in some coastal rectangles (Figure 5.1.1). In principle this means one sample per ½ ICES rectangle (east-west division). However when samples are obtained from ongoing research programmes the grid may deviate within the limitations of at least two samples per ICES rectangle. This results in a total of minimum 374 stations (Table 5.2.1).

Table 5.2.1. Overview of the number of ICES rectangles and stations within the survey area and an estimation of the minimum days at sea required to sample all stations at least once.

Region		Number of ICES rect.	Number of stations	Days required for single coverage*
Area A	VIId	11	18	2
Area B	51'00" - 53'30"	18	33	4
Area C	53'30" - 55'30" west	22	43	5
Area D	53'30" - 55'30" east	19	36	4
Area E	55'30" - 58'30" west	35	66	7
Area F	55'30" - 58'30" east	28	55	6
Area G	58'30" - 62'00"	64	123	13
Total area	VIId + IV	197	374	41

** approximately 10 samples per day*

If weather conditions are favourable the surveys will be expanded by extending the area covered by each cruise or by increasing the numbers of samples per ICES rectangles. PGEGGS recommend that the preferred option should be to undertake additional sampling in areas of high egg concentration if possible.

5.3 Temporal coverage

The major goal of PLACES is to map the spawning distribution of plaice and cod. At a minimum only a single survey coincident with peak spawning is required. However, as the spawning period is progressively later from south to north and there is some uncertainty in when peak spawning will occur, the egg surveys should cover more than one period. Table 5.3.1 indicates when ship time is available and when the cruises are planned. The highest priority is to cover each region at least once. PGREGGS Kiel made some minor adjustments to sampling coverage. A more detailed calendar of sampling times is shown in Figure 5.3.1 giving exact cruise dates.

Table 5.3.1. Overview of the planned shipping time. The hatched cells indicate when a vessel is not available. The number of days available specifically for PLACES are indicated and for each cruise the sampling regions (see Figure 5.1) are shown in parentheses.

week	Germany Alkor	Germany W. Herwig	England Corystes	Norway H. Mosby	Denmark Dana	Netherlands Tridens II	Netherlands Tridens I
51						IHLS (A+B)	
52							
1							
2	IHLS (A+B)						
3	IHLS (A+B)					IHLS (A+B)	
4	Dedicated 4 days (B)					Dedicated 5 days (C+D)	
5							
6							
7							Dedicated 5 days (B-C)
8	GLOBEC (D)						
9	GLOBEC (D)	MAL. (D)	7 days (C)		3 days (F)		
10	GLOBEC (D)	MAL. (D)	7 days (E)		7 days (F)	Dedicated 5 days (B+C)	
11			7 days (E)	7 days (G)[1]			
12				7 days (G)[1]			
13				NSHL(G)			
14				NSHL(G)			
15	GLOBEC (B+D)			NSHL(G)			
16	GLOBEC (B+D)						
17	GLOBEC (B+D)						

IHLS – International Herring Larval Surveys.
NSHL-Norwegian spring Spawning Herring Larval Survey.

[1]Survey may take place in weeks 10–11.

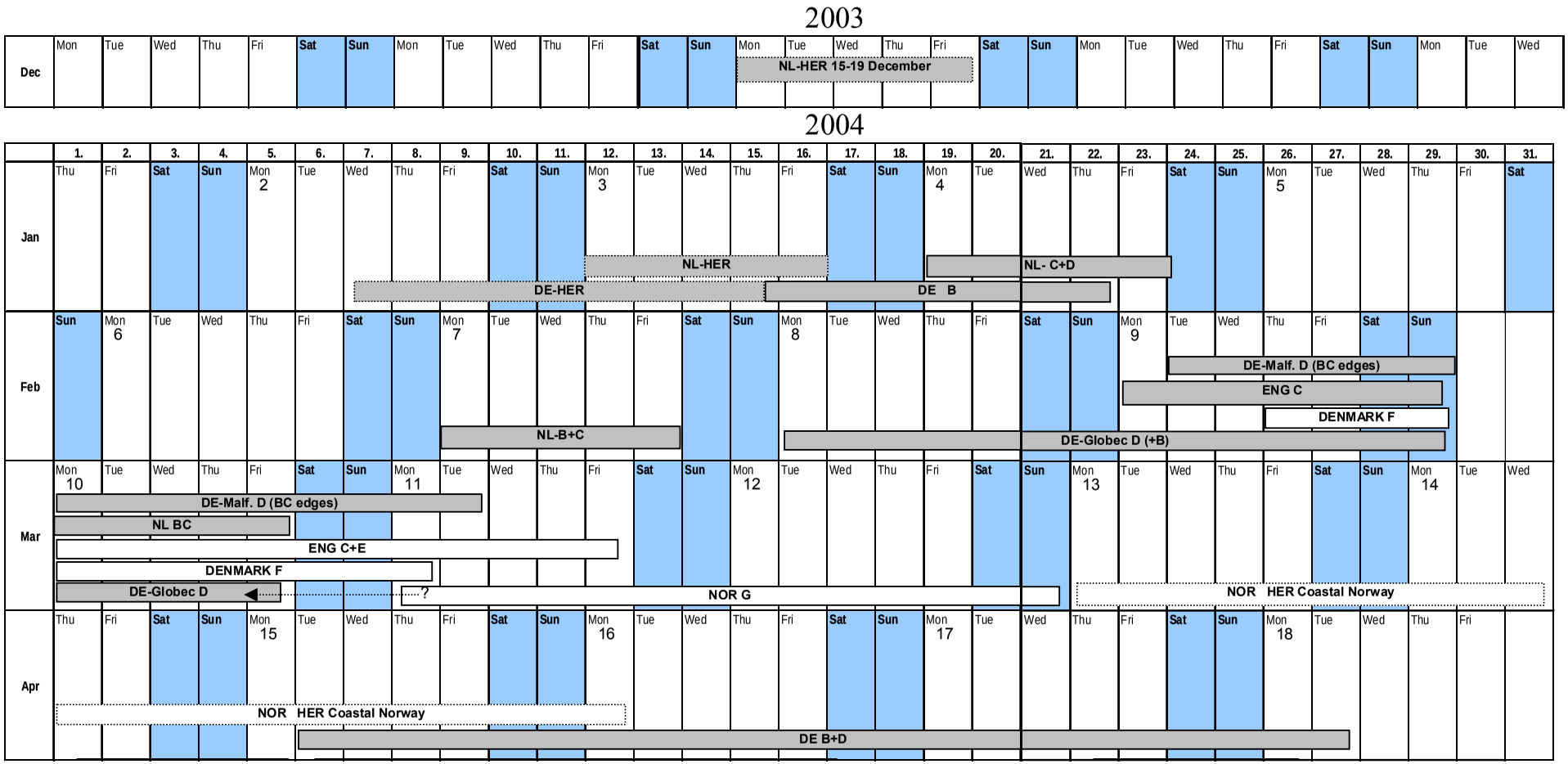


Figure 5.3.1. Calendar of sampling dates.

5.4 Individual cruise plans

Below we describe individual cruise plans as far as is practical at present.

5.4.1 Netherlands international herring larval survey

Dates:- 15 December 2003 – 19 December 2003
Gear:- Gulf III
Sampling region:- A+B
Purpose:- Sort fish eggs from formaldehyde fixed samples only
Proposed sampling grid:- Figure 5.4.1.1

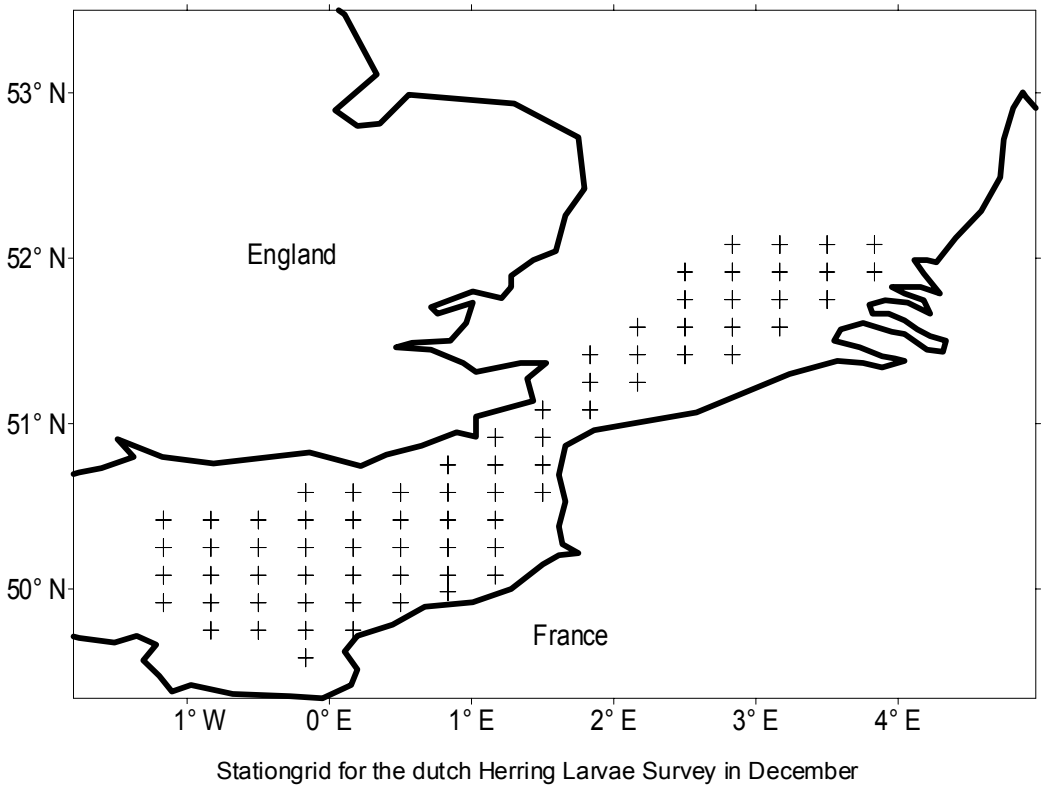


Figure 5.4.1.1. Station grid for Netherlands herring larval survey.

5.4.2 German international herring larval survey

Dates:- 7 January 2004 – 15 January 2004
Gear:- Gulf III + Bongo
Sampling region:- A+B
Purpose:- Sort fish eggs from formaldehyde fixed samples only from Gulf III hauls; Pre-sort cod-like eggs into ethanol from Bongo hauls at sea, subsequently sort all fish eggs and larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- Figure 5.4.2.1

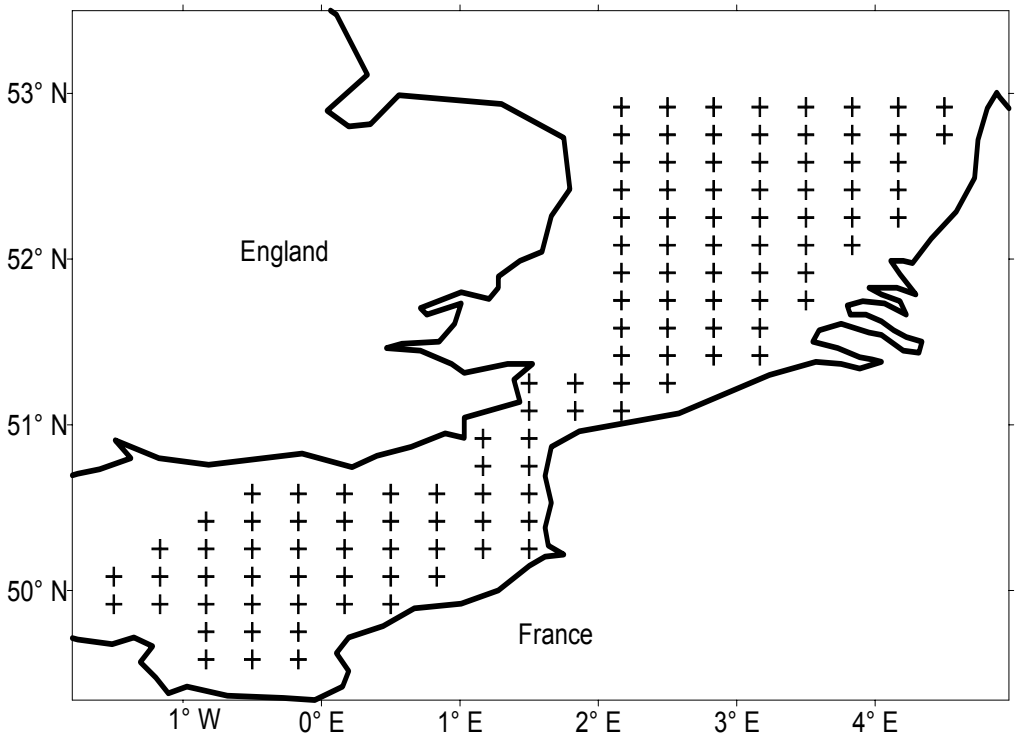


Figure 5.4.2.1. Station grid during the German herring larvae surveys in the Southern North Sea.

5.4.3 German dedicated PLACES survey

German dedicated PLACES survey
Dates:- 15 January 2004 – 22 January 2004
Gear:- Bongo
Sampling region:- B
Purpose:- Pre-sort cod-like eggs into ethanol from Bongo hauls at sea, subsequently sort all fish eggs and larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- to be arranged

5.4.4 Netherlands international herring larval survey

Dates:- 12 January 2004 – 16 January 2004
Gear:- Gulf III
Sampling region:- A+B
Purpose:- Sort fish eggs from formaldehyde fixed samples only from Gulf III samples
Proposed sampling grid:- Figure 5.4.4.1

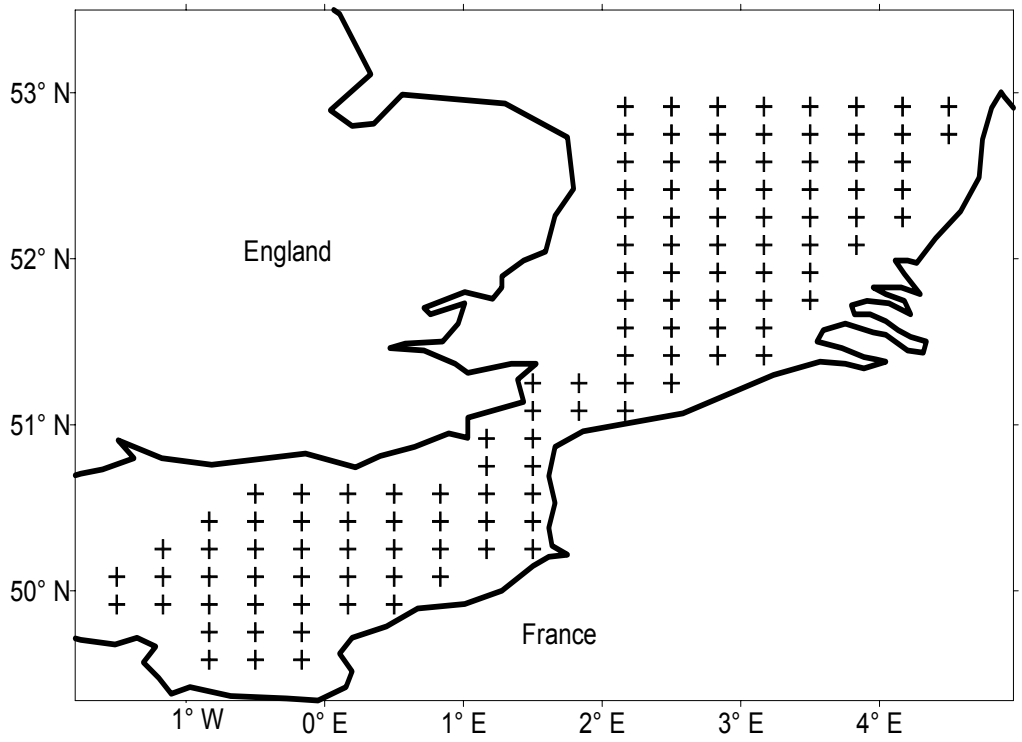


Figure 5.4.4.1. Station grid during the herring larvae surveys in the Southern North Sea.

5.4.5 Netherlands dedicated PLACES survey

Dates:- 19 January 2004 – 23 January 2004
Gear:- Gulf VII
Sampling region:- C+D
Purpose:- Pre-sort cod-like eggs into ethanol from Bongo hauls at sea, subsequently sort all fish eggs and cod and plaice larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- to be arranged

5.4.6 Scottish groundfish survey

Dates:- mid January – mid February 2004
Gear:- ?
Sampling region:-
Purpose:- Pre-sort cod-like eggs into ethanol from Bongo hauls at sea, subsequently sort all fish eggs and cod and plaice larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- Figure 5.4.6.1

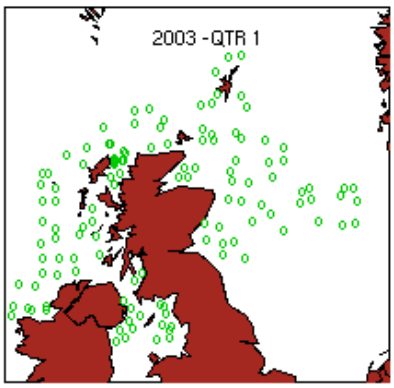


Figure 5.4.6.1. Coverage on Scottish Q1 groundfish survey.

5.4.7 Netherlands dedicated PLACES survey

Dates:- 9 February 2004 – 13 February 2004
Gear:- Gulf VII
Sampling region:- B+C
Purpose:- Pre-sort cod-like eggs into ethanol from Bongo hauls at sea, subsequently sort all fish eggs and cod and plaice larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- to be arranged

5.4.8 German GLOBEC survey

Dates:- 16 February 2004 – 15 March 2004
Gear:- Bongo
Sampling region:- D
Purpose:- Pre-sort cod-like eggs into ethanol from Bongo hauls at sea, subsequently sort all fish eggs and larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- Figure 5.4.8.1

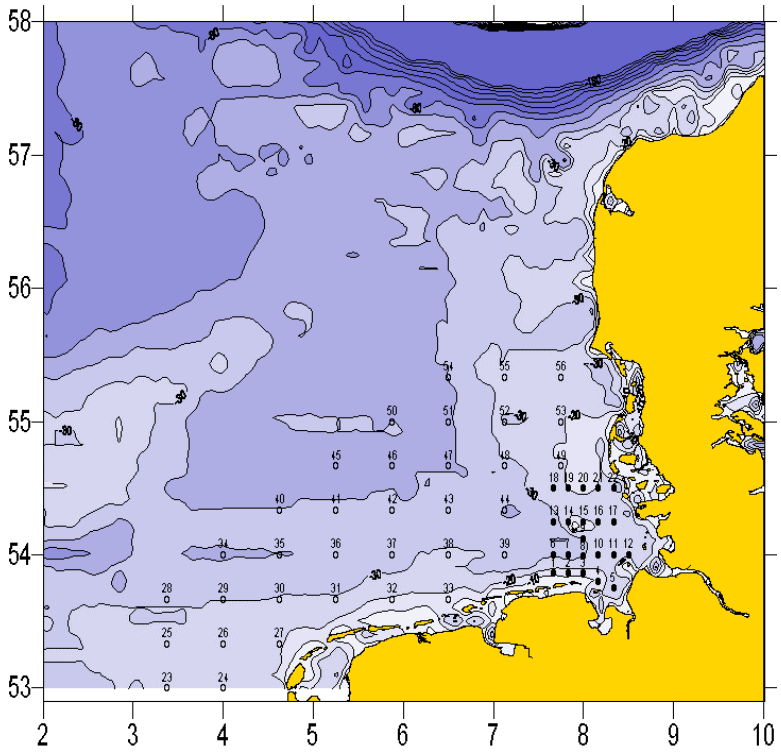


Figure 5.4.8.1. Planned sampling grid for the German GLOBEC program in the North Sea in 2004 (56 stations, 20 nm grid point distance).

5.4.9 German fish larval malformation survey

Dates:- 24 February 2004 – 9 March 2004
Gear:- to be arranged
Sampling region:- D
Purpose:- to be arranged
Proposed sampling grid:- Figure 5.4.9.1

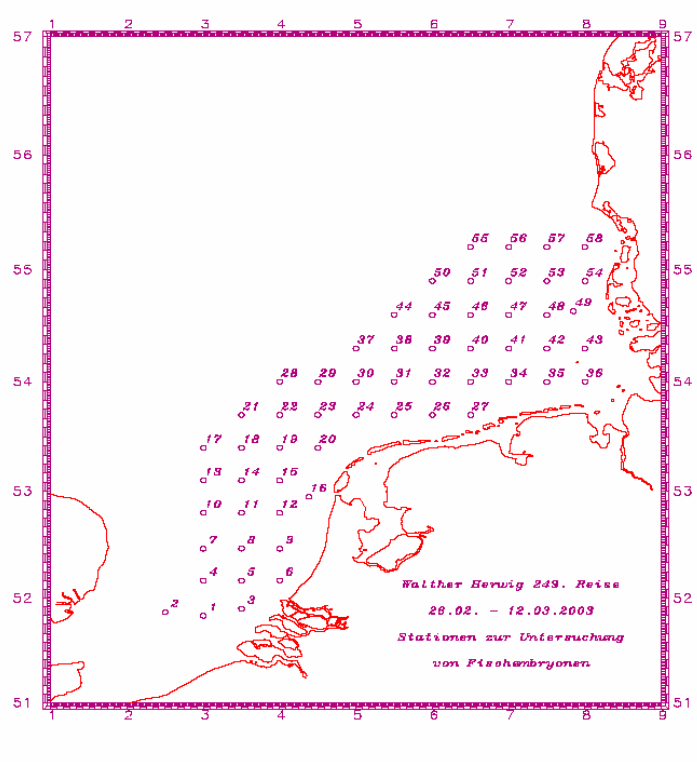
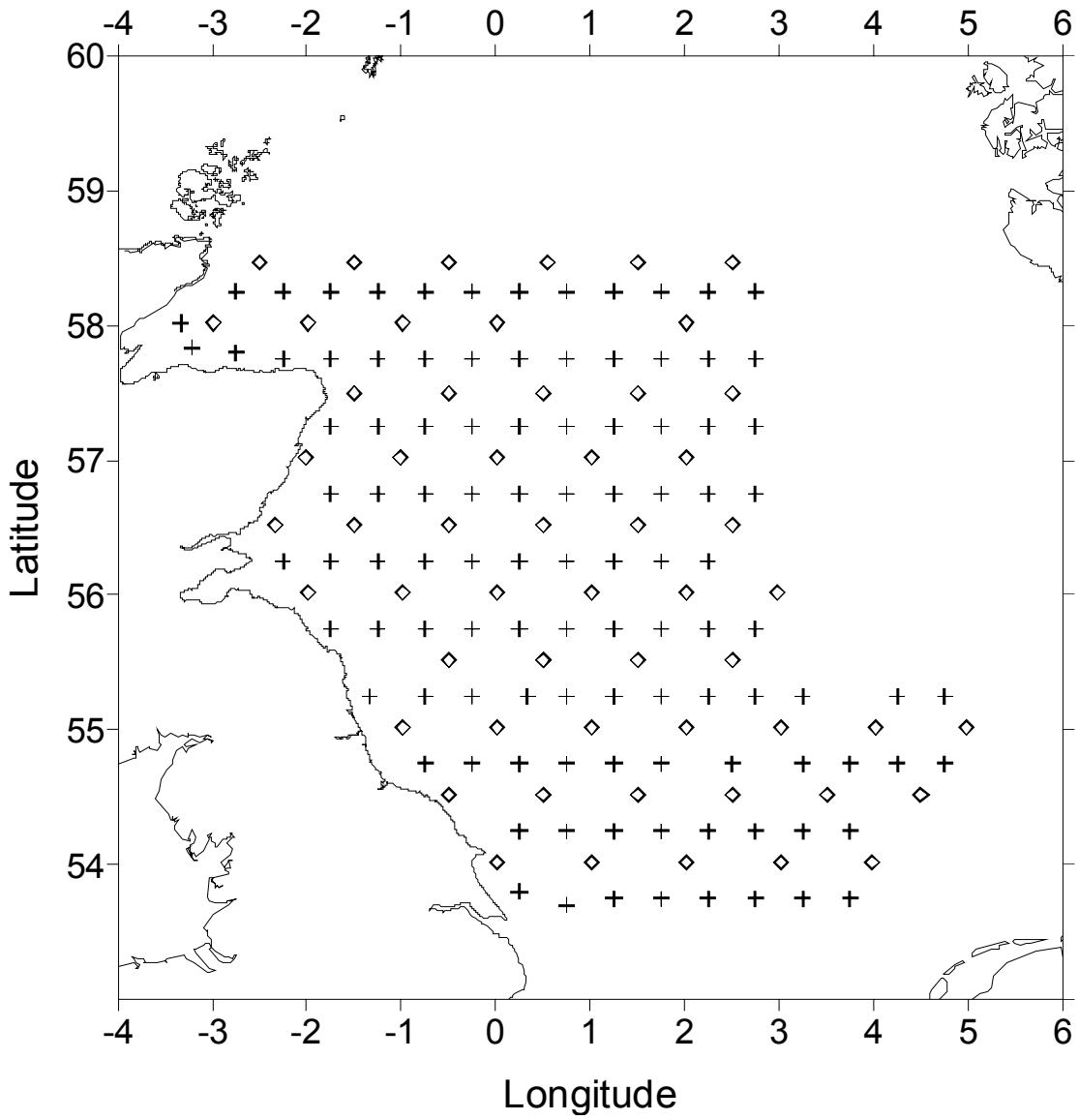


Figure 5.4.9.1. Station grid for the examination of malformations in embryonic fish larvae.

5.4.10 English dedicated PLACES survey

Dates:- 17 February 2004 – 9 March 2004
Gear:- Gulf VII
Sampling region:- C+E
Purpose:- Pre-sort cod-like eggs into ethanol at sea, subsequently sort all fish eggs and larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- see below



Primary stations are crosses; secondary (supplemental) stations are diamonds.

5.4.11 Danish dedicated PLACES survey

Dates:- 25 February 2004 – 7 March 2004
Gear:- Gulf VII
Sampling region:- F
Purpose:- Pre-sort cod-like eggs into ethanol at sea, subsequently sort all fish eggs and larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- Figure 5.4.11.1

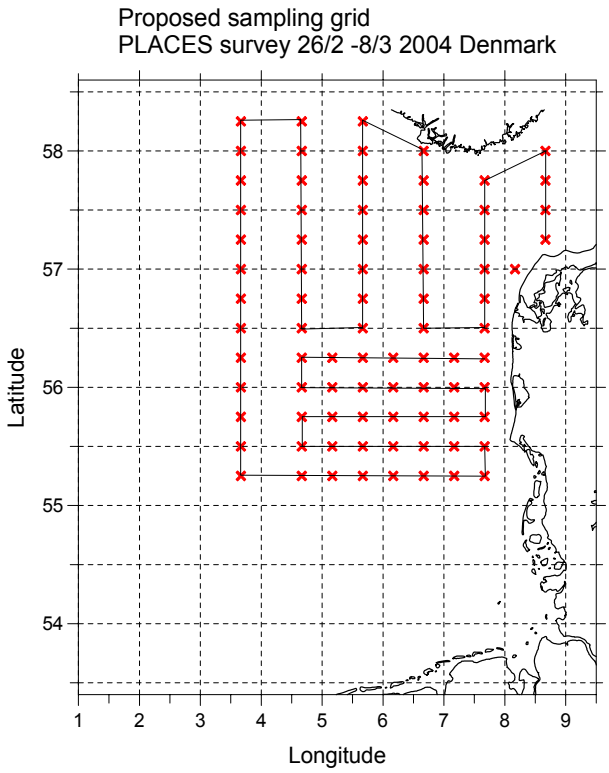


Figure 5.4.11.1. Sampling grid for Danish dedicated PLACES survey.

5.4.12 Norwegian dedicated PLACES survey

Dates:- 4 February 2004 – 21 March 2004
Gear:- Gulf VII if loan of gear can be arranged
Sampling region:- G
Purpose:- Pre-sort cod-like eggs into ethanol at sea, subsequently sort all fish eggs and larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- to be arranged

5.4.13 Norwegian spring spawning herring larval survey

Dates:- 22 March 2004 – 12 April 2004
Gear:- Gulf III
Sampling region:- G coastal areas
Purpose:- Samples will be scanned for occurrence of cod-like eggs
Proposed sampling grid:-

5.4.14 German GLOBEC survey

Dates:- 6 April 2004 – 27 April 2004
Gear:- Bongo
Sampling region:- D
Purpose:- Pre-sort cod-like eggs into ethanol from Bongo hauls at sea, subsequently sort all fish eggs and larvae from the rest of each sample fixed in formaldehyde
Proposed sampling grid:- Figure 5.4.14.1

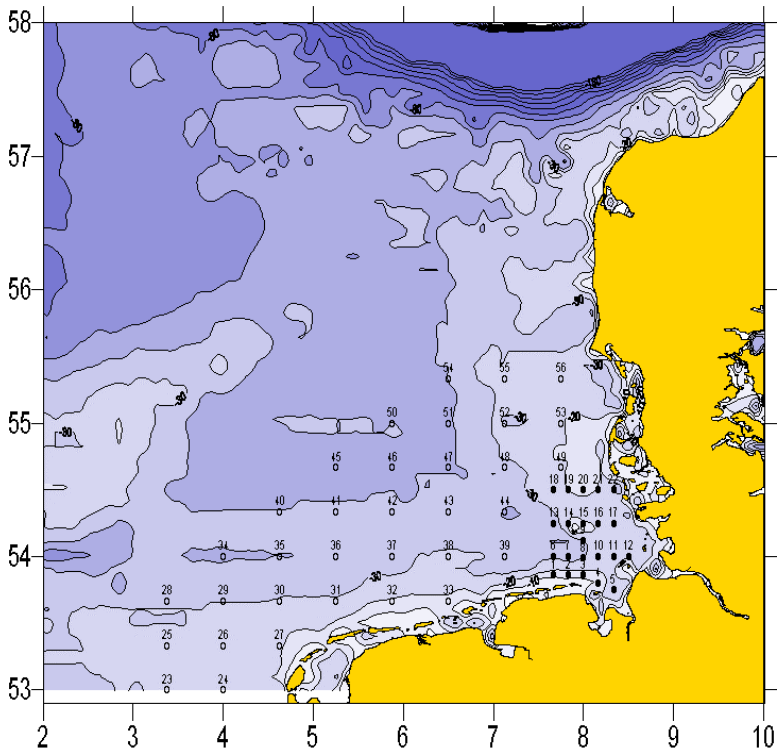


Figure 5.4.14.1. Planned sampling grid for the German GLOBEC program in the North Sea in 2004 (56 stations, 20 nm grid point distance).

6. AGREED PROTOCOLS

This section summarises the agreed protocols for hydrographic and plankton sampling, pre-sorting of eggs at sea, plankton sample analysis, preparation of data for entry into the central database.

Detailed protocols and operating guides are located in Appendices I (sampling at sea), II (laboratory analyses), Appendix III (data handling). These appendices can be used as a stand-alone series of standard operating manuals for PLACES.

6.1 Hydrographic sampling

The standard method for hydrographic sampling will be a vertical profile using a calibrated CTD, measuring at a minimum of 0.5 meter depth intervals to less than 2 meters above bottom. Optionally, the CTD profile may be supplemented by measurements of fluorescence and other physical and biological parameters. However, these supplementary measurements will not form part of the core hydrographic measurements required for PLACES.

If vertical profiling is not possible (due to time or equipment constraints) measurements by a calibrated CTD mounted on the plankton-sampler should be used.

Calibration protocols will follow those set by participating institutes. The collection of a set of surface water samples during each survey with subsequent salinity measurements being made with a high precision salinometer (e.g., Guildline AutoSal 8400) is encouraged.

Processing of hydrographic data will be the responsibility of the participating institutes. Summary data will be prepared in line with the guidance in Appendix I and passed to the central database coordinator in accordance with the guidance contained in Appendix III.

6.2 Plankton Sampling

Excepting for sampling conducted during the International Herring Larval Surveys and the Norwegian Spring Spawning Herring Larval Surveys which have their own established protocols, the standard method for plankton sampling within PLACES will be a double oblique tow made using a Gulf III, Gulf VII or Bongo net system (see Section 3.3.2).

The preferred option will be to use a Gulf VII design sampler with a 76 cm diameter un-encased body fitted with a 40 cm diameter aperture, conical nosecone. The standard net will be made of 270 μm aperture mesh.

The Bongo sampler has a 60 cm diameter opening and a net made of 330 μm aperture mesh. A 500 μm mesh net can also be used but will probably not be needed as substantial clogging is not anticipated during PLACES cruises.

The speed of the sampler through the water should be in the range 2.5 to 3 knots. The bottom of the profile should be to within 3 m of the seabed or reach a depth of 100 m (whichever is the greater). The minimum total towing time should be 20 mins. (this may necessitate multiple oblique tows in shallow areas) that should yield a volume of water filtered in the range 100 – 200 cubic meters.

Care should be taken to ensure equal volumes of water are filtered from each depth band. If possible, the dive profile should be monitored in real time to ensure this is achieved.

The degree of clogging due to phytoplankton should be monitored using a comparison of external and internal flowmeters in real time if possible. Should severe clogging be encountered, the net may be replaced with one having a 500 μm mesh aperture. Clogging is unlikely to be a significant problem except on later surveys.

Processing of plankton samples will be the responsibility of the participating institutes and will comply with the guidance given in Appendices I and II.

6.3 Data entry, exchange and database

PGEGGS agreed to follow the protocols used at CEFAS for data entry and database construction. This will be facilitated by the use of a standard data entry program that will be supplied by CEFAS.

Data will be prepared in line with the guidance contained in Appendix III and passed to the central database co-ordinator.

7. DATA OWNERSHIP, DISSEMINATION AND DELIVERABLES

PLACES will be a joint collaborative project between participating institutes. It is sponsored by ICES but funding comes from the individual programs of the participating institutes.

7.1 Data ownership

The cod and plaice egg data in 2004 will be collected during various surveys that serve other national objectives complemented with specific, nationally funded cruises. While the ownership of all data collected as well as the intellectual property rights regarding their scientific use remain within the national research institutes, the egg and larval plankton data and summary hydrographic data will be collected into a central database. This database will be freely accessible by all participants in a joint effort to meet all objectives described in the project plan.

If institutes involved in the project or from outside are interested in using the data for additional analyses they must seek approval from all institutes involved in the surveys, via the chairman of the planning group, before such use can be granted.

7.2 Dissemination of data

The project coordinator will be responsible for compilation of data from individual institutes, for supervising the management of the central database and for providing prompt access to the central database to participating institutes.

7.3 Publication rights

Results on abundance of cod and plaice eggs and larvae will be published as a coordinated report and possibly submitted to a peer reviewed journal. All participating institutes will be co-authors on such a report or paper.

Additional publications arising from the data produced from PLACES may be produced by the participating institutes or other institutes. Participating institutes will not have automatic rights to be co-authors on such publications although the project PLACES must be fully acknowledged.

The term participating institutes means institutes (or their named representatives) that have actively taken part in the 2004 plankton surveys.

7.4 Deliverables

Information on survey success including temporal and spatial grid coverage and results available in late summer 2004 will form the basis for a preliminary report to be presented to Living Resources Committee at the ICES Annual Science Conference in October, 2004.

Data will be combined and initially presented in the form of bubble plots for the occurrence of cod and plaice eggs by developmental stage in the North Sea. The data will be available for more complex statistical analysis (e.g., GAMs, geostatistical methods) by participating or other institutes.

PGEGGS will attempt to produce an egg production estimate for areas where sufficient data exist by June 2005.

A final report including the combined data submitted by participants will be prepared for presentation at the ICES ASC in 2005. The final report will include distribution maps of cod and plaice eggs for the entire North Sea up to 62° North.

8 CONCLUSIONS

8.1 Autumn 2004 coordination meeting

PGEGGS recommend a meeting in late 2004 to ensure a proper synthesis of survey results and to set up guidelines for preparation of the final report to be delivered to Living Resources Committee for the ASC 2005.

8.2 Training for surveys, exchange of expertise, funding of coordination

Sections 3.7–3.9 have already noted un-resolved problems in some areas. These are namely:

- Lack of identified source of funding for training of plankton sorting staff in some institutes and for inter-laboratory quality control
- Lack of funding to place experienced staff on initial cruises of some institutes
- Quality control of sorting and identification of plankton samples remains a serious un-resolved issue
- Lack of funding for project coordinator role beyond April 2004.
- Lack of funding for database management role beyond April 2004.
- Shortage of sea-going staff for some institutes.

These items will need to be addressed in the period up to April 2004.

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APPENDICES

The Appendices contain detailed protocols for sampling, sample handling, plankton sorting and data handling. Taken together they provide a manual of procedures for the PLACES surveys.

APPENDIX I: SAMPLING AT SEA

I.I. Hydrography

I.I.I. Description of samplers

The type of CTD used by each participating institute will vary but should be to oceanographic specification. Participants are encouraged to make a vertical cast at each sampling station if possible. Alternatively, CTDs may be mounted on the plankton sampler and deployed in double-oblique fashion.

I.I.II. Data to be recorded

CTD profiles will be processed in accordance with the procedures in the participating institutes. The recommended procedures will include:

- Pre-cruise calibration of the CTDs
- Visual inspection of profiles and trimming of spurious data, particularly at the surface
- Comparison of surface or depth stratified results with water bottle sample results for quality control
- Correction of data for sensor lag
- Pressure-averaging of data

Only summary data are required for the central database. The summary data will be:

- Ship and cruise number, station number
- Surface temperature
- Surface salinity
- Bottom temperature
- Bottom salinity
- Depth integrated temperature
- Depth integrated salinity

Profiles pressure averaged to 0.5 m bins are required for examination of egg and larval distribution patterns in relation to hydrography. This will be coordinated by Peter Munk to whom individual CTD profiles should be sent.

I.II. Plankton sampling

I.II.I. Description of samplers

The standard plankton sampler for CEFAS will be a Gulf VII high-speed plankton sampler (Nash *et al* 1998). This sampler has a 76 cm diameter unencased body fitted with a 40 cm diameter aperture, conical nosecone. The standard net will be made of 270μm aperture mesh.

The CEFAS sampler is fitted with a 'Guildline' conductivity, temperature, and depth (CTD) sensor unit. This has been modified to relay 'real-time' flowmeter (and environmental) data back to a shipboard display unit. A Valeport BFM 001 type flowmeter (with a blade diameter of 12.5 cm) will be centrally mounted inside the sampler nosecone with its boss 2.5 cm back from the leading edge. Another BFM 001 flowmeter will be mounted externally on the sampler frame to provide an accurate measure of distance travelled (D) and sampler speed through the water. The ratio between internal and external flowmeters will provide an index of clogging (C).

The Netherlands Institute for Fisheries Research (former RIVO) may be able to borrow a GULF VII PRO-NET system (Spartel Ltd, Totnes, Devon) together with CTD and flow monitoring system. This system uses Valeport BFM002 type

flowmeters (with a blade diameter of 5 cm) which will be mounted in similar positions to the larger flowmeters mounted on the CEFAS sampler.

A theoretical volume filtered can be calculated on each tow by multiplying the area of the nosecone aperture (A) by the distance travelled by the sampler (D). This theoretical volume can then be compared with actual volume filtered (calculated from flowmeter readings) and a sampler efficiency calculated for each station.

Where clogging occurs (i.e., sampler efficiency falls below 70% of A x D or where the index C falls below 0.6), nets of 400μm aperture mesh will replace the standard 270μm net. If clogging continues to be a problem, then a reduction in nosecone size to a 30 cm diameter aperture is advised.

The others Institutes involved may use other gears, e.g., GULF III type samplers or BONGO nets. Procedures should be standardized as much as possible.

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 centre of the net-opening. When operating the BONGO net, additional CTD profiles should be made available by e.g., Rosettes.

I.II.II. Deployment of samplers

The plankton samplers will be deployed on a double oblique tow, at 3 knots, from the surface to within 2 metres of the bottom (or as near as bottom topography will allow) and return to the surface. The requirement is an even, 'V' shaped dive profile, filtering the same volume of water per unit of depth. The aim will be to shoot and haul at the same rate with the sampler spending 10 seconds in each 1 metre depth band. 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 the end-bags and plankton samples into the ship's laboratory and proceed with the pre-sorting of cod-sized eggs.

I.II.III. Pre-sorting cod-like eggs for genetic analysis at sea

- Rinse the contents of the end-bags into a second, **formaldehyde free** jug using seawater. Take care to thoroughly rinse the mesh of the end-bags, a squeezy bottle is useful for this.
- Place the jug holding the plankton sample on ice, this will keep the sample cool and help reduce any DNA degradation.
- **Gently mix** and remove a small sub-sample into a large dish (this is important otherwise early stage eggs which are often damaged by the net will sink and you will not get a representative sub-sample)
- Pick out eggs by eye using a wide-mouth pipette and transfer onto a petri dish set up with small drops of water.
- Once around 10–15 eggs have been picked out move the petri-dish onto a low power microscope equipped with a calibrated eye-piece graticule or linked to an image analysis package.
- Examine each egg in turn and select 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 each egg into an individual, 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 a sequential number identifying the egg.
- Continue taking sub-samples until the desired number of cod-like eggs has been sorted.
- With a little practice it is possible to pre-sort up to 70 eggs in an hour.
- It is not recommended to continue egg sorting beyond one hour as DNA degradation may occur.
- After pre-sorting is complete, return the rejected eggs to the bulk of the plankton sample which should then be fixed and labelled as below.

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.

I.II.IV. Fixing plankton samples

The standard fixative for use on these surveys will be a 4% solution of buffered (pH 7–8) formaldehyde in either distilled or fresh water. (CEFAS 10 litres of 30% formaldehyde to make a buffered stock solution. The stock solution is then diluted to 4% using distilled water and 250g of sodium acetate trihydrate is dissolved in to raise the pH). **It is strongly recommended to check the pH of the solution periodically**. This solution is approximately iso-osmotic with sea-water and will minimise damage and distortion of the eggs. It has been demonstrated that shrinkage of eggs in this solution compared with fresh material is also minimal compared with other formulations.

- Top up the jar with 4% buffered formaldehyde, making sure that the volume of plankton does not exceed 50% of the volume of the jar.
- Any excess sample should be fixed separately in additional jars.
- Put water-proof labels containing station details in pencil into all jars.

Recipe for 4% buffered formaldehyde for fixing plankton samples at sea

This procedure will produce 10 litres of buffered 4% formaldehyde solution for use at sea when preserving plankton samples.

- Take 10 litres of 30% formaldehyde solution as commercially supplied.
- Place 1.33 litres of this solution in a new container and make up to 10 litres using deionised water.
- Add 420 g of sodium acetate trihydrate (note using 420 g give final w/v of 2.5% sodium acetate taking into account the trihydrate).
- Mix thoroughly.

This will create 10 litres of 4% formaldehyde solution buffered with 2.5% sodium acetate.

Note that the pH must be kept above 7.0 to prevent damage to DNA. At present we are not planning to undertake routine genetic analysis on formaldehyde fixed samples since reliable methods are still in development. Sodium acetate is not an ideal long-term buffer for formaldehyde as the pH will gradually drop (7.5 to 6.5 in three years). We are currently evaluating sodium glycerophosphate as an alternative buffer but do not have sufficient experience yet to recommend its routine use.

I.II.V. Recording data at sea

Participating institutes will follow their own procedures for data recording at sea. The following information for each station will be the minimum required by PLACES.

An accurate log of sampling will be kept detailing the following:

- Ship name
- Cruise number

- Station number
- Date
- Time (in GMT)
- Brief description of gear deployed e.g., Gulf VII, CTD vertical cast etc.
- Brief description of haul profile e.g., single double-oblique
- Decimal latitude and longitude at start of haul
- Decimal latitude and longitude at end of haul
- Decimal latitude and longitude at mid-point of haul
- Depth of water (sounded depth plus any correction for depth of transducer beneath the hull) in metres
- Maximum depth sampler reached in metres
- Internal flow-meter count
- External flow-meter count
- Water volume filtered

Longitudes should be notated as negative sign for west of Greenwich meridian (e.g., -001.435).

Data from CTDs will be logged and processed as described in Appendix I.I.II. Only summary data are required for the central database. The summary data will be:

- Ship and cruise number, station number
- Surface temperature
- Surface salinity
- Bottom temperature
- Bottom salinity
- Depth integrated temperature
- Depth integrated salinity

Profiles pressure averaged to 0.5 m bins are required for examination of egg and larval distribution patterns in relation to hydrography. This will be coordinated by Peter Munk to whom individual CTD profiles should be sent.

An accurate log of cod-like eggs pre-sorted at sea will be kept. A sample log-sheet is given below. The data will be transferred to the electronic records system as described in Appendix III.

SHIP **CRUISE**

Logsheets for cod-like eggs pre-sorted at sea and fixed in ethanol

No oil globules, 1.0<x<1.75 mm

Station	
Date	
Time hauled	

Watch leader

Tube no	Size (mm)	Stage	Species(IV, V eggs only
1			
2			
3			
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9			
10			
11			
12			
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Tube no	Size (mm)	Stage	Species(IV, V eggs only
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Total

APPENDIX II: PLANKTON ANALYSIS IN THE LABORATORY

Plankton samples fixed in 4% buffered formaldehyde will be returned to participating laboratories for the sorting and identification of fish eggs and larvae.

II.I. Overview of procedure

It is recommended that the 4% formaldehyde is drained from the sample immediately before analysis through a 275 µm mesh sieve. The sample can then be made up to a known volume using an odourless 'observation fluid' (Steedman 1976). This solution will act as a preservative on fixed material and enables the sample to be sorted without toxic formaldehyde fumes building up in the laboratory.

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 150 cm^3 Propylene phenoxetol and 1500 cm^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.

II.II. 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 folsom 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 (cod and plaice) 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 1/4 or less of the sample then 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 sub-sampled and all the larger eggs sorted from the remainder of 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.

II.III. 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).

Figure II.III.I and Table II.III.I. gives keys for identifying fish eggs.

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. 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 1.16–1.89 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. During PLACES, genetic methods will be employed to distinguish early stage cod and haddock eggs.

Table II.III.I also shows that for eggs without oil globules many species overlap in their size ranges. It is therefore not possible to assign species identification to them. 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).

Figure II.III.II, Table II.III.II and Plates II.III.I.–IV show the appearance of these developmental stages. In fresh samples stage identification is relatively easy but it becomes more difficult in fixed samples. From experience with mackerel and Irish Sea surveys, it is clear that reliability of staging increases with experience of the analysts. This is likely to be a problem in PLACES where many of the analysts will be relatively in-experienced.

Figure II.III.I. Flow chart for egg identification.

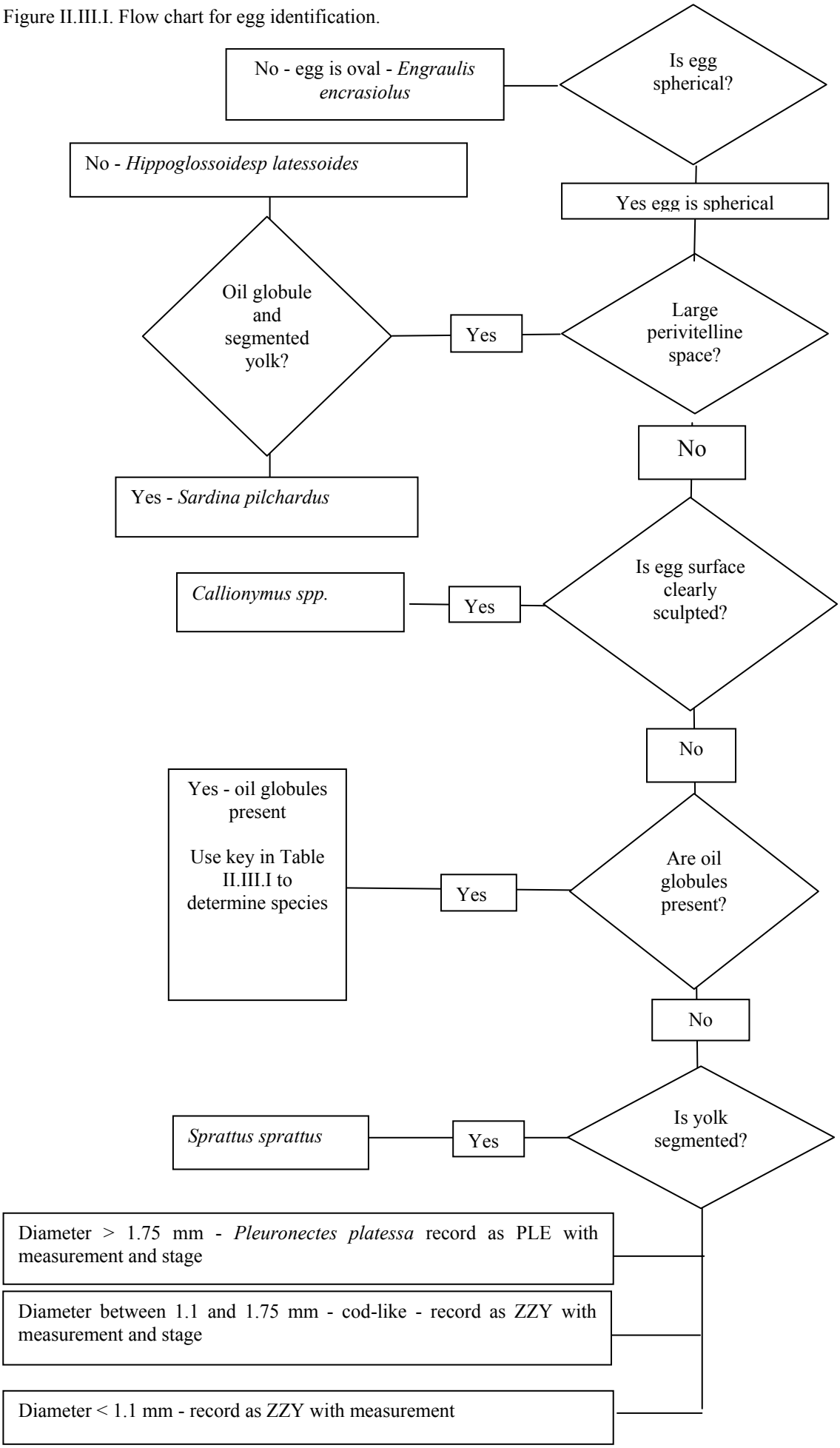

Table II.III.I. Key to identification of pelagic eggs (size diameter).

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

Table II.III.I. Continued. Key to identification of pelagic eggs (size diameter).

Pelagic spherical eggs con/td		
	Egg diameter (mm)	
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

Figure II.III.II. Diagram of egg development stages.

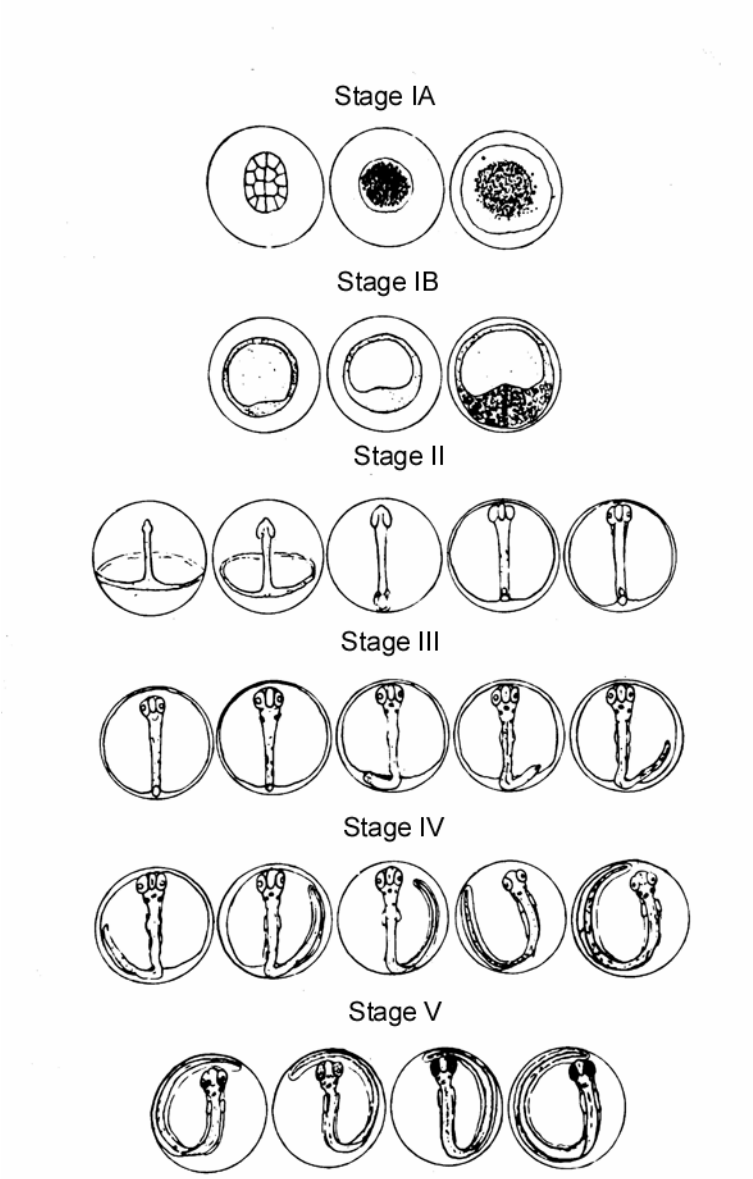

Table II.III.II Criteria for egg staging following Thompson and Riley (1981) and Riley (1973).

Stage	Criteria
IA	Blastula stage lasting from fertilization until successive cleavages produce a cellular mass in which individual cells are not visible
IB	Continuing development of the blastodisc, which becomes visible as a signet ring, up to the first indication of the primitive streak
II	Gastrulation stage lasting from the first sign of the primitive streak until the closure of the blastopore.
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.
IV	Growth of the tail occurs until the embryo fills the whole egg with the tail touching the head.
V	Growth of the tail past the head. Pigmentation of the eyes begins. At the end of this stage the larva hatches.

Plate II.III.I

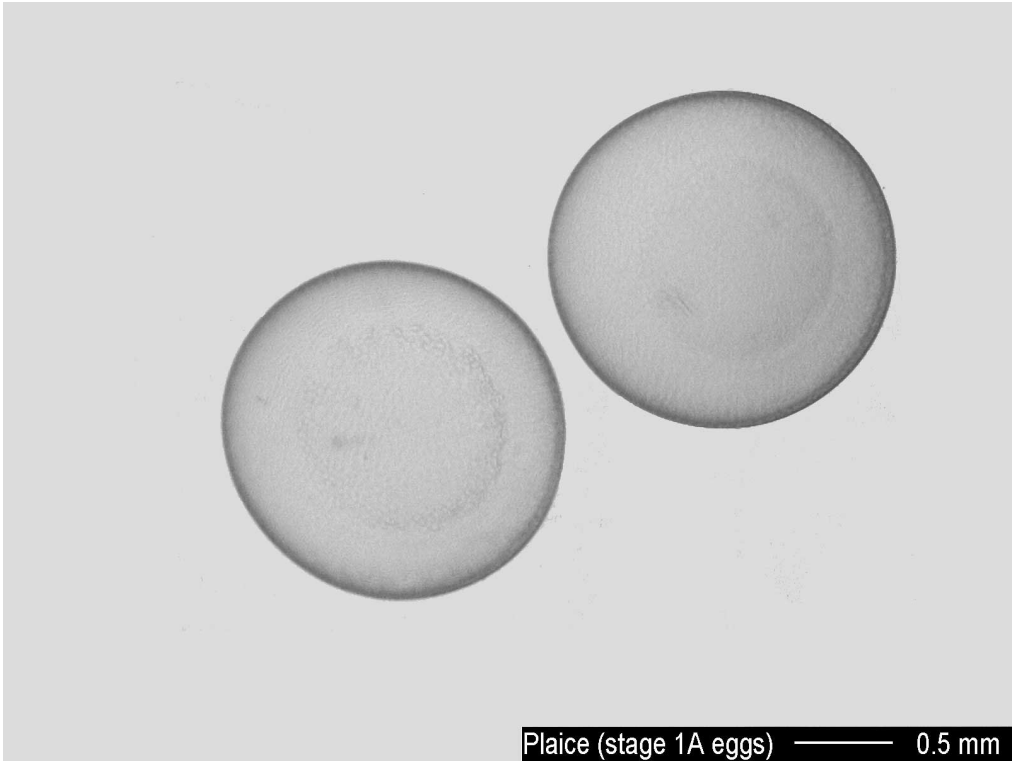

Plate II.III.II

Plate II.III.III

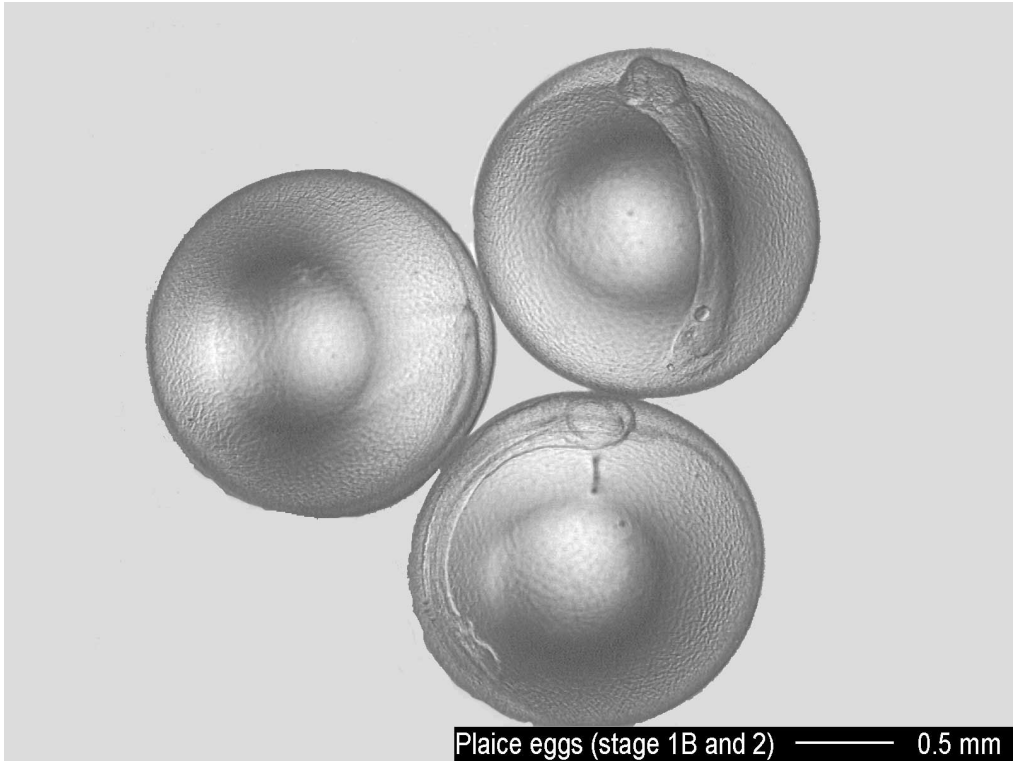

Plate II.III.IV

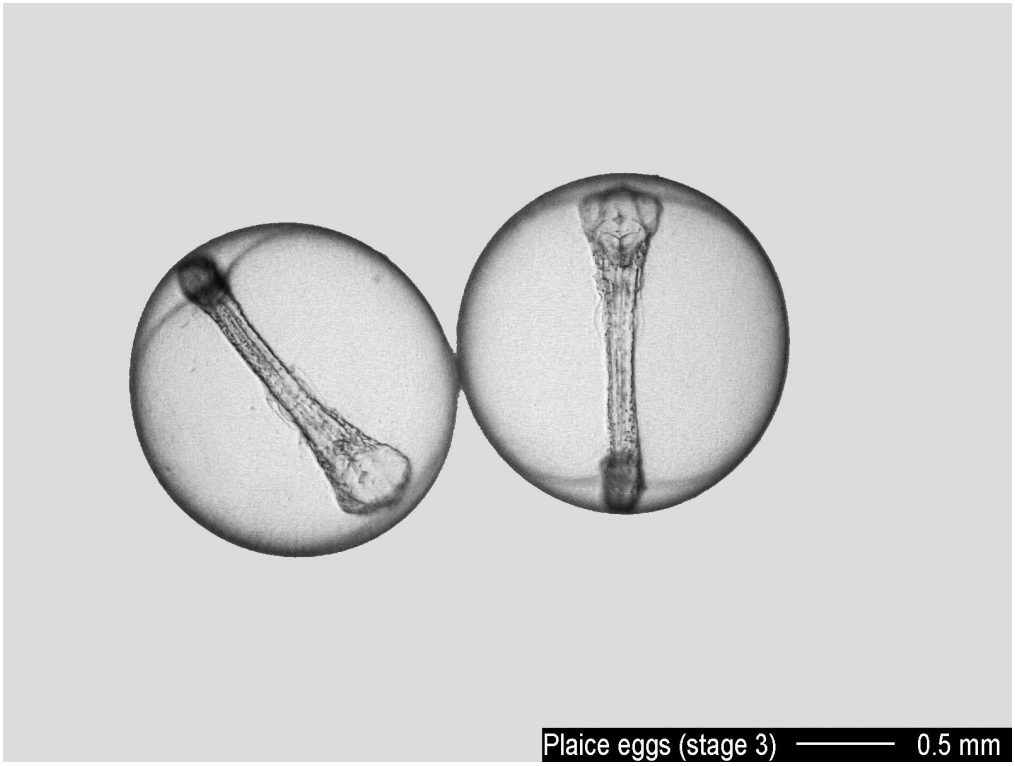

Plate II.III.V

Plate II.III.VI

II.IV 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 remains Russell (1976). Unfortunately this is now out of print but copies should be available via national libraries. A CD-ROM covering fish larvae is in preparation through the ETI-Biodiversity Center, University of Amsterdam but is not yet available.

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 a 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 described in Appendix III 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. See Figure II.IV.I for larval appearance.

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. See Figure II.IV.II for larval appearance.

Figure II.IV.I. Cod egg with late stage embryo and larvae.

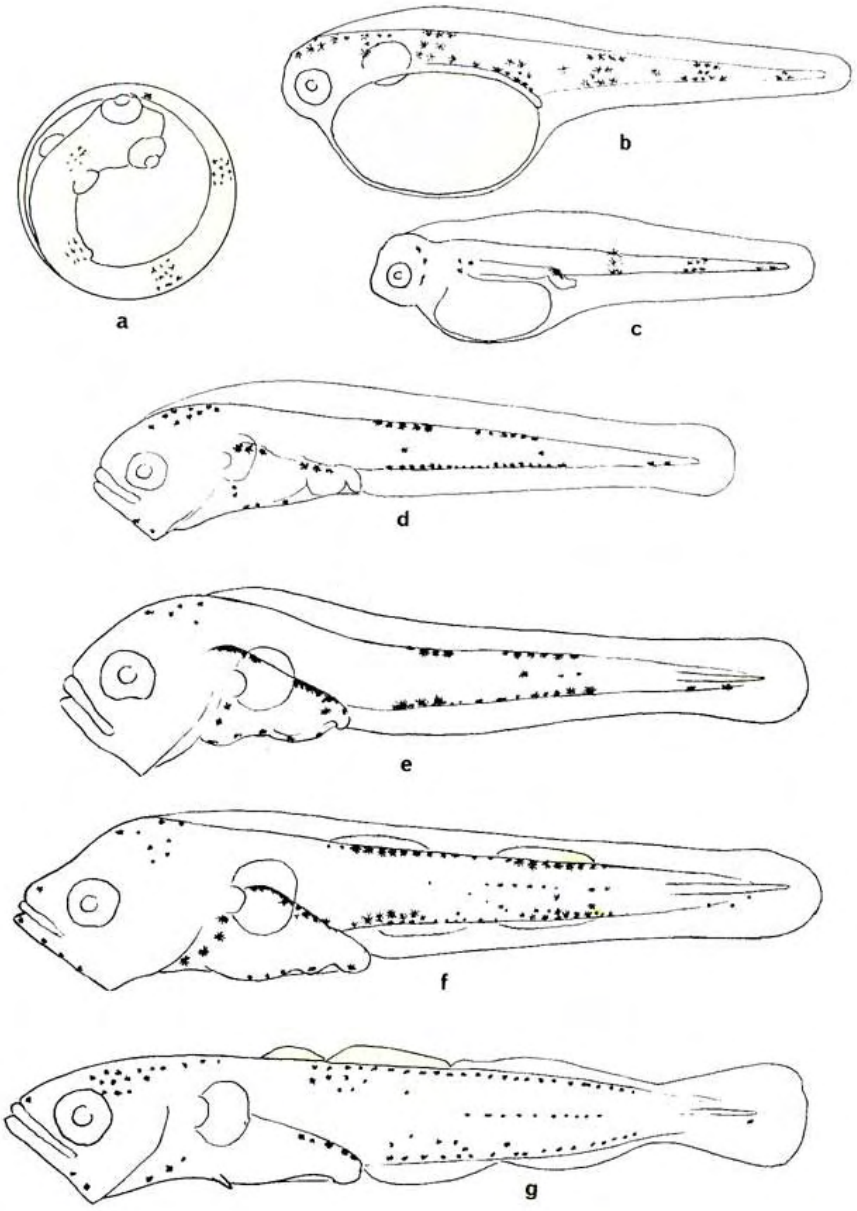

Fig. 19. *Gadus morhua*.

(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.

Figure II.IV.II. Plaice egg with late stage embryo and larvae.

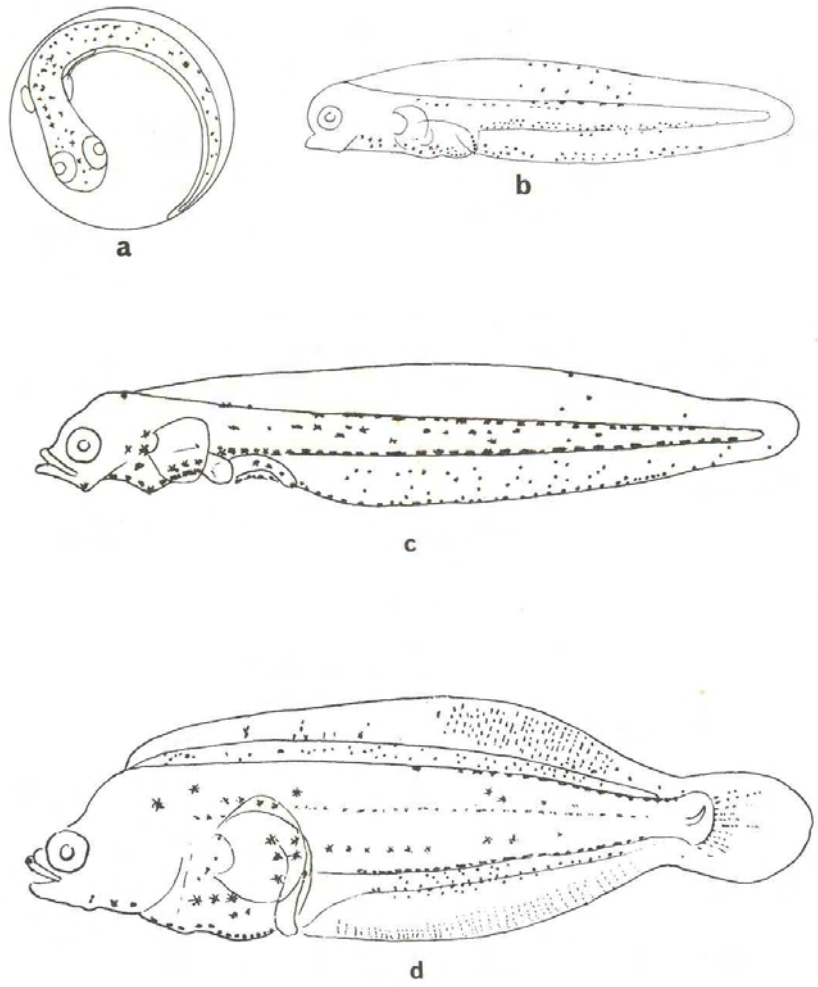

Fig. 121. *Pleuronectes platessa*

(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.

II.V. Storage of plankton samples (post analysis)

Samples should be archived for at least four years. Archiving of samples is the responsibility of each participating institute.

Sorted eggs and larvae should be placed in small glass vials containing preservative and stored in the larger jars used to hold the remainder of the sample to avoid wasting time re-sorting material if re-analysis is required.

Although the 'observation fluid' (see B2 above), as recommended by (Steedman 1976), does act as a preservative, it is recommended that a small amount of formaldehyde is added to this solution once each sample has been processed. To make up 10 litres of a stock solution for long-term storage of the samples, the following quantities of chemicals are recommended.

Recipe for storage fluid

50 cm^3 Propylene phenoxetol
500 cm^3 Propane-1, 2-diol
700 cm^3 30% formaldehyde
Make up to 10 litres with distilled water

This will give a final concentration of approximately 2% formaldehyde in the 'preservative' solution. This will prevent any deterioration of the plankton samples for a number of years.

Once the samples are in the 'preservative' solution it is recommended that the jars are dipped in molten wax to prevent evaporation from around the lid.

Responsibility for archiving samples will rest with the institutes that collected the samples.

II.VI. Health and safety

Formaldehyde has recognised health risks. Institutes will follow their own guidelines in handling this substance. The COSHH (Control of Harmful and Hazardous Substances) risk analysis from CEFAS is given below as an example (Table II.VI.I).

Ethanol (used for preserving eggs for genetics) is highly flammable but not particularly toxic unless ingested in reasonable quantities. Bulk quantities should be stored at sea in flammable containment lockers and small quantities decanted for use.

Remaining chemicals used are of low toxicity.

Table II.VI.I. COSHH assessment for formaldehyde.

CEFAS COSHH ASSESSMENT

Please refer to the guidance notes for additional information.

COSHH Reference: COSHH/LOW/FB REC/SPM/01 (Formally FSMG-SM02)

1.

1.1. Assessor	1.2. Laboratory
Stephen Milligan	Lowestoft

Part A: Potential to cause harm

2.

2.1. Location of process Preparation Room of the Plankton Laboratory (Rm 146) and at sea

2.2. Work / process description

Fixing plankton and young fish samples and their permanent storage.

30% buffered formaldehyde is provided for use at sea. This is diluted with distilled water to a working strength of 4%. The solution is normally made up at sea, 30 litres at a time, as required. Plankton samples are fixed in 4% formaldehyde at sea and transported to the laboratory.

The dilution of 30% formaldehyde with distilled water is potentially the most hazardous operation. It is always done in the open air using goggles and gloves and with a copious supply of water readily available from a deck hose.

Samples (in 4% formaldehyde) collected at sea are stored in the preparation room (Rm 146) prior to analysis. Before analysis, the 4% formaldehyde is filtered from each sample and washed down the sink (in Rm 146) with copious amounts of seawater. There is a large, externally vented, extraction fan continuously running in this room. The samples are then transferred to a sorting fluid (see COSHH/LOW/FB REC/SPM/02) prior to analysis in the plankton laboratory (Rm 147).

The Plankton laboratory (Rm 147) is a formalin free room although it is possible for some fumes to penetrate via the connecting doorway from room 146. An extraction fan is in continuous use in room 146 and levels of formaldehyde fumes, although detectable, are never high.

2.3. The work is routine

3.

Chemical / reagent / organism	Constituents of chemical / reagent (if prepared in CEFAS)	MEL/OES mg/m^{-3} Short/long term	State	Quantity	Hazard Classification
Formaldehyde	N/A	MEL 2.5 mg/m^{-3} 15min exposure 2.5 mg/m^{-3} 8 hour exposure	Liquid. Concs from 30% to 4%	Maximum container size handled 10 litres of 30%	Flammable Toxic Irritant Sensitizer

Additional sheets used No*

4. Assessment of exposure

4.1 Duration of process: <10mins to carry out dilution from 30% to 4%. <5mins to dispose of 4% solution from samples.
4.2 Frequency of process: Dilution carried out twice per week at sea (maximum). Disposal of 3 litres of 4% formaldehyde a week in lab (max)
4.3 Other comments: Most hazardous exposure will occur with accidental spillage in labs at sea or in the Laboratory. Container size has been reduced to 10 litres and 30% formaldehyde is only handled outside. Chemical spill kits are available both at sea and in the lab. Protective clothing including gloves, goggles and masks are available. Regular monitoring of formaldehyde fumes in Rm 146 is carried out by HSQ team.

5. Is elimination or substitution reasonably practicable for any of these chemicals / biological agents. No *

Part B: Control measures

6.

Controls:	Control required Yes/No	Comments
6.1. General ventilation	Yes	The dilution of 30% formaldehyde should only be carried out outside, preferably on the open deck of a ship in fine weather.
6.2. Local exhaust ventilation	Yes	Externally vented extraction fan should be used where formaldehyde is used in laboratories
6.3. Personal protective equipment	Yes	Goggles and strong gloves should ALWAYS be used. At sea, waterproofs and wellington boots should be worn.
6.4. Other	Yes	Copious amounts of water should be available to wash away spills. Specific spill kits should be available nearby.

7.

Monitoring	Control required Yes/No	Substance requiring additional surveillance or exposure monitoring
7.1 Health surveillance (in addition to that already provided)		
7.2 Exposure monitoring	Yes	Monitoring of fumes in Rm 146 should continue at regular intervals. (e.g., Every 4–6 months).

8.

Information/training	References / notes
8.1. Specific Training	Everybody made aware of the need for care and the use of PPE when handling Formaldehyde.
8.2. Labelling	All containers holding solutions of formaldehyde are labelled accordingly and stowed safely.
8.3. First aid	Remove from exposure and flush affected area with water.
8.4. Accidental spillage	Spill kits available in Rm 146 on shelf above sink.
8.5. Disposal	N/A
8.6. Additional safety data	See Hazards data sheet No. 100 (in this folder).
8.7. Stowage requirements (SIC's must give details)	Plankton samples in 4% formaldehyde stored in Rm 146 on racking or in strong trays on the floor. 2 litre containers of various strength solutions stored in flammables cupboard in Rm 146. At sea 10 litre containers of 30% formaldehyde stowed in a chemicals cabinet on the deck. The 4% solution for addition to samples is stowed in the fish room close to the door and with copious supplies of water available.

9.

Actions Required	By whom	By when
N/A		

10.

Date completed: 5 January, 2001	
Authorised by: Stephen Milligan (Team Coordinator)	Date: 5 January, 2001

APPENDIX III: DATA HANDLING AND DATABASES

III.I. Timing of data submission

Data from PLACES surveys will be compiled in a central database by CEFAS. Participants will be supplied with guidelines for data submission and programs to facilitate the production of the data files needed for database construction.

The PLACES field sampling should be completed by the end of April. As soon as possible after each cruise is completed, all participants will provide a cruise report and physical data summary to the coordinator (C Fox, CEFAS). Cruise reports will be promptly distributed among all involved institutes to ensure rapid information exchange. Based on submitted cruise reports adjustments in area coverage may be made for subsequent cruises. The physical summaries will be compiled into the central database as described below. This will provide a prompt and useful tool for assessing survey coverage whilst surveys are in progress.

As soon as possible after each cruise is completed, all participants will also send to the coordinator an electronic file detailing the cod-like eggs pre-sorted at sea. This electronic file will be prepared using the data entry program supplied by CEFAS.

It is intended that laboratory analysis of sampled plankton be completed before summer break 2004 by the majority of participants. However, England and Germany have indicated that they will probably not be able to provide results before late autumn.

As soon as possible after analysis is completed, each participant will supply to the coordinator (C Fox, CEFAS) an electronic file of the results prepared using the data entry program supplied by CEFAS.

All data will be compiled into a central database as described in the following sections.

III.II. Introduction to data handling protocols

PLACES will generate large amounts of data from both electronic instruments such as CTDs and from the sorting of plankton samples. The success of the project depends upon the accurate and reliable compilation and conversion of this information to provide meaningful output. The data handling protocols are designed to ensure

- An audit trail for data from the final output back to the bench source (allows tracing and correction of errors and checking of unusual observations)
- A consistent framework for recording and handling data
- A readily understandable set of databases and algorithms for data handling
- Built in checks and safeguards to trap incorrect data

Full implementation of these protocols will also help future-proof the data and aid investigators in coming years who might wish to analyse data further. In the past this has been extremely difficult when systems of data handling tended to be inconsistent from project to project. With the advent of electronic storage systems the situation has changed. However, the exclusive use of electronic media brings new problems principally associated with the speed of development and the lack of long-term archival solutions.

Figure III.II.I shows the way plankton data are handled within CEFAS. Different institutes in PLACES will have their own protocols but the underlying framework and database structure described in this section will be used.

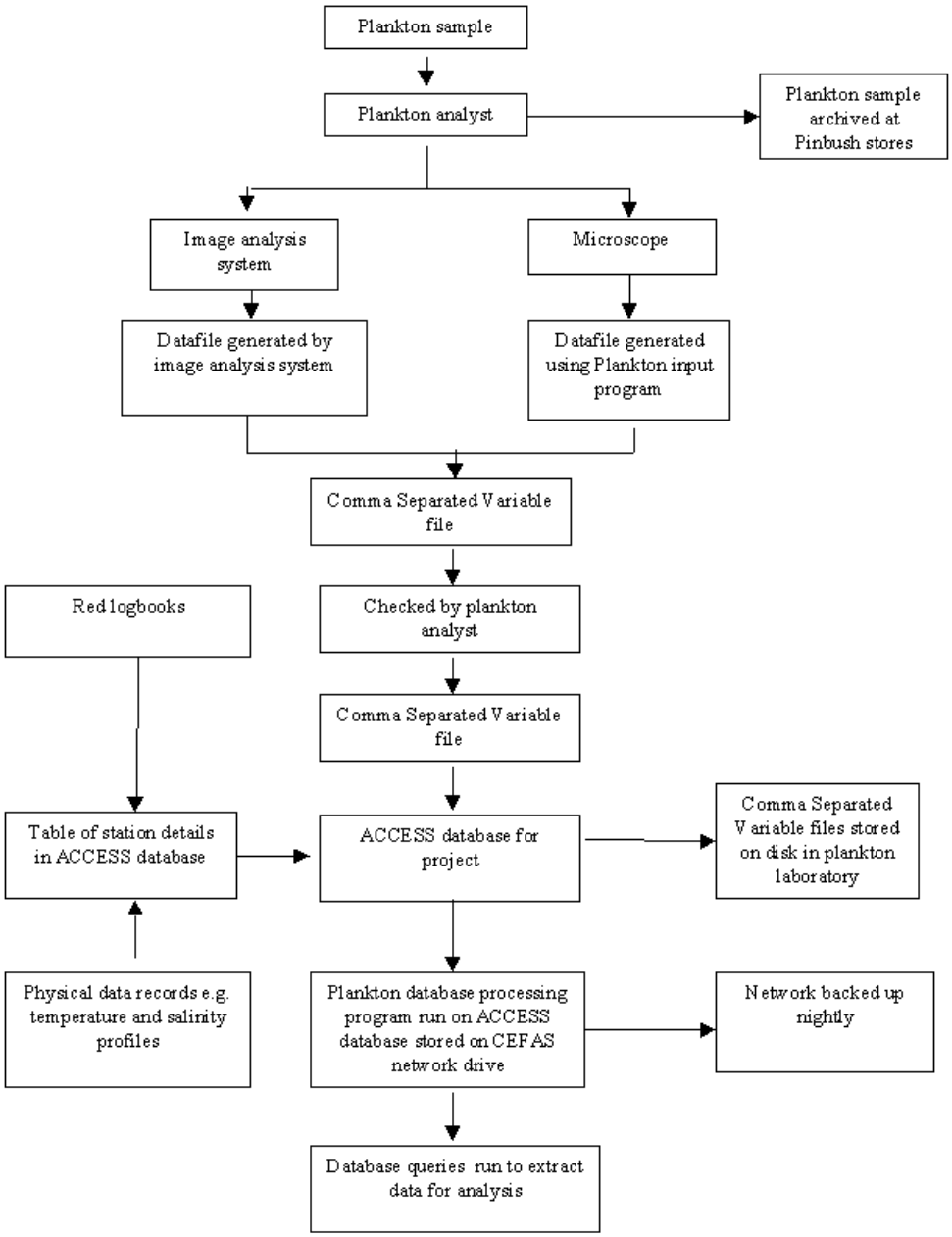

Figure III.II.I. Overall flow of plankton data.

III.III. The raw data-files used for building databases

The building blocks for the plankton databases are comma separated variable files (CSV) containing the zooplankton analysis data. These are combined in the database with information on each station (location, date, time etc). The CSV plankton files can be generated by the plankton analysts via two routes, either manually using the plankton input program or from image analysis systems. Within PLACES the manual entry method will be used.

III.III.I Physical data

The physical table in the database provides information on station location, time of collection, water depth etc. All participants should supply this information to the project coordinator in the form of an Excel file. The database manager will convert these files as necessary for the database. The Excel files **must** contain the following information for each station (including CTD stations).

a) Ship name
b) Cruise number
c) Station number
d) Date (dd/mm/yyyy)
e) Time (in GMT)
f) Brief description of gear deployed - e.g., Gulf VII, CTD vertical cast etc.
g) Brief description of haul profile e.g., single double-oblique
h) Brief description of level plankton samples will be analysed to e.g., fish eggs plus cod and plaice larvae, fish eggs plus all larvae
i) Decimal latitude and longitude at start of haul
j) Decimal latitude and longitude at end of haul
k) Decimal latitude and longitude at mid-point of haul
l) Depth of water (sounded depth plus any correction for depth of transducer beneath the hull) in metres
m) Maximum depth sampler reached in metres
n) Internal flow-meter count
o) External flow-meter count
p) Water volume filtered
q) Surface temperature
r) Surface salinity
s) Bottom temperature
t) Bottom salinity
u) Depth integrated temperature
v) Depth integrated salinity
w) Any other comments pertaining to the station

III.III.II. Plankton input program – ZooplanktonDatabaseInput.exe

This program allows the analyst to input data manually and is used where samples have been analysed under the microscope or pre-sorted at sea. It produces 'Comma Separated Variable' files that are used by the Database manager to construct the ACCESS database.

On starting the program, the analyst is presented with an input form (Figure III.III.II.I).

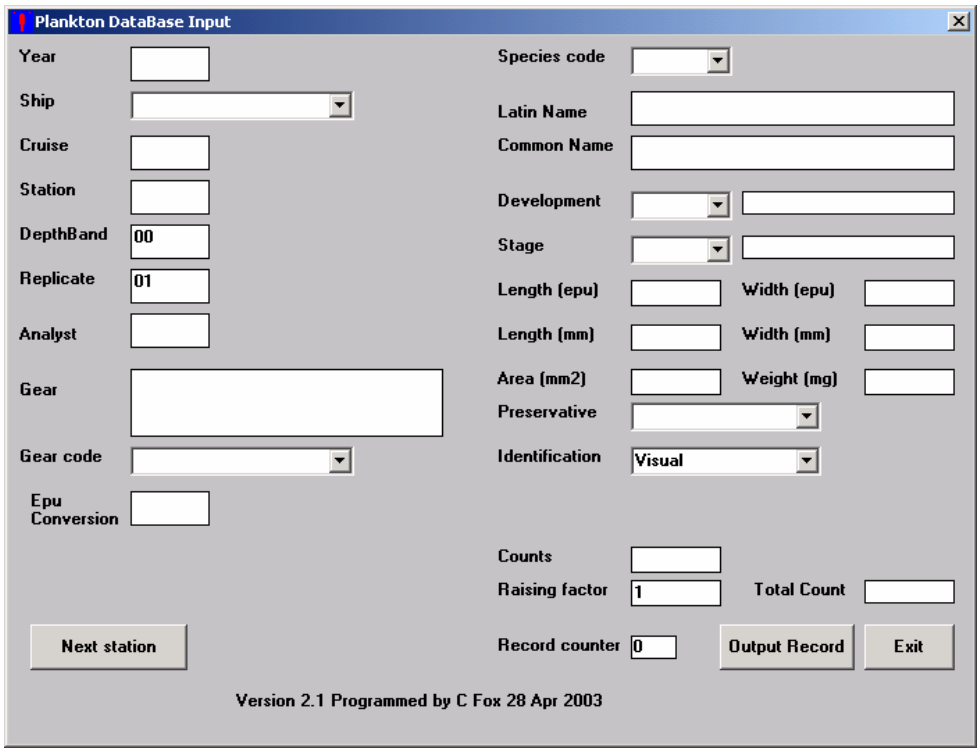

Figure III.III.II.I. The plankton data input form.

- Entry begins by filling in the fields for Ship, Cruise, Station, Depthband, Replicate, Analyst, Gear code and Epu Conversion (see table below for descriptions).
- Species codes can be entered manually or using a drop down list. The equivalent Latin name and Common name will appear in the boxes below the species code. You cannot type into the Latin and Common name boxes. The program will detect meaningless codes and issue a warning.
- Development codes are entered manually or using the drop down list. A description appears alongside.
- Stage codes are entered manually or using the drop down list.
- Lengths and widths either in eye-piece graticule units or in millimetres are entered manually.
- The preservative type is entered manually or selected from a drop down list.
- The method of identification of the object is selected using the drop down list.
- The counts are entered manually.
- The raising factor can be changed from the default value of 1 if necessary.
- A Total count appears in the box alongside.
- When happy with the results press the “Output record” button (Figure 3).
- The first time this is done file dialog will appear asking form the location to save the output file to (see description of comma separated files below)

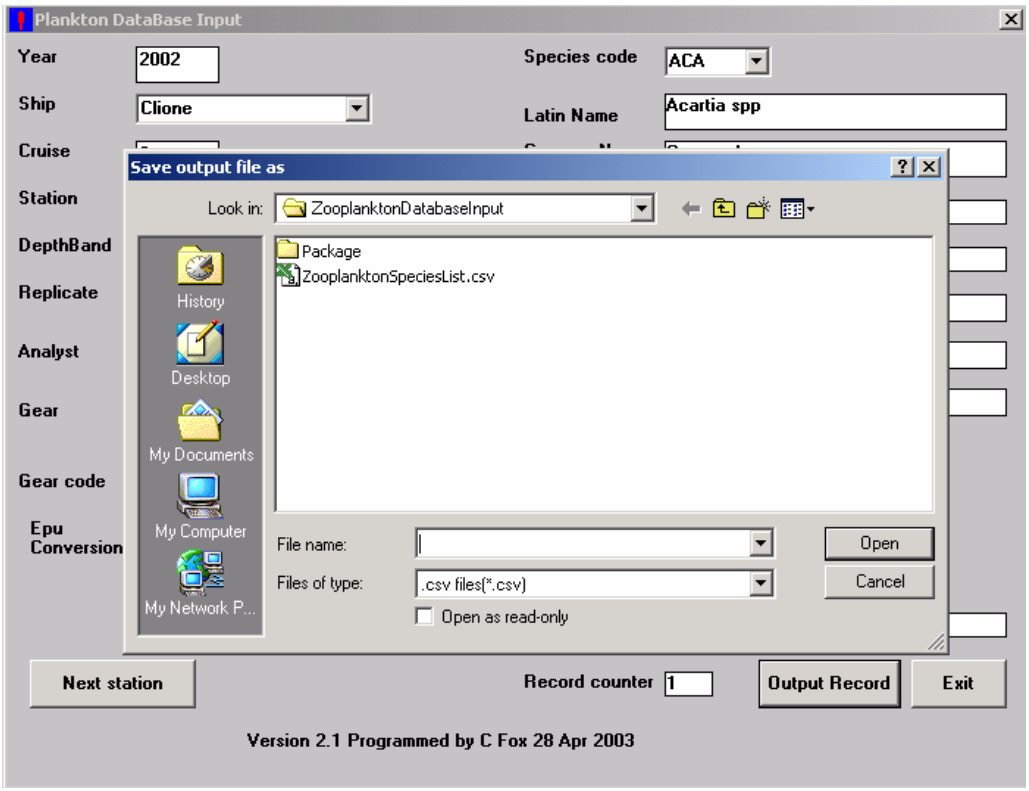

Figure III.III.II. Plankton lab input form, save file location.

- Enter a location (this is normally set up as C:/Data/Operator name or similar) and continue.
- Successive records are written to this file until the "Next Station" button is pressed. At this point the file is closed. The analyst is asked if they wish to continue with a new station or end the program.

Note that because the ship, gear and species codes are relatively complex, entry is facilitated by a drop down menu listing the available choices. INFORM C Fox, CEFAS IF A CODE YOU NEED IS NOT LISTED. THE PROGRAM WILL TRAP UNKOWN CODES LATER ON AND INSIST THEY ARE CORRECTED SO ENTERING ADDITIONAL CODES BY HAND WILL ONLY PRODUCE ERROR WARNINGS. THIS SYSTEM IS IN PLACE TO PREVENT ERRONEOUS CODES APPEARING OVER TIME NOT JUST TO ANNOY YOU!

Table III.III.I. Description of fields in data entry program.

Entry Field	Mandatory	Description
Year	Yes, 4 digits	Year sample collected e.g., 1999
Ship	Yes, generated by program	Ship from which sample was collected
Cruise	Yes, up to 2 digits	Cruise number
Station	Yes, up to 3 digits	Station number
Depthband	Yes	Set to 00 for oblique hauls, incremental for multi-depth hauls e.g., LHPR or MOCSNESS
Replicate	Yes	Can be incremented for replicate samples collected on same station
Analyst	Yes, 3 letters	Initials of analyst
Gearcode	Yes	A numerical code describing the sampling gear, see below for details
Epu conversion	Only if sizes entered in eye piece units	Conversion factor for eye piece graticule to millimetres
Species code	Yes, 3 letters	CEFAS organism identifier
Development	Yes, up to 3 letters	CEFAS development code e.g., egg, nauplius etc.
Stage		Egg stage, 1A, 1B, 2, 3, 4, 5, 6, 7, 8, 9
Length (epu)	No	Length in eye piece units
Width (epu)	No	Width in eye piece units
Length (mm)	No	Length in millimetres
Width (mm)	No	Width in millimetres
Area (mm2)	No	Area in square millimetres
Weight (mg)	No	Weight in milligrams
Preservative	Yes	Preservative used to fix the sample (note there is no entry for the plankton laboratory storage medium), default is set to 4%Formaldehyde but other options include IMS, Bouins and DMSO
Identification method	Yes	Method used to identify the object – default is set to Visual but other options include Geneprobe and IEF
Counts	Yes	Number of observations in sub-sample
Raising factor	Yes	Sample volume/sub sample volume to include apportioning of un-staged eggs (normally where > 100 in a sample)

III.IV. Structure of the Comma Separated Variable (CSV) files

The plankton data input program program outputs. CSV files. After checking these files, they should be printed out and a hard-copy stored in the plankton lab. The CSV files are then passed on floppy disk (or CD-ROM) to the database manager who imports them into the ACCESS database. After importing the files the disks are returned to the plankton lab and stored (in PLACES the disks will be archived at CEFAS in addition to participating laboratories). This system is designed to ensure that data are not lost or replicated during the construction of the ACCESS database. Storing the CSV files on disk and as hard copy in the plankton lab acts as a back up in case of major failure of the ACCESS databases on the CEFAS network system. In this case it should be possible to reconstruct the databases from the CSV files.

CSV files being checked should only be opened with a text editor such as Wordpad. Opening CSV files in **Excel can corrupt the data** as Excel by truncating long numbers such as the SampleID. CSV files must be saved after editing as **text files** only with the extension.csv

A typical csv file contains the following structure:

"Database input sheet"
"Year",1999
"Ship",11
"Cruise",2
"Station",105
"DepthBand",0
"Replicate",1
""
"Record","SampleID","Species","Dev","Stage","Preservative",”Identification”,"Epu_Conv","Len_epu","Width_epu","Len_mm","Width_mm","Area_mm2","Weight_mg","Count","Raise","Total","Gear","Analyst","Notes"
1,199911021050001,"PLE","E","16CELL","4%Formaldehyde",”Visual”,"","","","","","","",1,1,1,1202040270,"RH"
2,199911021050001,"PLE","E","1A","4%Formaldehyde","Visual”,””,"","","","","","","",99,1,99,1202040270,"RH"
3,199911021050001,"PLE","E","1B","4%Formaldehyde","Visual",””,"","","","","","","",16,1,16,1202040270,"RH"

The CSV file consists of a header section giving information about the sample, then a row showing column headings, then successive rows of data.

Table III.IV.I. Fields within CSV plankton datafiles.

Field	Mandatory	Description
Record	Yes	An incremental counter, reset to 1 at beginning of each sample
SampleID	Yes	Described below
Species	Yes	Three letter code identifying organism
Dev	Yes	Single letter code describing the development state of organism
Stage	No	Number or letter showing development stage for eggs only
Preservative	Yes	Preservative method used on object
Identification	Yes	Identification method used on object
EpuConv	No	Conversion factor for eyepiece graticules to millimetres
Len_Epu	No	Length of organism in eye piece units
Wid_Epu	No	Width of organism in eye piece units
Len_mm	No	Length of organism in millimetres
Width_mm	No	Width of organism in millimetres
Area_mm2	No	Area of organism if measured in square millimetres
Weight_mg	No	Weight of organism if measured in milligrams
Count	Yes	The number of organisms of that type and size observed in the sub-sample
Raise	Yes	The fraction of the total sample analysed (sample volume/sub-sample volume) **including any un-staged eggs apportioned across the analysed fraction**
Total	Yes	The total number of organisms of that type and size in the sample (=Count*Raise)
Gear	Yes	A numeric code describing the gear used to collect the sample (see below)
Analyst	Yes	Initials of the analyst, enables track back to lab notebooks for codes which need clarifying or correcting
Notes	No	Any notes

Note that since the files are generated automatically from the Plankton input program there are a controlled range of options for many fields.

III.V. Codes automatically generated by the database input program

III.V.I. SampleID

In order to track data through the system each plankton sample analysed is given a unique sample identifier. Since construction of the SampleID is quite complex (and thus prone to errors) the Plankton data input program automatically generate this code based on information supplied at the start of the session (see description of these programs). Although the SampleID may appear overly complex it has proved to be extremely flexible accommodating samples from a wide variety of investigations. It can also be understood by breaking the code down into its constituent bits.

The sample identifier is an 11 digit numeric code constructed from the following bits:

Year (4 digits) + Ship (2 digits) + Cruise (2 digits) + Station (3 digits) + Depthband (2 digits) + Replicate (2 digits)

e.g., 200109050230001 is a sample collected in 2001 on 'Cirolana' cruise '5' station '23' by double-oblique haul with no replication (or sample 1 of replicated samples)

The depth-band is coded '00' for all double-oblique hauls and in integer increments for a multi-depth sample e.g., LHPR or MOCNESS

The replicate number can be incremented if more than one sample is collected on a particular station.

The currently used ship codes are shown in Table III.V.I.I.

Table III.V.I.I. Ship codes (additional codes will be assigned as ship names for cruises in PLACES are confirmed).

Ship	Numeric code
Gill (FV)	02
Oceanus (FV)	03
Clione (CEFAS)	07
Cirolana (CEFAS)	09
Corella (CEFAS)	10
Corystes (CEFAS)	11
Endeavor (CEFAS)	12
Sir Lancelot	15
Ernest Holt	20
Dana (Denmark)	21
Tridens I (Holland)	22
Tridens II (Holland)	23
Scotia (Scotland)	24
Edward Forbes	25
Tellina	30
INA-K	31
Ocean Crest	35
Platessa	40
Cuma	45
John Beardsworth	50
Philomena	55
Beach sampling	60
Celtic Voyager	65
Roagan	70
FV Resolute	71
Lough Foyle	75
Bernicia	80
Lough Beltra	85
Prince Madog II	98
Prince Madog I	99

These codes include ships used historically and currently at CEFAS. There is no special significance in the ordering or numbering.

III.V.II. Gear codes

Because of the wide variety of sampling gear used by the plankton lab, a multipart code has been designed to describe sampling equipment. The code is made up of 4 fields: Sampler (2 digits) + Casing (2 digits) + Nosecone aperture (3 digits) + Net mesh size in um (3 digits) BUT note handling of mm meshes in juvenile beam trawls.

Current codes in use are listed in the table below:

Table III.V.II.I. Construction of gear codes.

Sampler	Code	Casing	Code	Nosecone aperture diameter (cm)	Code	Mesh size	Code
53 cm Rocket HSTN (old design)	05	Encased	01	20	020	35 µm	035
53 cm Gulf VII HSTN	10	Naked	02	20	020	275 µm	275
76 cm Gulf VII HSTN	12			40	040	275 µm	275
Gulf III	13	Encased	01	20	020	500 µm	500
Fine mesh auxiliary net on HSTN (Pup)	15			10	010	64 µm	064
Bongo	16	Naked	02	60	060	330 µm	330
Hensen net	17			15	015	Etc	Etc
Ringnet (WP nets)	20			etc	Etc	BUT	
Opening closing net	22			etc	Etc	2 mm	002
Pump	25					6 mm	006
LHPR	30						
MIK net	50						
1.5 m beam	55						
2.0 m beam	57						

Thus "1201033270" represents a 76 cm Gulf VII HSTN which is encased and has a nosecone with a 33 cm diameter opening and is fitted with a 270 µm mesh net.

NOTE the shift in codes for mesh sizes above 1 mm. The coding system was developed for plankton nets but now needs to cover MIK nets and fine mesh beam trawls (mesh size codes may be upgraded to 4 digits in the future).

III.V.III. Species codes

At present CEFAS use a three letter code to identify organisms. This system is somewhat cumbersome as the list of available codes is not exhaustive for all organisms that are found in our samples. In addition, extra codes have been generated by various sub-sections of CEFAS often with different meanings i.e., the code ADA might represent different organisms in a fishing survey and plankton database. This is clearly not satisfactory and the plan is to gradually replace over-lapping codes with unique identifiers. However, there is currently no CEFAS wide system in place for ensuring that duplicate codes are not generated.

Alternative coding systems are available (MCS codes, NODC codes) but have their own disadvantages (for example two versions of MCS codes exist, a printed listing and an electronic database but the codes assigned to particular organisms appear to be different between the two versions). The solution presently being developed in the plankton laboratory is to retain the three letter codes for data input since analysts are familiar with them but include facilities within the ACCESS databases for converting between alternative coding systems. This facility will be especially useful where projects require data sharing between different organisations.

Codes for common species we are likely to find on PLACES are shown below (Table III.V.III.I). A full list of available codes can be found in the electronic file ZooplanktonSpeciesList.csv. This file will be automatically installed when the Database input program is installed. If you are unsure which code to use or think a new code is needed please contact C Fox, CEFAS. A new version of the database input program will be shipped to all participants in this event.

Table III.V.III.I. Three letter codes for common species.

Scientific name	Common English name	Code
Agonus cataphractus	Pogge	POG
Ammodytidae	Sandeels	SAX
Argentina sphyraena	Lesser silver smelt	LSS
Arnoglossus laterna	Scaldfish	SDF
Buglossidium luteum	Solenette	SOT
Callionymidae	Dragonets	DTX
Chirolophis ascanii	Yarrel's blenny	YBY
Clupea harengus	Herring	HER
Clupeidae		CLU
Cottidae	Bullheads and sculpins	CDY
Cyclopterus lumpus	Lumpsucker	LUM
Dicentrarchus labrax	Seabass	ESB
Diplecogaster bimaculata	2 spotted clingfish	TSC
Gadidae		GAD
Gadus morhua	Cod	COD
Gaidropsarus spp.	Rocklings	ROL
Glyptocephalus cynoglossus	Witch	WIT
Gobiesocidae	Clingfishes	CFX
Gobiidae	Gobies	GPA
Hippoglossoides platessoides	Long rough dab	PLA
Limanda limanda	Dab	DAB
Liparis spp.	Sea snails	LPS
Lumpenus lampretaeformis	Snake blenny	SBY
Melanogrammus aeglefinus	Haddock	HAD
Merlangius merlangus	Whiting	WHG
Merluccius merluccius	European hake	HKE
Microchirus variegatus	Thickback sole	TBS
Microstomus kitt	Lemon sole	LEM
Molva molva	Ling	LIN
Myoxocephalus scorpius	Bullrout	BRT
Pholis gunnellus	Butter fish	BTF
Phynorhombus norvegicus	Norwegian topknot	NKT
Platichthys flesus	Flounder	FLE
Pleuronectes platessa	Plaice	PLE
Pleuronectidae spp.	Right eyed flatfish	PNX
Pollachius pollachius	Pollack	POL
Sardina pilchardus	Pilchard	PIL
Scomber scombrus	Mackerel	MAC
Scopthalmus maximus	Turbot	TUR
Scorpaenidae	Scorpion fishes	SCO
Solea solea	Dover sole	SOL
Soleidae	Soles	SOX
Sprattus sprattus	Sprat	SPR
Taurulus bubalis	Sea scorpion	SSN
Trachinus draco	Greater weaver	WEG
Trachinus vipera	Lesser weaver	WEL
Trachurus trachurus	Scad	HOM
Triglidae	Gurnards	ROX
Trisopterus luscus	Bib	BIB
Trisopterus minutus	Poor cod	POD
Zeugopterus punctatus	Topknot	TKT
Unidentified spp.	See detailed notes on use of this code with eggs (Section II.III and note below)	ZZY

Note that there are two addition ZZ* codes available. ZZG is for use following genetic analysis only and codes for an object which was analysed by genetic probes but gave a null reaction, ZZZ is for unidentifiable objects and is normally only used for damaged material.

III.V.IV Development and stage codes

The following development codes are available; one must be assigned to every object identified

"A"	ADULT – object is mature adult but has not been sexed
"B"	BROKEN – the object is broken
"C"	COPEPODITE – copepodite stage, applies to copepods only
"C&A"	COPEPODITE & ADULT – object is either a copepodite or adult stage of a copepod
"E"	EGGS –an egg
"F"	FEMALE – an adult sexed as female
"F&E"	FEMALE WITH EGGS – an adult sexed as female bearing eggs
"G"	MEGALOPA –crabs and lobsters etc.
"J"	JUVENILE –used mainly for fish
"L"	LARVAE – used for various organisms, for fish also see P and Y
"M"	MALE – adult sexed as male
"N"	NAUPLII – copepod nauplius, also used for Balanus etc.
"P"	POSTYOLK-SAC – larval fish only
"S"	CYPRIS – mainly applied to bryozoa
"X"	MIXED – used where development stages not determined
"Z"	ZOEA – crabs etc.
"Y"	YOLK-SAC – fish larvae only

The following stage codes are used for fish eggs only

"1cell"
"2cell"
"4cell"
"8cell"
"16cell"
"32cell"
"64cell"
"1A"
"1B"
"1", "2" through "11"
"U" – unstaged

In PLACES we will only use stages 1A, 1B, 2, 3, 4, 5 and U.

III.VI. Database structure

The aim of a standardised structure for the databases is to allow rapid construction, facilitate error checking and allow the sharing of queries for data extraction between databases. This considerably reduces the time required for database development and helps future-proof the data by providing a common organisation across datasets.

The system we currently use is Microsoft ACCESS. This is a relational database where data are stored in a number of tables. These are linked through fields common to each table via "relationships". "Queries" are used to extract data from the database.

WARNING: THE STRUCTURE OF THE DATABASES SHOULD ONLY BE CHANGED IF YOU KNOW WHAT YOU ARE DOING! EVEN SMALL CHANGES TO FIELD NAMES, CHARACTERISTICS OR RELATIONSHIPS CAN PREVENT QUERIES AND DATABASE PROCESSING PROGRAMS FROM OPERATING. FOR THIS REASON PERMISSION TO MODIFY DATABASES IS RESTRICTED AND DATABASES ARE BACKED UP REGULARLY.

BECAUSE MICROSOFT ACCESS IS A LIVE DATABASE SYSTEM, ANY CHANGES YOU IMPLEMENT ARE IMMEDIATELY REFLECTED IN THE UNDERLYING DATABASE. IN EFFECT THIS MEANS THAT ANY CHANGES ARE IMMEDIATELY SAVED TO THE DATABASE AND THERE IS NO SEPARATE SAVEFILE OPTION AS IN WORD OR EXCEL. ALTHOUGH THIS FEATURE MAKES THE DATABASE EXTREMELY RESPONSIVE IT IS ALSO QUITE DANGEROUS IN THAT CHANGES CAN INADVERTANTLY BE MADE AND YOU WILL PROBABLY RECEIVE NO WARNING OF POSSIBLE PROBLEMS THAT MIGHT ARISE.

III.VI.I. Underlying database structure

The underlying common structure of the databases is shown below.

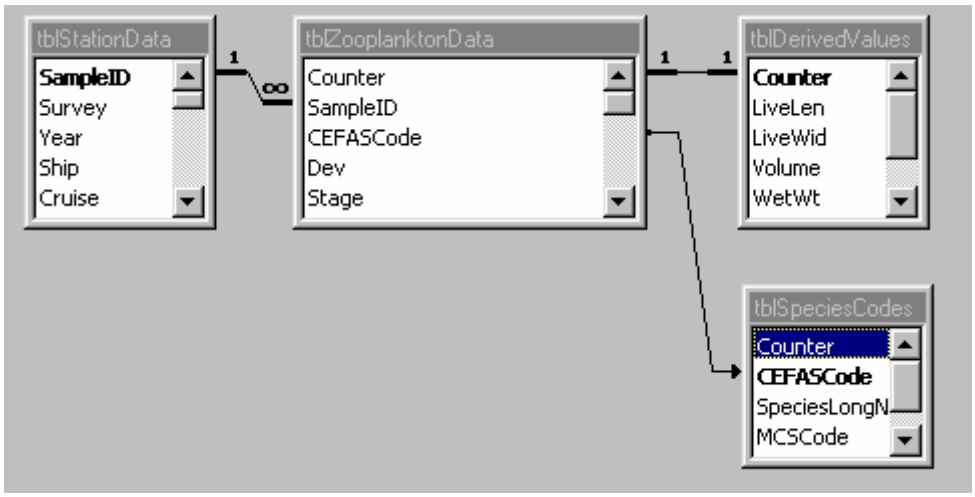

Figure III.VI.I.I Plankton lab database structure.

All tables begin with the letters “tbl” and each table is described below:

The “tblStationData” typically contains details about sampling location, time, date etc. It is compiled by the database manager based on information supplied through the cruise logbooks.

Field Name	Data Type	Description
SampleID	Number	SampleID= Year(4)+Ship(2)+Cruise(2)+Station(3)+DepthBand(2)+Replicate(2)
Survey	Text	Survey type - beach or plankton
Year	Number	Year YYYY
Ship	Number	Ship code
Cruise	Number	Cruise number
Stn	Number	Station number
SoundDepth	Number	Depth in metres below ship hull
TotalDepth	Number	Total depth = Sound depth + ? m
SampledDepth	Number	Max depth sampled in metres (from FSI-CTD) - This field is mandatory otherwise calcualtion of zooplankton per m2 will fail
TowLen	Number	In minutes for plankton surveys, in metres for beach
MainIntCount	Number	Count from main internal flowmeter
PupIntCount	Number	Count from pup internal flowmeter
ExtCount	Number	Count from external flowmeter
MainVol	Number	Main net volume filtered in m^3: Samples collected on Prince Madog and Resolute - Calibration for gear 1202040270 equipped with
PupVol	Number	Pup net volume filtered in m^3: Samples collected on Prince Madog and Resolute - Calibration for gear 1502005080 equipped with
Depthband	Number	Depthband
Replicate	Number	Replicate
Site	Text	Name of beach, or ICES area for plankton surveys
Lat	Number	Position of tow: decimal latitude (midpoint for plankton surveys)
Long	Number	Position of tow: decimal longitiude (midpoint for plankton surveys)
Date	Date/Time	Date sample collected
Time	Date/Time	Time gear in water at start of tow GMT
SurTemp	Number	Surface water temperature °C recorded for beach sampling
IntTemp	Number	Integrated water column temperature °C from depth averaged FSI-CTD record
Notes	Text	

Figure III.VI.I.II. Plankton lab database Station data table structure.

NOTE that there is no “Gearcode” field in this table. This is because several gear types may be deployed on a single sampling event e.g., the Gulf VII sampler may carry a main net and an auxiliary fine-mesh pup net. Gearcodes are therefore stored in the ZooplanktonData table.

The table “tblZooplanktonData” holds the actual plankton analysis results. It is compiled by the database manager from the relevant CSV files supplied by the plankton analysts. The fields are described in Section III.VI.

Field Name	Data Type	Description
Counter	AutoNumber	Record counter
SampleID	Number	SampleID = Year(4)+Shipcode(2)+Cruise(2)+Station(3)+DepthBand(2)+Replicate(2)
CEFASCode	Text	Three letter organism identification code used in CEFAS
Dev	Text	Development code
Stage	Text	Egg Stage code (fish eggs only)
Preserve	Text	Preservative, 70%IMS, 4%Formalin, 100%IMS, Bouins
EpuCon	Number	Eye piece units conversion factor
LengthEpu	Number	Length in eye piece units
WidthEpu	Number	Width in eye piece units
Length	Number	Length (mm)
Width	Number	Width (mm)
Area	Number	Area (mm2)
Weight	Number	Weight (mg)
Count	Number	Counts of observation
Raise	Number	Raising factor (1/fraction of whole sample analysed)
Total	Number	Total counts in record =Count*Raise
Gearcode	Number	Gear code
Analyst	Text	Analyst initials
Notes	Text	Any notes on record

Figure III.VI.I.III. Plankton lab database Zooplankton data table structure.

NOTE THAT CHANGING FIELDS IN THIS TABLE WILL PROBABLY LEAD TO SERIOUS PROBLEMS IN THE DATABASE

The tables “tblDerivedValues” and “tblSpeciesCodes” are produced by the database manager running the Plankton database processing program on the ACCESS database.

Field Name	Data Type	Description
Counter	AutoNumber	Record counter
LiveLen	Number	Estimated live length from Len_mm * shrinkage
LiveWid	Number	Estimated live width from Wid_mm * Shrinkage
Volume	Number	Volume computed from length and width data using rotational formulae
WetWt	Number	Wet weight in g derived from volume * 1.025
DryWt	Number	Dry weight in g derived from wet weight * percentage
Carbon	Number	Carbon content derived from dry weight * percentage carbon

Figure III.VI.I.IV. Plankton database Derived values table structure.

The derived values table holds information computed from data in the zooplankton data table. It is most relevant where measurements have been taken since the parameters estimated are volume, wet weight, dry weight and carbon content.

Field Name	Data Type	Description
Counter	AutoNumber	Record counter
CEFASCode	Text	Three letter organism identifier used in CEFAS
SpeciesLongName	Text	Scientific name
MCSCode	Text	MCS Species Directory code
CommonName	Text	Common English name

Figure III.VI.I.V. Plankton database Species codes table structure.

The information in the species codes table allows ready conversion between different organism coding conventions.

IF YOU DISCOVER AN INCORRECT CODE, PLEASE DO NOT ALTER IT. INFORM THE PLANKTON DATABASE MANAGER OR PROGRAMMER, CORRECTIONS REQUIRE CHANGES TO PROCESSING PROGRAMS AND THAT THESE PROGRAMS ARE RUN ON ALL THE PLANKTON DATABASES. THE RATIONALE IN CONTROLLING THE GENERATION OF THESE TABLES VIA EXTERNAL CODE IS THAT IT HELPS ENSURE CONSISTENCY ACROSS DATABASES. THE CHANGES ONLY NEED TO BE IMPLEMENTED ONCE IN THE PROGRAM CODE. RUNNING THE CODE ACROSS ALL OF THE DATABASES IS RELATIVELY QUICK COMPARED WITH OPENING EACH DATABASE AND MAKING CHANGES MANUALLY.

The structure shown above is the basic one. Some databases contain additional tables for non-standard work e.g., tables of stomach content data, tables for beach sampling results. These will vary with the project but will still be linked to the station data table via a “SampleID”.

III.VI.II. Getting data out of a database

There are two ways to do this. You may use the standard queries included in the database and described below. However you may need to modify the queries to get data in the exact form you require. Since only the Plankton database manager, programmer and Plankton lab manager have permission to modify the databases you will need to copy the database onto your local drive. You can then play with the copied version without any danger of affecting the copy stored on the network drive.

IF YOU THINK A MODIFICATION NEEDS TO BE MADE TO THE ORIGINAL DATABASE, TELL THE DATABASE MANAGER OR PROGRAMMER.

III.VI.III. Queries

Queries are what Microsoft ACCESS uses to extract data. They are based on Sequential Query Language (SQL). Luckily ACCESS includes a nice graphic interface that allows the user to produce queries without having to understand SQL. The plankton databases contain a number of pre-written queries for undertaking common tasks. All queries begin with the three letters “qry”. If you need help running, modifying or designing new queries to extract data consult the database manager or programmer.

Queries are accessed by the tab in the database as shown below:

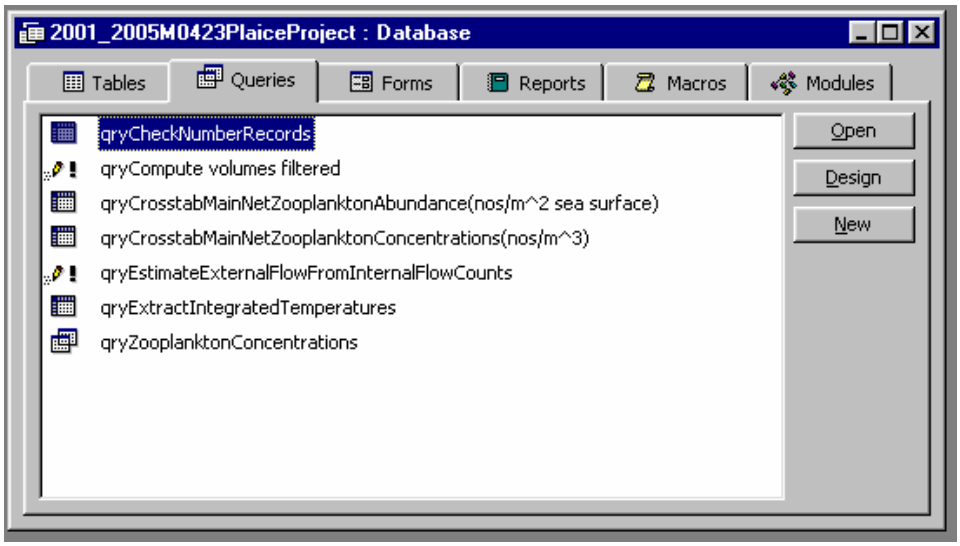

Figure III.VI.III.I. Plankton database queries.

To run a query simply highlight it and press "Open". Alternatively, to modify the query or check its parameters press "Design".

Two of the essential queries included in all the plankton databases are now described.

Most queries which extract data from the plankton databases are actually based upon an underlying query which is run in the background. This query is called "qryZooplanktonConcentrations".

It includes fields called Concentration(MAIN) or Concentration(PUP) or Concentration(PUMP) etc. up to the number of gear-types deployed in the study. The formula in this field computes the concentration for that observation per m^3. NOTE THAT THIS IS NOT THE CONCENTRATION OF THAT ORGANISM IN THE SAMPLE, THAT CALCUALTION IS UNDERTAKEN BY THE NEXT QUERY.

Running on top of the query just described is a "Crosstabulation" query, usually called "qryCrosstabMainNetZooplanktonConcentrations(Nos/m^3)" or similar. This query groups the concentration data from the query described above by station (or sample) and by organism identifier. It is normally this type of query which you will want to run to extract data from the database. An example of output from this type of query is shown below:

Microsoft Access - [qryCrosstabMainNetZooplanktonConcentrations(nos/m^3) : Crosstab Query]

File Edit View Insert Format Records Tools Window Help

SampleID	Year	Ship	Cruise	Stn	Depthband	Replicate	Lat	Long	Date	Site	BIB L	BRT L
200171010010001	2001	71	1	1	0	1	53.368	-4.101	03/04/01	35e5/4s		
200171010020001	2001	71	1	2	0	1	53.443	-4.359	03/04/01	35e5/3		0.0141295372
200171010030001	2001	71	1	3	0	1	53.450	-4.142	03/04/01	35e5/4n		
200171010040001	2001	71	1	4	0	1	53.459	-3.884	03/04/01	35e6/1n		
200171010050001	2001	71	1	5	0	1	53.376	-3.860	03/04/01	35e6/1s	0.0739244555	
200171010060001	2001	71	1	6	0	1	53.368	-3.626	03/04/01	35e6/2s		
200171010070001	2001	71	1	7	0	1	53.459	-3.617	03/04/01	35e6/2n		
200171010080001	2001	71	1	8	0	1	53.459	-3.367	03/04/01	35e6/3n		
200171010090001	2001	71	1	9	0	1	53.567	-3.368	03/04/01	36e6/7s		

Figure III.VIII.II. Example of output from a crosstab query giving concentrations of organisms per m^3 by station.

Statistical analysis of the data is not included in the database. We feel it is preferable to use the ACCESS database purely to hold information. Once extracted, the data can be exported on to statistical packages in a variety of formats from the query.