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Manual for egg survey for winter spawning fish in the North Sea

Version 2

The Working Group on North Sea Cod and Plaice Egg Surveys 2 (WGEGGS2)

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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1 Introduction

Ichthyoplankton surveys, specifically targeting stage 1 (i.e. recently spawned) eggs provide invaluable data on the distribution of spawning. In the North Sea two such surveys were undertaken to examine the winter/early spring spawning of various fish species in 2004 and 2009 (Fox et al. 2008; Damme et al. 2009; Munk et al. 2009; Höffle et al. 2017). The target species for these surveys were cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*). The survey in 2004 covered the North Sea a number of times, however, due to financial and vessel constraints the 2009 survey provided only a single coverage of the entire North Sea (ICES 2005, 2010).

The results of these surveys indicated that spawning grounds were shifting and the density of eggs varied, suggesting a shift in the demographics of spawning in the North Sea. It was decided that regular surveys on a 5 year basis be undertaken to 'map' the spawning grounds of cod and plaice (ICES 2008). The intention was to determine if changes in spawning locations were occurring or whether the relative contribution from substocks was changing. It was envisioned that these surveys would be 'dedicated' egg sampling surveys with a primary goal of determining egg abundances and distributions. In 2004 and 2009 a number of these surveys were, combined with ongoing surveys e.g. the 1st Quarter IBTS and the ICES Herring larvae survey (IHLS) (see ICES 2009). While dedicated surveys are less likely to be available for research on the distribution of eggs and larvae such a combination of surveys is a feasible way to cover the important task of distribution of cod and plaice eggs and spawning locations.

As a consequence, the ICES Working Group (WGEGGS) looked at the feasibility of obtaining egg samples from standard surveys. The surveys need to cover the whole North Sea at an appropriate time of year to capture the spawning locations of cod and plaice and other fish species. The only survey to cover the whole North Sea at the appropriate time (in the window January to early April) is the 1st quarter IBTS-MIK. During 2009 the egg surveys were undertaken during the Norwegian and Danish 1st quarter IBTS. In both cases the surveys were accomplished without compromising the time schedule of the routine bottom-trawl sampling or the MIK sampling for herring larvae, however, the deployment of an extra piece of equipment e.g. Gulf VII high speed sampler (Nash et al. 1998) did add a significant amount of time to the survey.

There was a need to move to a North Sea egg survey which could be repeated periodically in the future to map and track the winter spawning locations and intensity of fish species. Using the IBTS Q1 surveys as a platform would be ideal. As extra operations are often not possible on the existing IBTS due to time constraint, Bongo or Gulf nets were inappropriate. The midwater ring net (MIK) has a too large mesh size (1.6mm) to accurately sample fish eggs.

During the ICES WGEGGS meeting in Sète (October 2011) a discussion on possible sampling regimes for eggs resulted in a new ichthyoplankton net being devised, to complement the MIK, and collect fish eggs. The result was the addition of a small plankton net on the side of the MIK to collect egg samples with no additional hauling operation. The ring was dimensioned so as to filter about 20m^3 of water during an average MIK haul.

2 Methodology for fish egg sampling and identifying and staging of the eggs and larvae

2.1 Samplers

The standard sampling gear for the cod and plaice eggs survey is the MIKeyM attachment to the MIK net. The MIK net is used for night time sampling of herring larvae during the Q1 IBTS.

2.1.1 The MIKeyM net attachment to the MIK net

The MIKeyM net is a small mid water ring net and consists of a 20 cm stainless steel ring, which is attached to the outside of the 2 m MIK ring, with a cylinder-conical net of black 335 μm mesh. Distance between the large and the small ring should be approximately 10 cm. The ring has fixtures to place a flowmeter either slightly away from or in the center of the net-opening (see below). Either 1 or 2 MIKeyM nets can be attached to the MIK in various configurations according to figure 1.

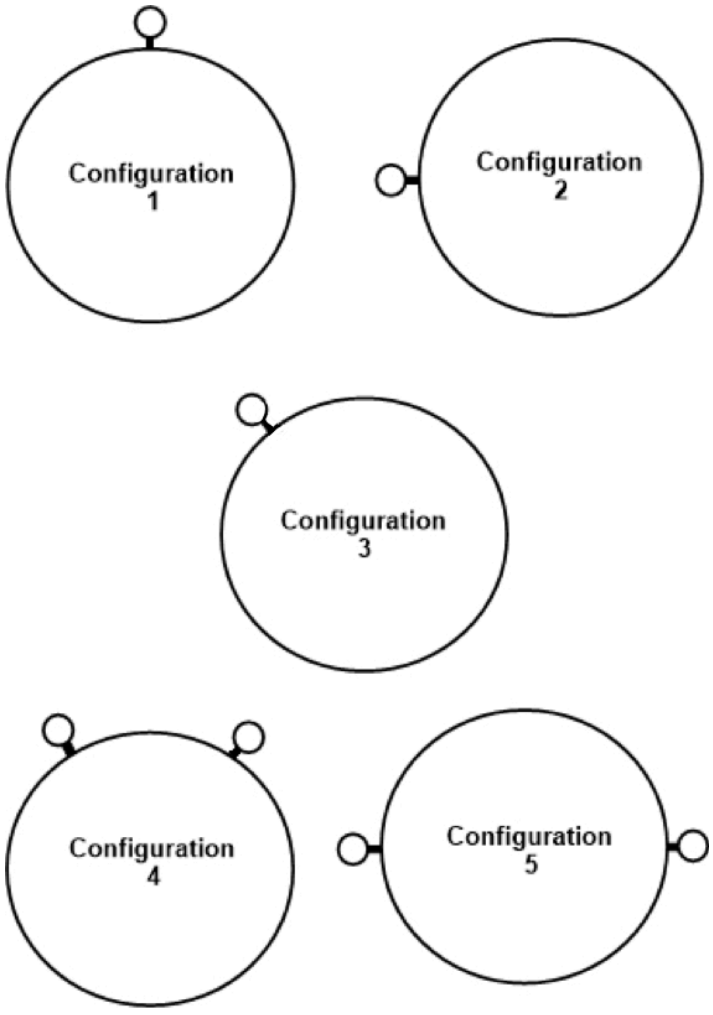

Figure 2.1.1.1 Placement of the accessory rings (20 cm) on the standard 2 m Ring Trawl (MIK). Configurations 2 and 3 can have the ring on either side. Configurations 4 and 5 give the options for two nets or alternatively one ring only has a flow meter for external flow and the option of estimating net efficiency or clogging. Configuration 1 utilized by IFREMER, France (an additional bridle attached to the top of the ring), configuration 2 utilized by DTU-Aqua, Denmark, Configuration 4 was utilized by Wageningen Marine Research, The Netherlands, but now uses Configuration 3, and Configuration 5 utilized by IMR, Norway.

The nets are cylindro-conical (figure 2.1.1.2) with a porosity of about 50% with a 20 cm diameter inlet. Due to the relatively high towing speeds of the MIK (up to 3 knots) and the often poor weather conditions and severe sea states during the early part of the year, which could induce short burst speeds for the nets in excess of 6 knots there is the addition of a 0.5-1 m tube in front of a 75 cm conical section. This gives an inlet to open area (i.e. the sum of the area of all pores of the net) ratio in excess of 10 which should be sufficient for most extreme sampling conditions.

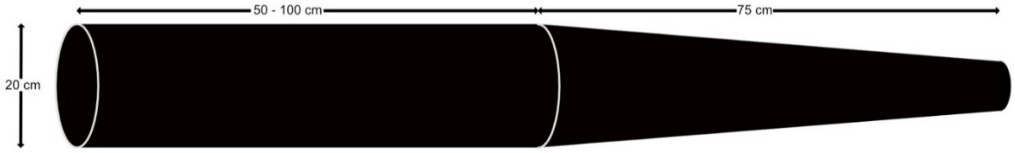

Figure 2.1.1.2: Sketch of the cylindro-conical black net for the MIKeyM net.

2.1.2 The MIKeyM net attachment description in the ICES Egg and Larvae database

In the ICES database the MIKeyM net attachment is called a midwater ring net. The gear description includes the position of the ring net on the MIK (MRN2) (Table 2.1.2.1).

Table 2.1.2.1. Gear descriptions for the MIKeyM surveys (for configurations see Fig. 2.1.1.1).

Gear (Sampler type (SMTYP))	Description
MRN0.2-1	Midwater ring net - 0.2m diameter – configuration 1 (one net on top of the MRN2)
MRN0.2-2	Midwater ring net - 0.2m diameter – configuration 2 (one net on the side of the MRN2)
MRN0.2-3	Midwater ring net - 0.2m diameter – configuration 3 (one net at an angle of approximately 45 or 315 degrees on the MRN2)
MRN0.2-4	Midwater ring net - 0.2m diameter – configuration 4 (two nets at an angle of approximately 45 and 315 degrees on the MRN2)
MRN0.2-5	Midwater ring net - 0.2m diameter – configuration 5 (two nets on either side of the MRN2)

2.2 At sea

2.2.1 Deployment of samplers

The plankton samplers should be deployed following the standard operational procedures given in the Manual for the Midwater Ring Net sampling during IBTS Q1 (ICES 2017). Briefly, sampling is done in a double oblique tow, defined here as, from the surface to within 5 metres of lower edge of the MIK ring from the bottom and return to the surface. Towing speed is 3 knots. At shallow stations, multiple double-oblique tow profiles 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 maximum. A minimum sampler deployment time of 10 minutes is recommended. A flowmeter should be placed either slightly away from middle or in the middle of the net opening. Due to the relative diameters of the flow meters (with their impeller) and the MIKeyM rings a centralposition is preferable because care should be taken to ensure that the functioning of the flowmeter is not obstructed. A mechanical flowmeter is read before and after each tow

and the readings noted to calculate the filtered volume of water. Electronic flowmeters revolutions are automatically saved and stored after each haul.

2.2.2 Calibration of the flowmeter

All flowmeters used in the survey should either be calibrated at least twice per survey, or under controlled conditions at land prior to each survey, to give revolutions per metre. The method during the survey is to tow the MIK with the MIKeyM net attached (without any codends) at a depth of at least 10 metre for a known distance and to make at least two measurements in opposite directions. The distance is measured between the points where the flowmeter goes into and comes out of the water. Calibration on land can be done e.g. in a tank towing the flowmeter over a defined distance at the towing speed of the survey.

If a flow meter is changed then a new calibration is necessary.

2.2.3 The standard procedure for recovery of the plankton sample

Gently wash down the net with 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 codend.

Remove the codend and place in the bucket for transfer into the wet lab on the ship. If genetic analyses are to be carried out on the eggs, this bucket **must** be kept free from formaldehyde and should be clearly labelled.

Make sure the net is clean, using more than one codend and repeating the above steps if necessary.

Check the plankton net for tears, and replace if necessary.

Make sure that a clean codend is left on the sampler ready for the next sampling station.

Move the bucket containing the codends and plankton samples into the ship's laboratory and proceed with sample analyses or preservation.

2.2.4 Fixing plankton samples

If genetic analysis is to be carried out on the fish eggs, it is required that the eggs are immediately sorted fresh from the samples and preserved in ethanol. To prevent quick deterioration of the eggs, larvae and other plankters, the sorting must be carried out on a bed of crushed ice, to keep temperatures cool. To speed up the sorting, all fish eggs without oil-globule can be washed through a stack of sieves (335 μm, 1.1 and 1.7 mm) to separate cod-like eggs from other fish eggs. The cod-like eggs or sub-sample of 50 of those will be staged and fixed separately in 96% pure ethanol for later molecular identification (for staging of fish eggs see chapter 2.3.3 “Egg staging criteria”). The remainder of the eggs and larvae will be fixed and preserved. See the following flow chart (Fig. 2.2.4.1), which illustrates the different steps of sample treatment onboard.

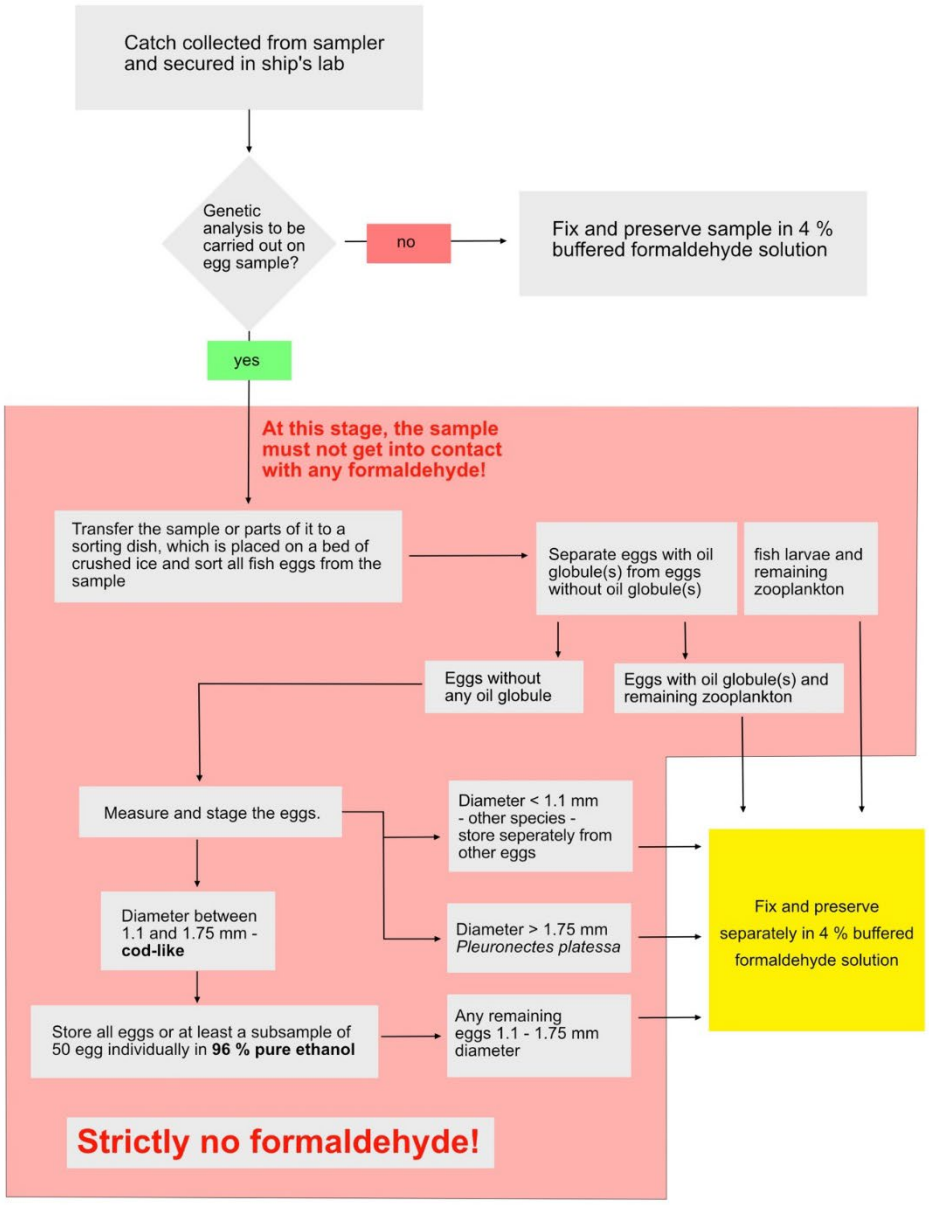

Figure 2.2.4.1: Flow chart showing the different steps for on board sample work up.

If genetic analysis will be carried out by France, the collected zooplankton will be fixed in a 1% formalin solution and transferred within the next two weeks in the conservation fluid following Lelièvre et al. (2010) to await identification, staging and counting.

Recipe for preservation fluid used by France (5 litres)

France uses the following recipe for preserving the samples:

A stock solution is made from the following ingredients and mixing procedure

- Ascorbic acid 2 g
- disodic EDTA 20 g
- BHA (buthylhydroxyanisol) 8 g
- Monopropylene glycol 1 l
- commercial formaldehyde solution (36 %) 2 l
- distilled or deionised water to make up 5 l
- buffer at pH 7 using sodium glycerophosphate (about 200 g)

Disolve BHA in 0.5 litres propylene glycol. Dissolve separately EDTA in 0.5 litres of distilled water, add ascorbic acid and buffer at pH 7 using sodium glycerophosphate (about 90 g). Into a recipient of at least 5 litres, pour the formaldehyde solution and, while mixing with a magnetic stirer, bring to pH 7 using the remainder of sodium glycerophosphate (about 110 g). Add the BHA solution, the remaining propylene glycol and fill up to 5 litres with the distilled water. Mix 1/2 hour.

Finally, the samples are fixed in sea water using 6 ml of this solution for every 100 ml sample volume. It is important to note that the resulting concentration of formalin in the sample is less than 1%.

Recipe for 4% preservation fluid (10 litres)

If no genetic analyses are carried out, the samples should be fixed and preserved in a 4% solution of buffered (pH 7– 8) formaldehyde. Also the remainder of the sample, after picking out eggs for genetic analyses should be fixed in this formaldehyde solution. The recipe for this is:

1. 1100 ml 37% formaldehyde
2. 8900 ml distilled (or fresh) water
3. 420 gr sodium acetate trihydrate

Add all ingredients together and stir well.

2.3 Working up samples at sea or in the laboratory

2.3.1 Transfer of fixed material.

Samples preserved in the solution used by France will remain in the same solution for analyses.

It is recommended that the material in the 4% preservation solution is transferred to the formaldehyde-free observation fluid (Steedman, 1976) between 24 h and 3 weeks from sampling for analyses. This solution will also act as a preservative on thoroughly formalin-fixed material.

Recipe for observation fluid (30 litres)

The following recipe according to Steedman (1976) will make 30 litres of observation fluid for use as medium for analysis and short-term storage of plankton samples in the laboratory.

1. Mix together 150 mL Propylene phenoxetol and 1350 mL Propane-1,2-diol. This must be done vigorously as the two chemicals are not very miscible.
2. Fill up that mixture to 30 litres with deionised water. Mix thoroughly again.

2.3.2 Sorting the samples for fish eggs and larvae

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

Sub - sampling protocol.

In the unlikely event, where large numbers of eggs and larvae occur in plankton samples, it becomes impractical to sort the total sample. The recommended method for sub-sampling is by using a plankton splitter, e.g. Motoda box (Motoda 1959) or a Folsom splitter (Smith and Richardson 1977, Griffith et al. 1984). In this way, samples can

be sub-divided repeatedly to achieve the optimum sampling level. It is recommended that at least 100 eggs are present in the sub-sample.

All fish larvae should be identified.

2.3.3 Identification and staging of eggs from plankton samples

Eggs will be identified on the basis of the presence/absence of oil globules, size of the egg and in some cases the characteristic appearance as described in (Russell 1976; Munk and Nielsen 2005).

All eggs will be identified, measured and classified into one of six developmental stages (IA, IB, II, III, IV, and V) following the development criteria described hereafter for cod (Thompson and Riley 1981) and plaice (Ryland and Nichols 1975) and summarised in Geffen and Nash (2012). Staged cod-like eggs that are determined for genetic analysis must be separately (by station and stage) stored in 96 % pure ethanol and clearly labeled.

Egg stage criteria

Stage IA

Primary characteristics: From fertilization until cleavage produces a cell bundle in which the individual cells are not visible.

Secondary characteristics: There are no signs of a thickening of cells around the edge of the cell bundle. NB. In preserved eggs, the edge of the cell bundle can sometimes fold over giving the appearance of a 'signet ring' seen in a stage Ib.

Stage IB

Primary characteristics: Formation of the blastodisc, visible as a 'signet ring' and subsequent thickening a one pole.

Secondary characteristics: The cell bundle has thickened around the edge giving a distinct ring appearance. Cells in the centre of the ring form a progressively thinner layer and eventually disappear. NB. At the end of this stage, the ring can become very indistinct as it spreads towards the circumference of the egg.

Stage II

Primary characteristics: From the first sign of the primitive streak, which begins as a cleft in the cell bundle, until closure of the blastopore. By the end of this stage, the embryo is half way round the circumference of the egg. However, the tail still tapers to end flattened against the yolk, in this stage.

Secondary characteristics: Early in this stage, the primitive streak can be difficult to see, only appearing as a faint line in the surface of the cell bundle. Late in this stage, the head is still narrow and the eyes are not well formed.

Stage III

Primary characteristics: Growth of the embryo from half way to three-quarters of the way around the circumference of the egg. The end of the tail has thickened, becoming bulbous in appearance, and has lifted clear of the yolk sac.

Secondary characteristics: Widening of the head and development of the eyes. Pigment spots develop on the embryo, usually close to the posterior end.

Stage IV

Primary characteristics: Growth of the embryo from three-quarters to the full circumference of the egg.

Secondary characteristics: Eyes continue to develop and the lenses become visible. Development of the marginal fin and the tail begins to separate from the yolk. Pigmentation of the body increases.

Stage V

Primary characteristics: Growth of the tail past the head. At the end of this stage the larva hatches

Secondary characteristics: Pigmentation develops in the eye.

Identification of fish larvae in the plankton samples

All fish larvae should be identified, if possible to the species, and measured to the nearest 0.1 mm SL using calibrated eyepiece graticules in the microscopes. Of particular interest are herring (*Clupea harengus*), cod-like (Gadidae), sandeel (Ammodytidae), and flatfish (Pleuronectiformes) larvae. Descriptions of clupeid, gadid, ammodytid and pleuronectiform larvae are given in the Annex 4.

2.3.4 Conservation of the samples

After analyses, eggs and larvae can be stored on observation fluid, or they can be transferred to conservation fluid. The remaining plankton of the sorted sample can be discarded.

Recipe for conservation fluid (10 litres)

1. Propylene phenoxetol 50 mL
2. Propane-1,2-diol 500 mL
3. 40% buffered* formaldehyde 500 mL or 30% buffered* formaldehyde 700 mL
4. Mix together and stir well.
5. Add distilled water or de-ionised water to make up 10 litres
6. Stir well

* buffering with either 52 g Borax or Sodium Glycerophosphate per litre to obtain a pH of around 8 or 7, respectively (Steedman 1974).

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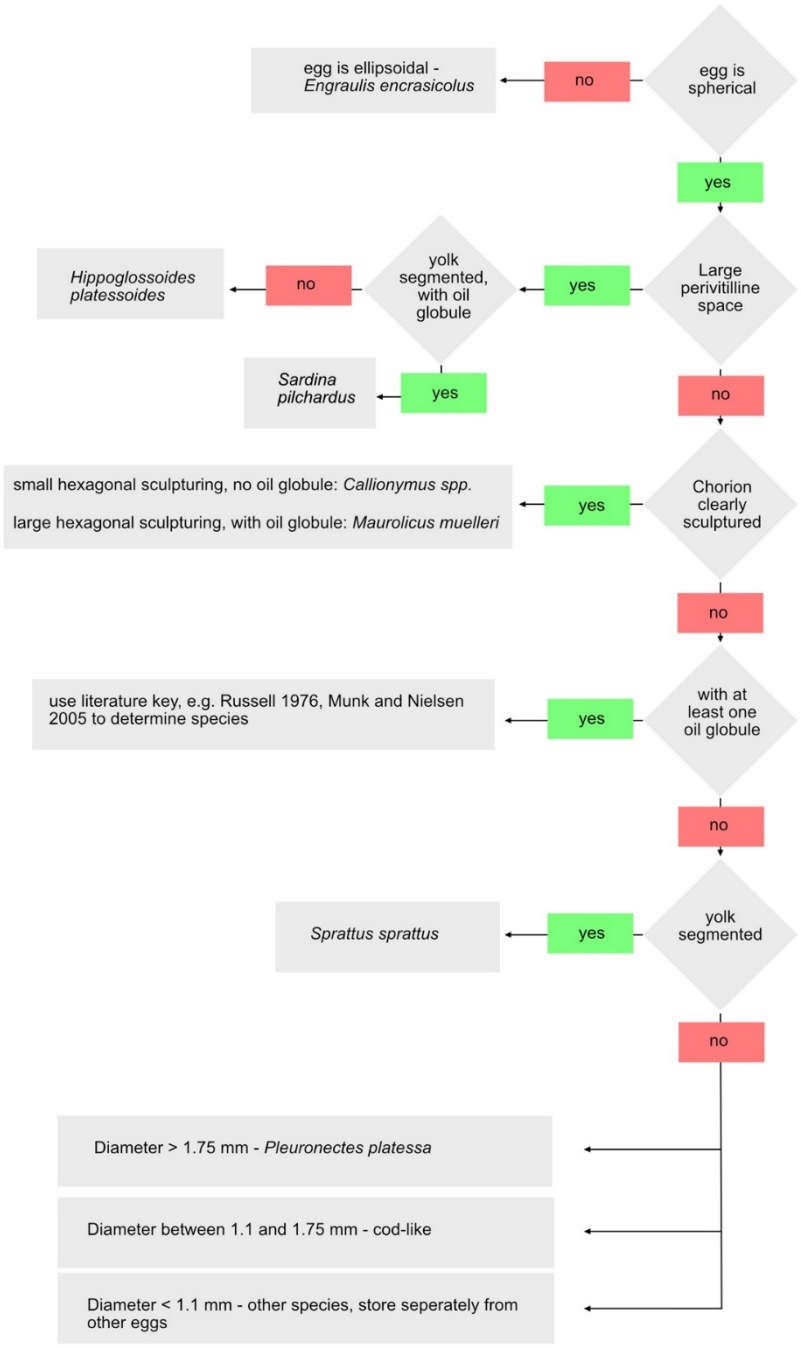
Annex 1: Flow chart for egg identification



Annex 2: Key to identification of pelagic eggs (from Russell 1976)

Pelagic spherical eggs		
	Egg diameter (mm)	
Large eggs with large perivitelline spaces		
Sardina pilchardus	1.30 - 1.09	With oil globule and segmented yolk
Hippoglossoides platessoides	1.38 - 2.64	No oil globule and unsegmented yolk
Small eggs with sculptured membrane		
Callionymus spp.	0.7 - 1.0	No oil globule
Eggs with several oil globules and yolk with peripheral segmentation		
Solea solea	1.00 - 1.60	Oil globules small and clustered
Buglosidium luteum	0.64 - 0.94	12-15 oil globules scattered
Pegusa lascaris	1.28 - 1.38	50 or more scattered oil globules
Microchirus variegates	1.28 - 1.42	50 or more scattered oil globules
Eggs with several oil globules and unsegmented yolk		
Trachinus vipera	1.00 -1.37	6-30 oil globules scattered
Eggs with one oil globule and segmented yolk		
Argentina sphyraena	1.70 - 1.85	Yolk wholly segmented
Trachurus trachurus	0.81 - 1.04	Yolk wholly segmented
Mullus surmuletus	0.81 - 0.91	Yolk with peripheral segmentation
Eggs with one oil globule and unsegmented yolk		
	Egg diameter (mm)	Oil globule diameter (mm)
Triglidae	1.10 -1.70	0.17 - 0.33
Zeus faber	1.96 - 2.00	0.36 - 0.40
Dicentrarchus labrax	1.20 - 1.51	0.36 - 0.46
Scophthalmus rhombus	1.24 - 1.50	0.16 - 0.25
Scomber scombrus	1.00 - 1.38	0.28 - 0.35
Lepidorhombus whiffiagonis	1.07 - 1.22	0.25 - 0.30
Scopthalmus maximus	0.91 - 1.20	0.15 - 0.22
Molva molva	0.97 - 1.13	0.28 - 0.31
Trachinus draco	0.96 - 1.11	0.19 - 0.23
Zeugopterus punctatus	0.92 - 1.07	0.17 - 0.20
Merluccius merluccius	0.94 - 1.03	0.25 - 0.28
Capros aper	0.90 - 1.01	0.15 - 0.17
Phrynorhombus regius	0.90 - 0.99	0.16 - 0.18
Serranus cabrilla	0.90 - 0.97	0.14 - 0.15
Phrynorhombus norvegicus	0.72 - 0.92	0.09 - 0.16
Raniceps raninus	0.75 - 0.91	0.14 - 0.19
Arnoglossus thori	0.72 - 0.74	0.12
Rocklings	0.66 - 0.98	0.14 - 0.19

Arnoglossus laterna	0.60 - 0.76	0.11 - 0.15
Eggs without oil globules		
With segmented yolk		
Sprattus sprattus	0.80 - 1.23	
With unsegmented yolk		
Pleuronected platessa	1.66 - 2.17	
Boreogadus saida	1.53 - 1.90	
Gadus morhua	1.16 - 1.89	
Melanogrammus aeglefinus	1.20 -1.70	
Microstomus kitt	1.13 - 1.45	
Merlangius merlangus	0.97 - 1.32	
Micromesistius poutassou	1.04 - 1.28	
Glyptocephalus cynoglossus	1.07 - 1.25	
Pollachius pollachius	1.10 - 1.22	
Pollachius virens	1.03 - 1.22	
Trisopterus luscus	0.90 - 1.23	
Trisopterus esmarkii	1.00 - 1.19	
Platichthys flesus	0.80 - 1.13	
Trisopterus minutus	0.95 - 1.03	
Ctenolabrus rupestris	0.72 - 1.01	
Limanda limanda	0.66 - 0.92	
Pelagic ellipsoidal eggs		
Engraulis encrasicolus	1.2 - 1.9 x 0.5 - 1.2	Segmented yolk

Annex 3: Identification of various taxonomic groups of fish larvae (Clupeoid, Gadoids, Ammodytidae, and Pleuronectiformes)

A compilation of the distinctive features of clupeoid larvae was done during the recend ICES workshop WKIDFL. The results are given in the following paragraphs and are copied from the respective workshop report (ICES 2011).

Clupeoids

General characteristics:

- Tubular, slender shape of body
- Long gut with anus near the tail end
- Number of myotomes in the trunk
- The body proportion changes during development thus the anus moves forward and the myotome count decreases with age
- The difference between clupeiods and sandeel is the position of the anus. In sandeel the anus is halfway the body, in clupeoids the guts is much longer and the anus is positioned close to the tail. Argentinids which also have a long gut tend to have a deeper body and different pigmentation than clupeids.

Primary characteristics of clupeoids (from Russell 1976)

Developement stage	Herring	Sprat	Pilchard/Sardine	Anchovy
Yolk sac	Yolk not segmented	Yolk segmented	Yolk segmented	Yolk segmented, oblong shape
10 mm				
No. myotomes in trunk	47	37	41-42	
10-20 mm				
No. myotomes in trunk	46-47	35-37	41-42	
Position pelvic fin	Not appaered yet	Appaers at 17.5-20 mm, 4-5 myotomes behind the pylorus	Appaer at 18-20 mm, level with the pylorus	
Dorsal fin				Rear edge of dorsal fin overlaps with the anal fin
20-40 mm				
No. myotomes in trunk	41-46	31-35	36-41	
Position pelvic fin	7-8 myotomes behind the pylorus	4-5 myotomes behind the pylorus	Level with the pylorus	
Length of tail from anus to base of caudal fin	Greater than 6 times in total length	Less than 6 times in total length		

Secondary characteristics:

Herring is always bigger at any developmental stage compared to the other species. Herring have pigmented eyes at hatching while other species do not gain pigment until later (5mm). Herring attain flexion stage later (17 mm) than other species so a larvae at 11-13 mm with flexion will not be herring (Munk & Nielsen 2005). The head of anchovy is bigger compared to the other species (not very useful).

Gadoids (Gadiformes – Merlucciidae, Gadidae and Lotidae

General characteristics:

• Typical fish like forms

• Anus opens sideways and may be difficult to see

• Head mostly rectangular

Gadoid larvae can be divided in two major groups:

A: Well developed pelvic fins in the early stages

Spiny pelvic fins:

- Rocklings comprising the genera *Ciliata, Gaidropsarus, Rhinonemus*, and *Raniceps*

Soft pelvic fins:

- Pelvic fins not reaching anus. Pigments more like big spots than bands. **Hake (*Merluccius merluccius*)**
- Pelvic fins reaching longer than anus. Two transversal pigment bands.
  - **Ling (*Molva molva*)** Pigment bands split laterally
  - **Torsk (*Brosme brosme*)** Pigment bands consistent. Rounded head.

B: Short pelvic fins do not appear until at least 10 mm

- With a medio-lateral streak (may be difficult to see in early stages):
- Melanophores in distinct rows
  - **Pollack (*Pollachius pollachius*):** double rows of melanophores along ventral and dorsal body contours
- Melanophores in patches
  - **Cod (*Gadus morhua*):** The two ventral spots may be bound together, especially in the later stages. Small melanophores near tip of tail that may be difficult to see, or missing.
  - **Saithe (*Pollachius virens*):** The two ventral spots never together and smaller than the dorsal. Medio-lateral streak appears at smaller size than in cod; first between the posterior pigment patches.
- Without a medio-lateral streak:
- Single row of melanophores along the ventral body contour pigment line
  - **Haddock (*Melanogrammus aeglefinus*)**: Ventral contour row, also pigmentation on abdomen and head.
- Single rows of melanophores along both, ventral and dorsal contours
  - **Whiting (*Merlangius merlangus*):** Dorsal line shorter than the ventral. Difference in length becoming less conspicuous in later stages. Haddock always stockier. 1st dorsal fin of whiting longer.
  - ***Trisopterus spp.*** Dorsal and ventral rows of almost equal length.

⇒ **Bib (*Trisopterus luscus*):** Dorsal and vertral melanophore rows reach only half-way towards the tail.
⇒ **Poor cod (*Trisopterus minutus*):** Dorsal and vertral melanophore rows reach the tail, large melanophores.
⇒ **Norway pout (*Trisopterus esmarki*):** Ventral melanophore row denser than the dorsal, gap in dorsal pigment line.

Ammodytidae larvae

While sandeel larvae share the same tubular and slender body shape with clupeid larvae, their gut is much shorter, reaching only to the midpoint of the body. For an untrained eye, however, they can be easily confused with clupeoid larvae. (see e.g. the description of slender fish larvae in Russell 1976). In the North Sea, 5 different species of sandeel occur: The two *Ammodytes* species *A. marinus* (Lesser or Raitt's sandeel) and *A. tobianus* (Common sandeel), two species of *Hyperoplus: H. lanceolatus* (Greater sandeel) and *H. immaculatus* (Corbin's sandeel), and one species of the Genus *Gymnammodytes*, the Smooth sandeel (*G. semisquamatus*). All sandeel species lay demersal eggs. Their larvae are pelagic. During the MIK survey, only the recently spawned larvae of *A. marinus* and of *H. immaculatus* may be found in the MIKeyM net because those are the only winter spawning sandeels species, while all other spawn between spring and autumn. Sandeel larvae can best be identified by their characteristic pigmentation patterns on head, ventral gut, along the dorsal midline, on the caudal fin and on the primordial finfold. The characteristic pigmentation pattern of the North Sea sandeel larvae is summarized in the following table. Other helpful characteristics can be found in Macer (1967), Russell (1976) and Munk and Nielsen (2005).

Pigmentation patterns of the different Ammodytidae species in the North Sea (extracted from Stevens et al. 1984): 0 = absent, + = present, ↑ = increasing with development, ↓ = decreasing with development, an = anterior, po = posterior. Sources: Cameron 1959, Einarsson 1951,1955 and Macer 1967

Species	Stage	Body length (mm)	Head				Ventral gut	Dorsal midline	Caudal	Finfold
			Jaws	Snout	Brain	Nape				
Gymnammodytes semisquamatus	preflex	4.8	0	0	0	0	+	0	0	+
	flex	7.0	0	0	0	+	+	near tail	+	+
	postflex	11.8 – 38.0	0	+	+, 0	+	+, ↓	↑, +	+	+
Hyperoplus lanceolatus	flex	6.0	0	0	+	+	+	po 1/3	0	0
	postflex	11.0 – 25.0	0, +	0, +	+	+	+, ↓	+	↑	
Hyperoplus immaculatus	preflex	5.5 – 9.0	0	0	0, ↑	0	an	po 1/4	0	0
	flex	13.0	0	0	+	0	an	↑	+	0
	postflex	26.0	+	+	+	0	an	+	+	
Ammodytes tobianus	preflex	4.0 – 5.0	0	0	0	+	an	0	0	0
	flex	7.5 – 12.0	0	0	+	+, 0	an	near tail	0	0

	postflex	16-0 – 27.0	0	0	+, 0	+, 0	0	↑	0, ↑	
Ammodytes marinus	preflex	4.5 – 6.0	0	0	0	0	an	0	+	0
	flex	7.5 – 11.0	0	0	+	0	an	po ¼	+	0
	postflex	19.0 – 33.0	0	0, ↑	+	0	+	↑	+	

In the MIKey M net during the Q1 IBTS, the small larvae of *A. marinus* and *H. immanculatus* are the most likely to be found. Both are very similar in appearance. However, the larvae of *A. marinus* show less pigmentation, particularly on the posterior part of the dorsal midline (see table above) and are always smaller at any developmental stage than *H. immaculatus*. Large ammodytid larvae and juveniles are very unlikely to be caught with the MIKey M net.

Flatfish (Pleuronectiformes)

General characteristics:

- Laterally compressed exhibiting asymmetry after metamorphosis
- Short, protruding, twisted gut
- Rounded head in younger larvae

Bothidae and Scophthalmidae are identified by spines on the head and migration of the right eye to left side of head. In Pleuronectidae and Soleidae the left eye migrates to the right side.

Pleuronectidae, plaice-like fish

Pleuronectidae have a more slender body form compared to soleidae. The pre-anal area is less than half the total area of the body (Munk and Nielsen, 2005).

Pleuronectidae with ventral pigmentation

	Plaice (*Pleuronectes platessa*)	**Dab (*Limanda limanda*)**	**Flounder (*Platichthys flesus*)**
Pigmentation of ventral body contour	Ventral double row of melanophores (less evenly spaced than those in dab)	Very evenly spaced double row of melanophores	Scattered small melanophores along both sides of the ventral part of body
Other pigmentation		Melanophores along the edges of pectoral fins (Munk and Nielsen 2005), though mostly difficult to see or lost during capture	Band of melanophores midway along post anal part of body (Russell 1976)
Size at hatching	7 mm	3 – 4 mm	2.25 mm

Size in relation to development	Larger than other Pleuronectidae at any developmental stage		
Body shape	Long and slender	Shorter and stockier than plaice	Gut ends closer to the middle of body compared to dab
Head	Smaller head	Larger head than plaice	Small head and relativel small eyes
Caudal fin rays (Russell 1976)	19 – 22	16 – 18	18
Onset of asymmetry	11 – 12 mm	10 – 12 mm	< 10 mm
			Caudal peduncle longer than broad (Nichols 1971)

Time and location of occurrence is important in distinguishing between Plaice and Dab!

Pleuronectidae with bars/bands of melanophores on the primordial fin and body

Asymmetry for long rough dab and lemon sole begins at a larger size (15mm; Munk and Nielsen, 2005).

	Long rough dab (*Hippoglossoides platessoides*)	**Lemon sole (*Microstomus kitt)***	**Halibut (*Hippoglossus hippoglossus*)**	**Witch flounder (*Glyptocephalus cynoglossus*)**
Pigmentation	Bands less clearly defined, with pigmentation between bands (Munk and Nielsen 2015)	More distinct bands compared to long rough dab (Munk and Nielsen 2015)	Less pigmented and lacks bars on primordial fins	
Body shape	Bigger mouth	High and round body with small head and mouth	Dpression above eyes in a relatively large head	Elongated appearance
Anal fin rays	< 85	< 85	< 85	85 – 102 (Munk and Nielsen 2015)

Soleidae

Soleidae have a deep abdominal region, a rounded vertical profile of the head and characteristic pigmentation (Russell, 1976).

	Sole (*Solea solea*)	**Solenette (*Buglossidium luteum*)**	**Thickback Sole (*Microchirus variegatus*)**
Pigmentation	Almost unbroken line of melanophores along ventral and dorsal body contour	Larger and evenly spaced melanophores along body contours	Smaller stellate chromatophores (Nichols 1976)
Other pigmentation	Pigmentation on primordial fin bigger and more distinct	Localized pigmentation on underside of abdomen	Pigmentation may be arranged into longitudinal rows.
Abdomen		Abdomen very prominent, particularly in the earlier stages	Anus just behind body mid point (Russell 1976)
Size at hatching	3 mm (Nichols 1976)	2 mm	2.5 mm

Scopthalmidae

All with upward pointed mouth and nose

Early stages with rectal and post anal pigment bar and dense pigmentation

	Brill (*Scophthalmus rhombus*)	**Turbot (*Scophthalmus maximus*)**
Size	Larger than turbot at early development (Russell 1976)	In later stages body depth larger than brill
Body shape	Longer and more slender in later stages	Short and stout compared to brill
Spines		Opercular spines are more strongly developed (Munk and Nielsen 2005)

Species with bands of melanophores at all stages

	Topknot (*Zeugopterus punctatus*)	**Norwegian topknot (*Phrynorhombus norvegicus*)**	**Megrim (*Lepidorhombus whiff-iagonis*)**
Pigmentation	Patches of melanophores on primordial fin in early stages (Munk and Nielsen 2005)	Pigmentation on edge of pectoral fin and row of melanophores along jaw and abdomen	Characteristic rows of melanophores along body contour and primordial fin margin.
Spines	Otocystic spines	No otocystic spines	Two large otocystic spines
Dorsal fin rays	75 – 94 (Munk and Nielsen 2005)	85 – 102	76 – 84)

Bothidae

Rounder head compared to Scophthalmidae. Only 1 genus (Scaldfish, *Arnoglossus sp.*) in the area. All scaldfish species have a tentacle on top of the head. Most commonly occurring is the species *A. laterna*.

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