Guidelines for ichthyoplankton Survey in the open part of the Baltic Proper

The ichthyoplankton survey is conducted in June every year. Its purpose is to quantitatively sample pelagic fish eggs and larvae to determine the abundance and composition of ichthyoplankton, as well as information on macrozooplankton, water blooms, and hydrographic data at key sites. The investigated area is the Latvian EEZ in the open part of the Baltic Sea. The most important parts of the area are over big depths and in the southern part, a region of significant importance due to the more intensive (and, in the case of cod, more successful) spawning of pelagophilic fishes.

Below are the main guidelines for conducting a scientific ichthyoplankton survey, as well as the analysis of collected samples in the laboratory:

Ichthyoplankton sampling in the Latvian EEZ (Eastern part of the Baltic proper) is carried out with the ichthyoplankton net IKS-80. Ichthyoplankton samples are collected on the standard set of stations (Figure 1, Table 1). Ichthyoplankton net IKS-80 is a conic ring-net ca. 4 m long. It has a mouth opening of 0.5 m^2 and a mesh size of 500 µm (Figure 2). This net is operated vertically from the bottom or 125 m depth to the water surface with a speed of 0.4 ms^{-1}. Low lifting speed prevents eggs from being damaged by mechanical forces. The filtration rate was determined to be approximately 70 % using a flow meter rotating in only one direction. The net is lowered down from the winch or gillnet machine. The speed of going down is not very important; the only thing the operator should care about is not to get the rope too loose and not to make knots on it. <u>During this operation, the vessel must be standing with the board from which the net is operated (usually it is portside) to the wind so that the ship drifts away from a rope with the net; otherwise, the rope gets under the vessel, and the net could be lost. Tying the end of the rope to the vessel is also vitally important.</u> The weight on the end of the net is 10 to 20 kg. More is better, especially in rough weather, but getting off the net aboard requires much strength. The angle of the rope should be measured during the net lifting. <u>Net must always be lifted on board by the ropes going from ring to cod-end, and never by the gauze!</u>

To sample the upper layers by circulation method, the net is towed on the water surface at a speed of ca. 2 knots for 10 minutes; the speed of the vessel is ca. 3 knots, and it is making circulations, so the net is going in the inner circus and not in the wake of the vessel. The condition of fish larvae sampled with this method is not ideal, like that of larvae sampled with Bongo net. Ichthyoplankton net must be washed rather gently from the water hose when raised from

the water to concentrate eggs and larvae in cod-end. After emptying the cod-end, it is attached again, and the lower part of the net is washed once more to ensure that practically all the stuff gets in the cod-end and thus cannot get in the next haul. Information on the collection of samples is recorded in paper format:

1. Date of collection of the sample;
2. Time of collection of the sample;
3. Station number (if any);
4. Coordinates of the station (latitude/longitude degrees and minutes);
5. The depth of the station;
6. Depth of sample;
7. Angle of rope;
8. The existence of comb jellies (*Ctenophora*) and their number (comb jellies dissolve in formalin and alcohol and thus can't be identified afterwards).

After collection, the samples are conserved in a 2 % unbuffered formaldehyde solution with seawater and processed later in the laboratory. All the eggs and larvae are meticulously counted. If the number of eggs is substantial, the sample is split for the most numerous species, usually sprat. The content of this sample is poured into a 150 ml measuring glass, thoroughly mixed, and a portion is transferred into a Bogorov tray using a special closing metal pipette. The remaining sample is then examined for less numerous species.

At least 100 eggs (or all of them if there are fewer than 100 in the sample) are processed to measure and determine the stages of development. Species are identified according to the keys for determining species provided by P. Munk, Kazanova, Horbowa, Fey, and Russel. In our institute, Kaznova's book is in Russian, Horbowa's is in Polish, and Russel's is available in PDF format only.

Table 1. Positions of ichthyoplankton stations in the Gotland Basin, Eastern part of the Baltic Sea.

Station	Lat°	Lat '	Long°	Long '	Long	Lat
1	57	23	20	36	20.6	57.38333
2	57	23	20	48	20.8	57.38333
3	57	23	20	24	20.4	57.38333
4	57	22	20	13	20.21667	57.36667
5	57	22	20	0	20	57.36667
6	57	33	20	12	20.2	57.55
7	57	45	20	25	20.41667	57.75
8	57	55	20	32	20.53333	57.91667
9	57	55	20	45	20.75	57.91667
10	57	52	20	59	20.98333	57.86667
11	57	48	21	9	21.15	57.8
12	57	45	21	24	21.4	57.75
13	56	55	20	43	20.71667	56.91667
14	56	55	20	25	20.41667	56.91667
15	56	55	20	10	20.16667	56.91667
16	56	55	19	53	19.88333	56.91667
17	56	55	19	43	19.71667	56.91667
18	56	42	19	32	19.53333	56.7
19	56	30	19	22	19.36667	56.5
20	56	31	19	39	19.65	56.51667
21	56	32	19	52	19.86667	56.53333
22	56	32	20	8	20.13333	56.53333
23	56	32	20	22	20.36667	56.53333
24	56	32	20	38	20.63333	56.53333
25	56	15	19	18	19.3	56.25
26	56	7	19	10	19.16667	56.11667
27	56	2	19	8	19.13333	56.03333
28	55	57	19	5	19.08333	55.95
29	55	59	19	22	19.36667	55.98333
30	56	1	19	38	19.63333	56.01667
31	56	3	19	56	19.93333	56.05
32	56	12	20	17	20.28333	56.2
33	56	22	20	38	20.63333	56.36667
34	56	22	19	20	19.33333	56.36667
35	57	8	19	48	19.8	57.13333
36	57	15	19	53	19.88333	57.25

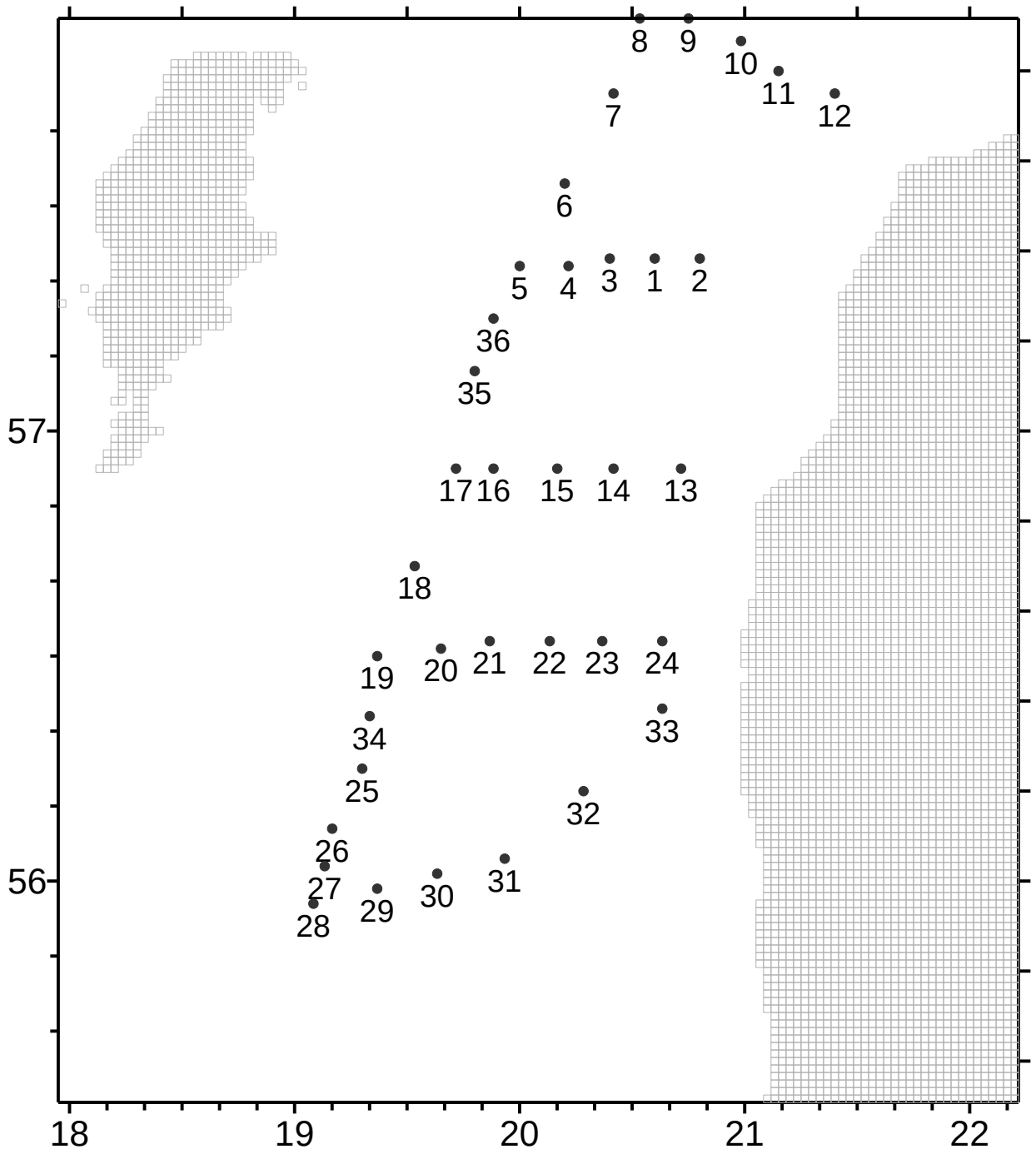

Figure 1. Positions of ichthyoplankton stations in the Gotland Basin, Eastern part of the Baltic Sea.

Eggs are measured under 40X magnification using a micrometre scale. One unit of this scale is equal to 0.025 mm. If the eggs have an irregular shape, a lesser diameter is measured. The stages of egg development are determined according to the 4-stage system by Rass and Kazanova (1965); additionally, the 1st stage is divided into substages IA and IB. Substage IA lasts until 64 cells of the embryo (including). Eggs in each stage are divided into alive and dead ones arbitrarily following morphological criteria (shape of egg and yolk) and the condition of chorion and embryo in the main (Rass and Kazanova, 1965). Total lengths of fish larvae are measured in 0.5 mm length classes (to the nearest value). Usually, all found larvae are measured, but in some cases, if the number of larvae exceeds 100, only 100 or 200 are measured. The reason for measuring all the larvae is that it is sometimes impossible to divide them into equal portions because many of them are mixed with numerous algae and dandelion seeds. The obtained results are written in an MS EXCEL spreadsheet. The data spreadsheet contains the following information:

1) number in the notebook;
2) date of sample collection;
3) month of sample collection;
4) year of sample collection;
5) time of sampling;
6) station number (if available);
7) geographical coordinates of station (latitude/longitude and minutes);
8) station depth;
9) depth of sampling for vertical haul and time of sampling for circulation;
10) angle of the rope;
11) wind direction;
12) speed of wind;
13) number on the bottle with the sample;
14) species name;
15) number of individuals in the sample;
16) number of individuals per square metre (N/m^2);
17) development stages for eggs/size groups for larvae;
18) number of macrozooplankton organisms for every species.

The data should be uploaded into the national database BIODATA and stored on the BIOR server.

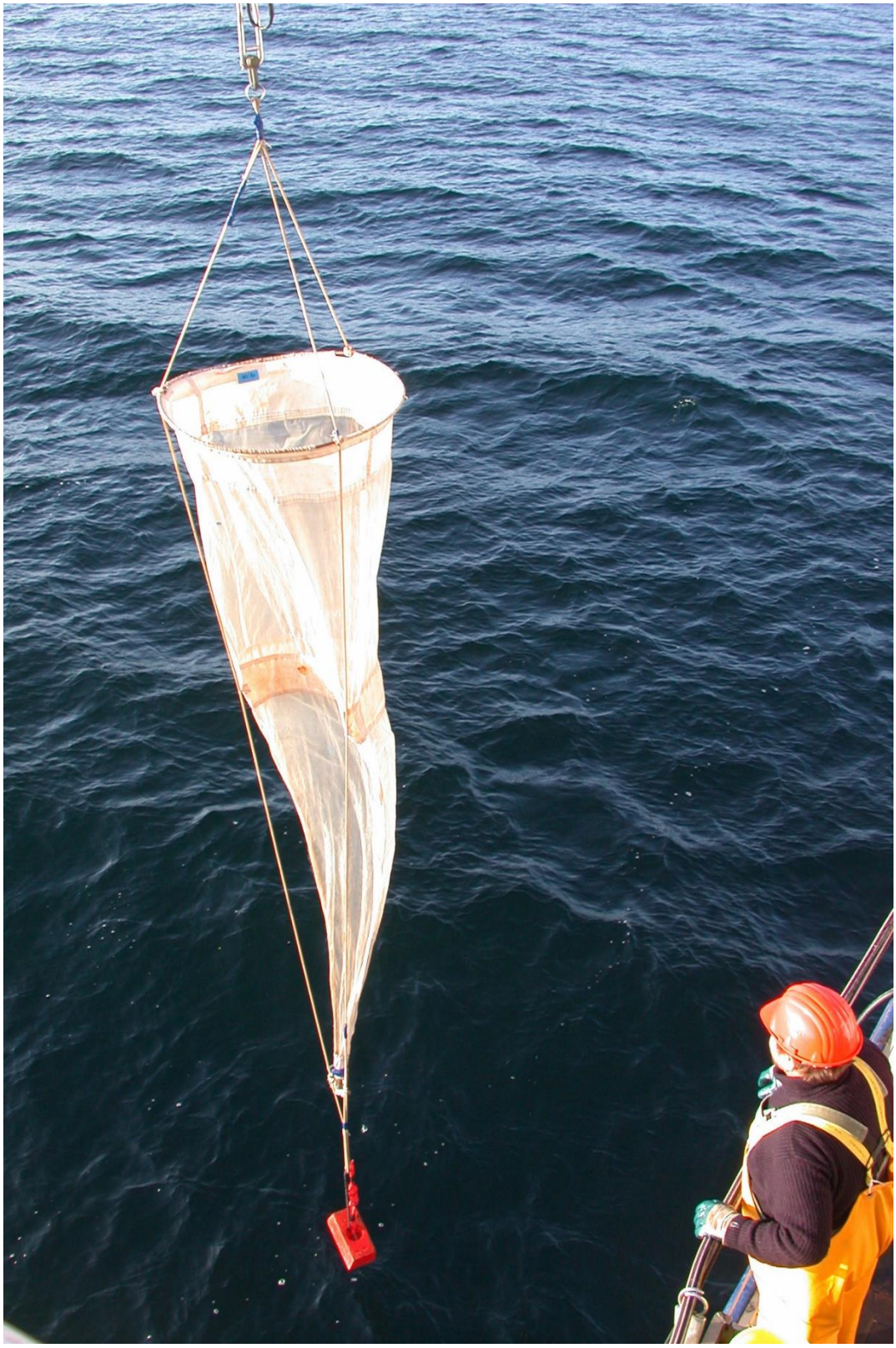

Figure 2. Ichthyoplankton net IKS-80.

Figure 3. Washing of ichthyoplankton net IKS-80.

Figure 4. Taking a sample from ichthyoplankton net IKS-80.

Appendix 1. Equipment in the sea survey.

- Two Ichtyoplankton nets IKS-80 (ring diameter 80 cm, mesh size 500 µm), one of them in reserve;
- Ichthyoplankton net IKS-80 for circulation (ring diameter 80 cm, 2 floaters attached to the ring, mesh size 500 µm);
- Ca. 80 bottles for sample storage (500 ml);
- 2.5 l of 37 % formaldehyde to fix the samples;
- 3 boxes for storage of bottles;
- Rope 8 –12 mm 200 m for vertical hauls;
- Rope ≥12 mm 30 m for hauls on the water surface;
- Secchi disk;
- Glue gun, threads and needles to repair nets in case of damage;
- Weight 10 –20 kg (more compact is better);
- Shekels and carabines;
- CTD.

Appendix 2. Equipment in the laboratory:

- Microscope (binocular) with ocular-micrometre scale;
- Measuring cups of 100 ml, 250 ml, 500 ml and 1000 ml volumes;
- Calibrated Stempel pipette (2ml);
- Bogorov tray 22 ml;
- Bogorov tray 100 ml;
- Sieves for rinsing the sample with a mesh size of 300 µm or less;
- Notebook to register the results of sample analysis;
- Preparatory needles, Paster's pipettes, Petri dishes (better from glass);
- Keys for identification of eggs and larvae.

Literature:

Kazanova, I.I. Key for identification of eggs and larvae of the Baltic Sea (In Russian). In: Trudy VNIRO, Vol. XXVI, 1954.

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Rass, T.S. and I.I. Kazanova 1965. Manual for sampling of fish eggs, larvae and fry (in Russian). Moscow, Pischevaya Promyshlennostj, 44 pp.

Russel. Eggs and larvae.