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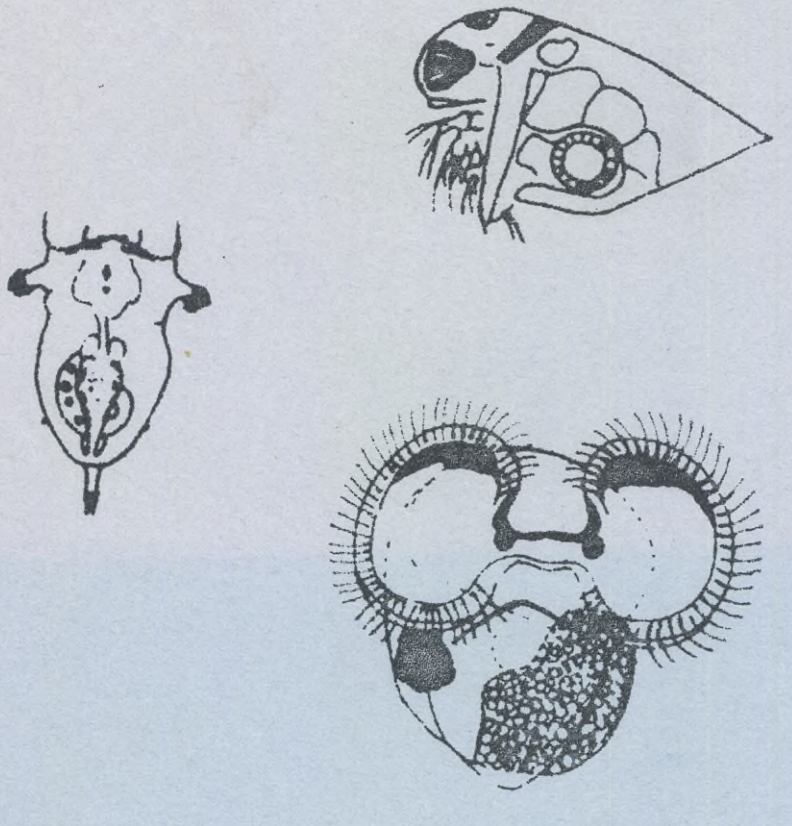

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Manual for zooplankton-ichtyoplankton investigations in the Baltic area

by

Hans Ackefors, Lars Hernroth, Odd Lindahl
and Ulf Persson

October 1974

MANUAL FOR ZOOPLANKTON-ICHTYOPLANKTON INVESTIGATIONS IN THE BALTIC AREA

by

Hans Ackefors
Lars Hernroth
Odd Lindahl
Ulf Persson

Preface

At the Second Baltic Symposium on Marine Biology in Stockholm, June 1971, six working groups for plankton studies and other subjects in the Baltic were proposed. Dr Hans Ackefors, Sweden, was appointed a convener for the Working Group 6 concerned with plankton studies inside the Baltic Marine Biologists. In January, 1972, the conveners met for a meeting in Rostock, GDR, and members for the working groups were proposed. After a circular letter sent out by the convener the Working Group 6 for plankton studies was established in March, 1972. 1-3 members from each country joined the group except from Denmark and U.S.S.R. Later a representative from Denmark was included in the group.

In October, 1972, a compilation of present studies of zooplankton and ichtyoplankton was made and sent out to the members of the group and others. In February, 1973, the convener sent by letter the first proposal for methods to be used for:

A. Ecological investigations
B. Secondary production studies
C. Samples taken for analysing heavy metals and chlorinated hydrocarbon.
D. Samples for fish eggs and larvae.

This proposal and the recieved replies from the members of the group were discussed in Helsinki, Finland, June, 1973, during the Third Baltic Symposium on Marine Biology.

The Working Group met in Gdynia, Poland, 1-3 October, 1973, where discussions about suitable methods took place. A new circular letter was sent out to the members of the group, where the results of the discussions were compiled and also a joint practical investigation program for zooplankton sampling was established.

At the request of the Working Group 6 the convener and his colleagues at the Institute of Marine Research, Lysekil, Sweden, have now prepared the first "Manual for Zooplankton-Ichtyoplankton investigations in the Baltic Area". In April, 1974, a preliminary manual was sent out to the members of the Working Group. The convener and his colleagues are especially grateful to Dr Sigrid Schnack, Kiel, FRG, who has sent us valuable remarks and suggestions for the final version of the manual.

Present studies at our institute about tests with different nets strongly emphasize the need to use UNESCO WP-2 net with 90-100 µ mesh size for ecological zooplankton studies instead of 200 µ mesh size.

Finally I want to thank all members of the Working Group 6 inside the Baltic Marine Biologists and especially my colleague mr Lars Hernroth for their co-operative help when preparing the present manual.

Lysekil September 27, 1974

Hans Ackefors
convener

The proposed recommendations are intended mainly for investigations in the open sea and in relatively deep coastal areas. In shallow coastal areas and in heavily polluted areas, the methods may be modified, or special methods used.

MICROZOOPLANKTON (<200 µ)

Sampling:

Microzooplankton should be collected with a 5-liter watersampler. The samples should be taken at the following depths: 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 and 200 m. The samples should be filtrated through a 20-µ filter. The filtrate is then washed into a bottle and diluted to approx. 190 ml with filtrated sea-water from the corresponding depth.

Preservation of the samples should be done with 10 ml formaldehyde (HCHO 40 %), giving a final concentration of 2 % and a volume of 200 ml. The pH of the formaldehyde ought to be adjusted to 8.0-8.2. This is done with di-sodiumtetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$). For special purposes the nonloricate ciliates should be preserved with 200 ml isotonic solution consisting of equal parts of ethylalcohol (C_2H_5OH 80 %) and formaldehyde (4 %).

Subsampling should be done according to the following method:
The preserved sample (200 ml) is turned upside-down continously for about one minute. A subsample is then poured into a 50 ml sedimentary tube (App. 1). After 24 hours the organisms have sedimented into the counting chamber.

When the subsample contains less than 100 specimens of the dominating species, the whole sample should be analysed in the following way: The rest of the sample (150 ml) is filtered through a sieve of approx. 3 cm diameter and a mesh-size of 20 µ. The content of the sieve is then washed into a 50 ml sedimentary tube. The washing should be done with filtered water from the sample in question.

Analysing:

Enumeration of microzooplankton should be done by an inverted microscope procedure based on Utermöhl (1958). Non-loricate ciliates should be analysed at 200 X magnification while the remaining taxonomic groups should

be analysed at 100 X.

When doing in vivo studies, the problem arises that many organisms move too fast to be examined properly. To prevent this, an anaesthetic like nickel sulphate can be used. A useful concentration is 0.01 % $NiSO_4 \cdot 6\ H_2O$ from which dilutions can be made. Exposure to the nickel sulphate for 15 minutes should suffice.

Biomass calculation:

The estimation of the biomass starts from the volume of the animal which is obtained according to the formulas for simple geometrical figures. The volume is expressed as ml/m^3 (V) and as ml/m^2 (T). The volume is identical with the wet weight or the biomass (B), assuming that the density of the organisms is equal to one.

$$V = ml/m^3$$

The total volume per 1 m^3 = V (B) = $\frac{1000}{5} \cdot x \cdot v \cdot 10^{-9}$

x = individuals per 5-liter (the volume of the sample)

$\because$ 200 x per 1000 liter or 1 m^3

v = the volume of the animal expressed as μ^3

$\because$ the volume of the animal expressed as ml = $\mu^3 \cdot 10^{-9}$

B = biomass

$$T = ml/m^2$$

The Trapeziodal formula [1] should be used for the estimation of the total volume from the surface to the depth of the deepest sample. The volume should be expressed as ml/m^2 (T).

$$T = \frac{1}{2}\left[\sum_{K=1}^{n-1} \left(V_K + V_{K+1} \right) \cdot h_K\right]$$

1) There is also a more exact formula called the Simpsons formula but it can only be used for an odd number of sampling depths. A time-consuming but more exact way to calculate the biomass is to use a planimeter.

$K = 1, 2, 3 \ldots n-1$

n = number of sampled depths

V_K = volume of the animals (ml/m^3) from depth K

V_{K+1} = volume of the animals (ml/m^3) from depth K+1

h_K = distance (m) between the sampled depths K and K+1

0 - 200 m

$$T = \frac{1}{2}\left[(V_1 + V_2)5 + (V_2 + V_3)5 + (V_3 + V_4)5 + (V_4 + V_5)5 + (V_5 + V_6)10 + \right.$$

$$+ (V_6 + V_7)10 + (V_7 + V_8)10 + (V_8 + V_9)10 + (V_9 + V_{10})10 + (V_{10} + V_{11})10 +$$

$$\left. + (V_{11} + V_{12})10 + (V_{12} + V_{13})10 + (V_{13} + V_{14})50 + (V_{14} + V_{15})50\right] =$$

$$= \frac{5}{2}\left[V_1 + 2(V_2 + V_3 + V_4) + 3V_5 + 4(V_6 + V_7 + V_8 + V_9 + V_{10} + V_{11} + V_{12}) + \right.$$

$$\left. + 12V_{13} + 20(V_{14} + V_{15})\right]$$

SMALLER MESOZOOPLANKTON (200 µ - 1 mm)

Sampling:

For ecological purposes the W.G. preliminary recommends vertical hauls with the WP-2 net (UNESCO 1968)(App. 2) with a meshsize of 100 µ. For more detailed studies a large planktonsampler should be used (Ackefors 1971) (App. 3). The towing-speed of the net should be kept at approx. 0.5 m/sec. When the hauls are fractioned the recommended depths are 25-0, 50-25, 100-50, 150-100, 200-150, 300-200 and 450-300 m.

For biomass studies the W.G. recommends oblique hauls with the Bongo-net (App. 4). The meshsize should be 300 µ and the net should be equipped with a flow-meter (General Oceanics) and a depth-recorder. The net has to be lowered at 50 m wire/min. and brought back at 20 m wire/min. while the ship moves at a constant speed of 3.5 knots.

Preservation of the samples should be done in formaldehyde (40 %) diluted with sea water in such a way that the final concentration is 4 %. The formaldehyde has to be buffered to pH 8.0-8.2 with di-sodiumtetraborate

($Na_2B_4O_7 \cdot 10\ H_2O$).

Subsampling: The W.G. recommends the whirling apparatus designed by Kott (1953)(App. 5).

Analysing

Two subsamples of each sample should be analysed. Each specimen should preferably be analysed to both sex and developmental stage.

Biomass calculation

As the water-content of the preserved specimens decreases very rapidly during the first month following preservation, the W.G. recommends that the samples should be stored for one month before the analyses (SCOR-UNESCO WG 23).
The biomass of the samples is calculated according to the displacement volume technique. It is recommended to use the modified Frolande method (App. 6) constructed by Lillelund and Kinzer (1966).

LARGER MESOZOOPLANKTON (>1 mm) AND ICHTYOPLANKTON

Sampling

The W.G. recommends the use of oblique hauls with the Bongo-net equipped with one net of 300 µ and one of 500 µ. The net should have a flow-meter (General Oceanics) and a depth-recorder. The towing speed should be 3.5 knots.

Preservation: See smaller mesozooplankton.

Analysing

Normally, no subsampling is needed for zooplankton larger than 1 mm. All fish larvae or a subsample of at least 100 specimens should be measured. In case of polymodal length frequencies the number of larvae measured should be at least n = 100 x number of modes. Standard length (from the tip of the snout to the end of the urostyle) should be taken to the mm below; which means that, for instance, all larvae between 8 and 9 mm length are recorded as 8 mm. Notice that to all mean values, calculated from these data, 0.5 mm have to be added. In case of very small larvae

a measurement to the 0.1 mm below is recommended.

REPORT OF THE SAMPLING

The W.G. recommends that all information should be reported on standard sheets (App. 8).

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Kott, P., 1953: Modified whirling apparatus for the subsampling of plankton. - Aust.J.Mar.Freshw.Res., 4(2):387-393.

Lillelund, K. & Kinzer, J., 1966: Absetz- und Verdrängungsvolumen von Planktonproben. Untersuchungen zur Metodik. - Int.Revue ges. Hydrobiol., 51(5):757-774.

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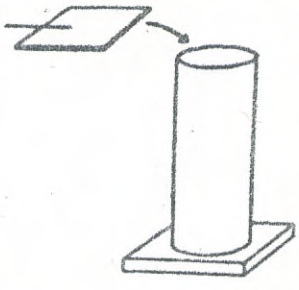
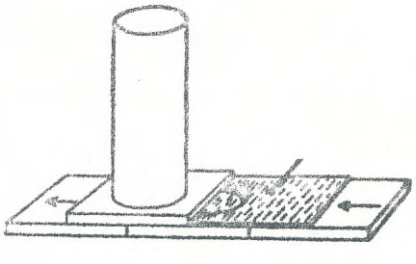
SUBSAMPLING OF MICROZOOPLANKTON

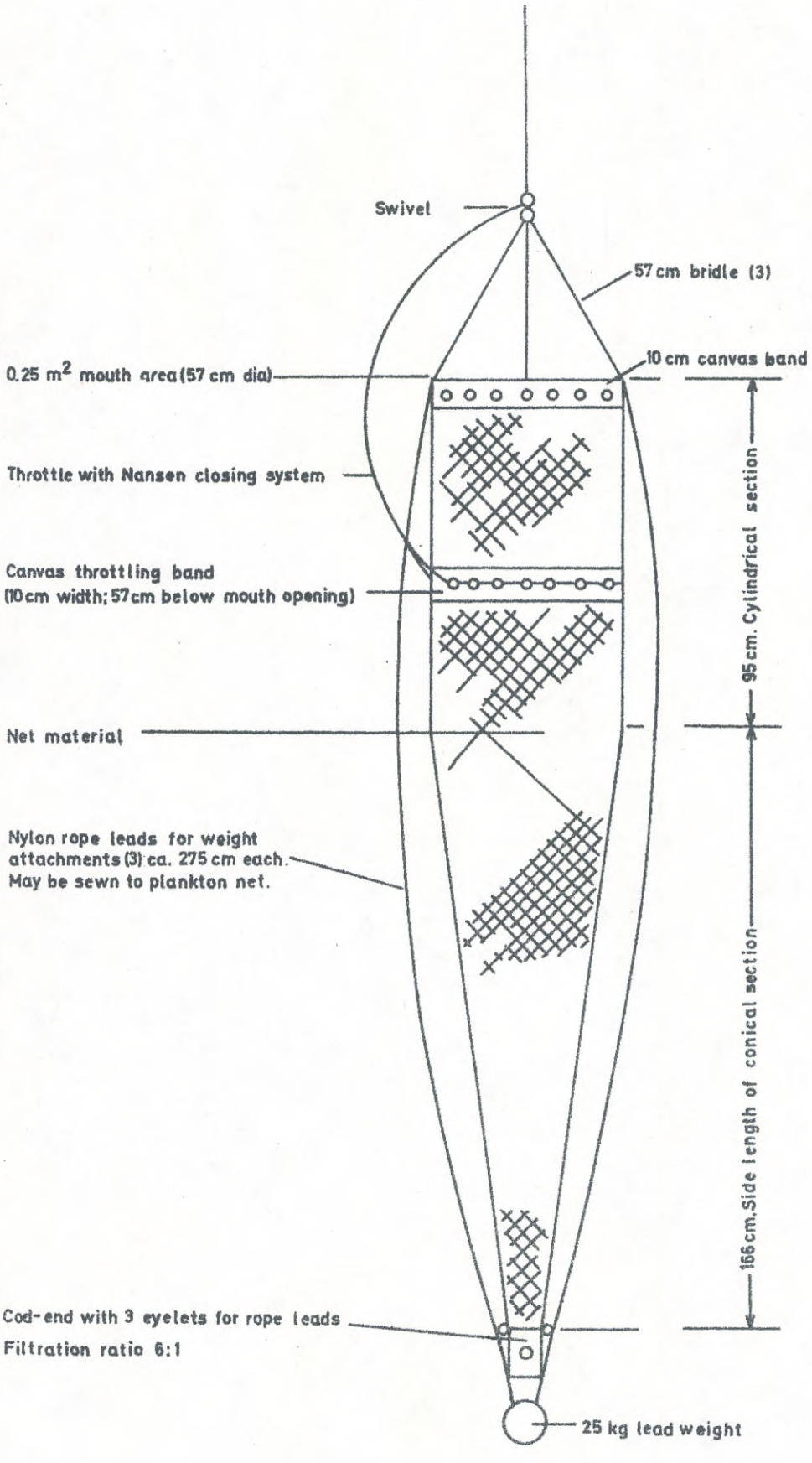
Counting chamber with a thin bottom of cover glas.



50 ml sedimentary tube. 1/4 of the preserved 200 ml sample is poured down for sedimentation.



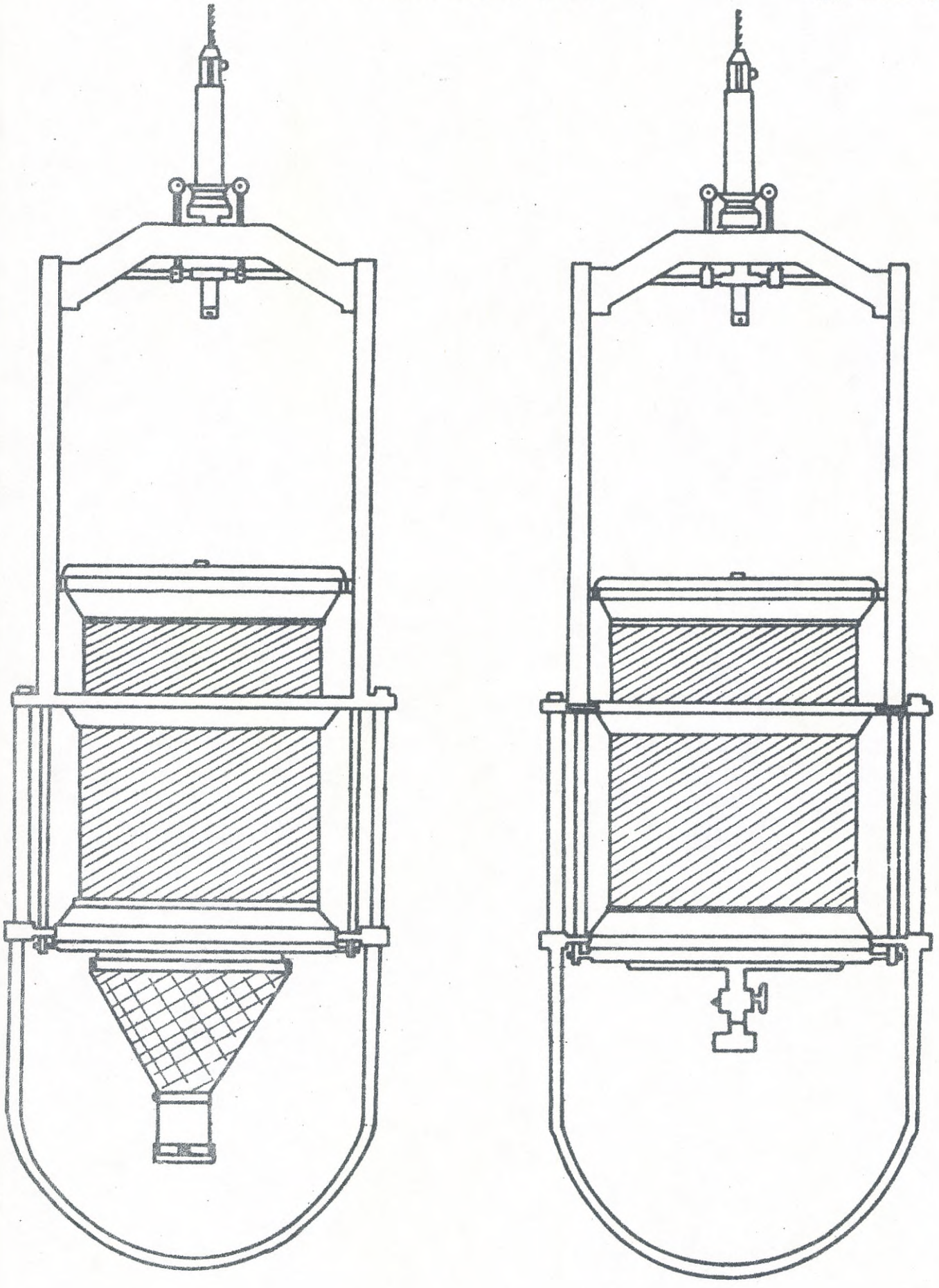
After 6 hours the "cleaned" water is removed according to the figure



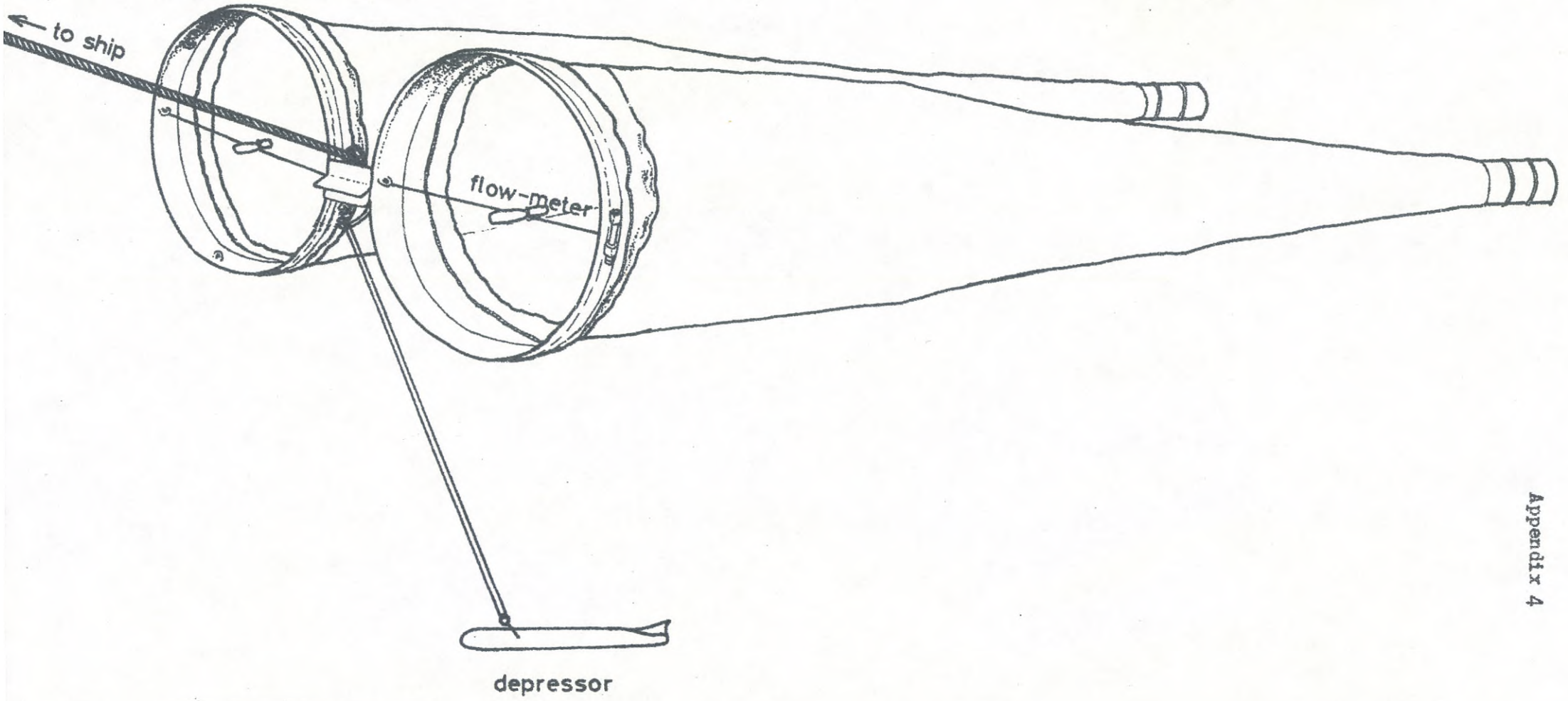
Ring: 57 cm internal diameter, 1.5 cm diameter thickness; with three eyelets, 120° apart, for bridles and rope lead attachments.

Ackefors-planktonsampler (23 l)

Appendix 3

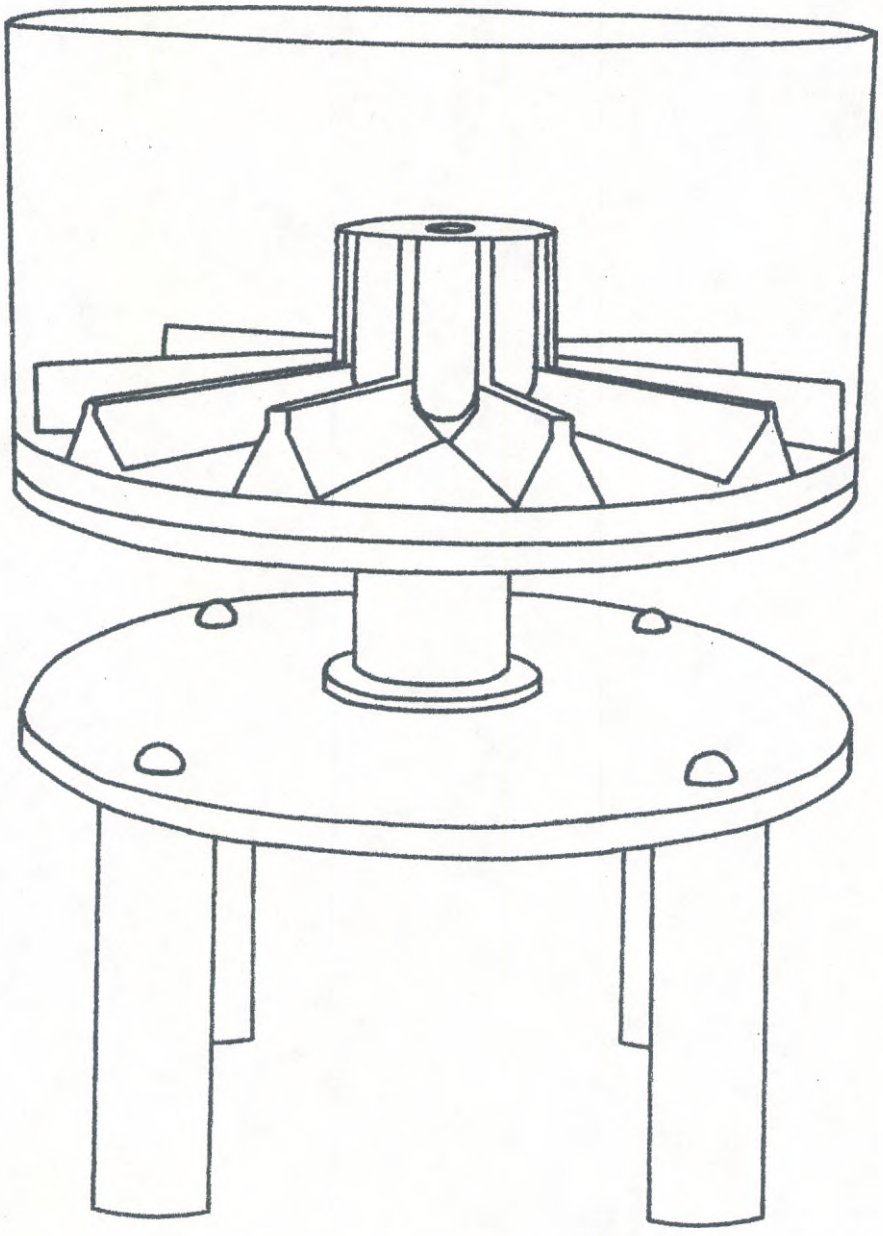


Bongo-net (cylindrical-conical)



Appendix 4

Subsampling apparatus



Displacement volume apparatus

(acc. Lillelund - Kinzer)

Calculation of ml/m^2 and ml/m^3 from oblique hauls

$$ml \text{ per } m^2 = \frac{B \times D}{V}$$

B = Biomass of sample (ml)

D = Maximum depth of sample

V = Volume of water sampled (m^3)

To calculate V

$$V = A \times S \times T$$

A = Mouth area of gear (m^2)

S = Speed of vessel (m. per sec.)

T = Duration of haul (sec.)

$$ml \text{ per } m^3 = \frac{B}{V}$$

The above formula gives the theoretical volume filtered. A correction for the efficiency of the net is obtained by using a flow-meter.

Vessel: Country: Cruise Period: Gear:

Mesh size:

SAMPLE No	STATION				TOW					WIND		WAVE HGT (m)	CLOUDE Cover (8 ths)	Secchi depth (m)	REMARKS
	No	POSITION	DEPTH (m)	DATE	Start Time (GMT)	Dura-tion (min)	Speed (knots)	Water Strnd (m^3)	Depth (m)	Knots	DRCTN				

Appendix 8:1

Sample No	FRCTN analysed	Biomass			MAIN GROUPS OF ORGANISMS (rel. abundance)
		ml/tow	ml/m^3	ml/m^2	