CHAPTER 3

Methods of collecting and processing samples, microscopic study, counts and determination of zooplankton biomass

3.1 Sampling: general aspects

Zooplankton composition, abundance, and distribution patterns depend on type and geographical location of the water body, season, time (considering daily vertical migrations), trophic status and a large number of other internal characteristics of the water body as well as numerous environmental (external) factors influencing the aquatic biota. Therefore, adequate sampling design should be developed prior to collecting zooplankton samples, relevant sampling methods should be selected, and appropriate sampling intervals chosen (for details see Telesh et al., 2009).

Zooplankton sampling techniques depend on the aim of the study and the targeted zooplankton fraction. For example, the *taxonomic survey* would benefit from the sample size: the larger the volume of water analyzed the more zooplankton species can be found in the sample; for this research, qualitative samples can be collected. Meanwhile, *numerical estimation* of zooplankton density, biomass and production can be performed only if quantitative samples are available.

For collecting various target groups of zooplankton, different sets of equipment are necessary. For example, ciliates are sampled by water bottles, meanwhile the larger organisms are only occasionally captured during such sampling; they must be caught by plankton nets with different mesh sizes depending on the size of the organisms.

3.2 Sampling, identification and quantification of meso- and macrozooplankton

Mesozooplankton in the sea is best sampled by the WP-2 UNESCO Standard net (UNESCO, 1968). It is a closing net suitable for vertical tows and stratified sampling. For effective sampling of the smaller mesozooplankton in the Baltic Sea, this net (Figure 3.1a) is recommended for the HELCOM Monitoring and Assessment program with a mesh size of 100 µm (HELCOM, 1988, 2005). In shallow coastal areas, the use of horizontally or oblique towed nets of a similar shape is suitable, like Bongo or Multiple nets (Figure 3.1b, c).

Macrozooplankton is collected by nets with larger openings and mesh sizes (Wiebe & Benfield, 2003, and references therein). Fractionation strategies of sampling

and examples of estimating the total plankton concentration can be found in Witek and Krajewska-Soltys (1989), Quinones et al. (2003), Postel et al. (2007).

The exact amount of water filtered by the net during towing should be determined by a flow meter. For the details of recommended flow meter types, their position, functioning, and efficiency calculation see Telesh et al. (2009).

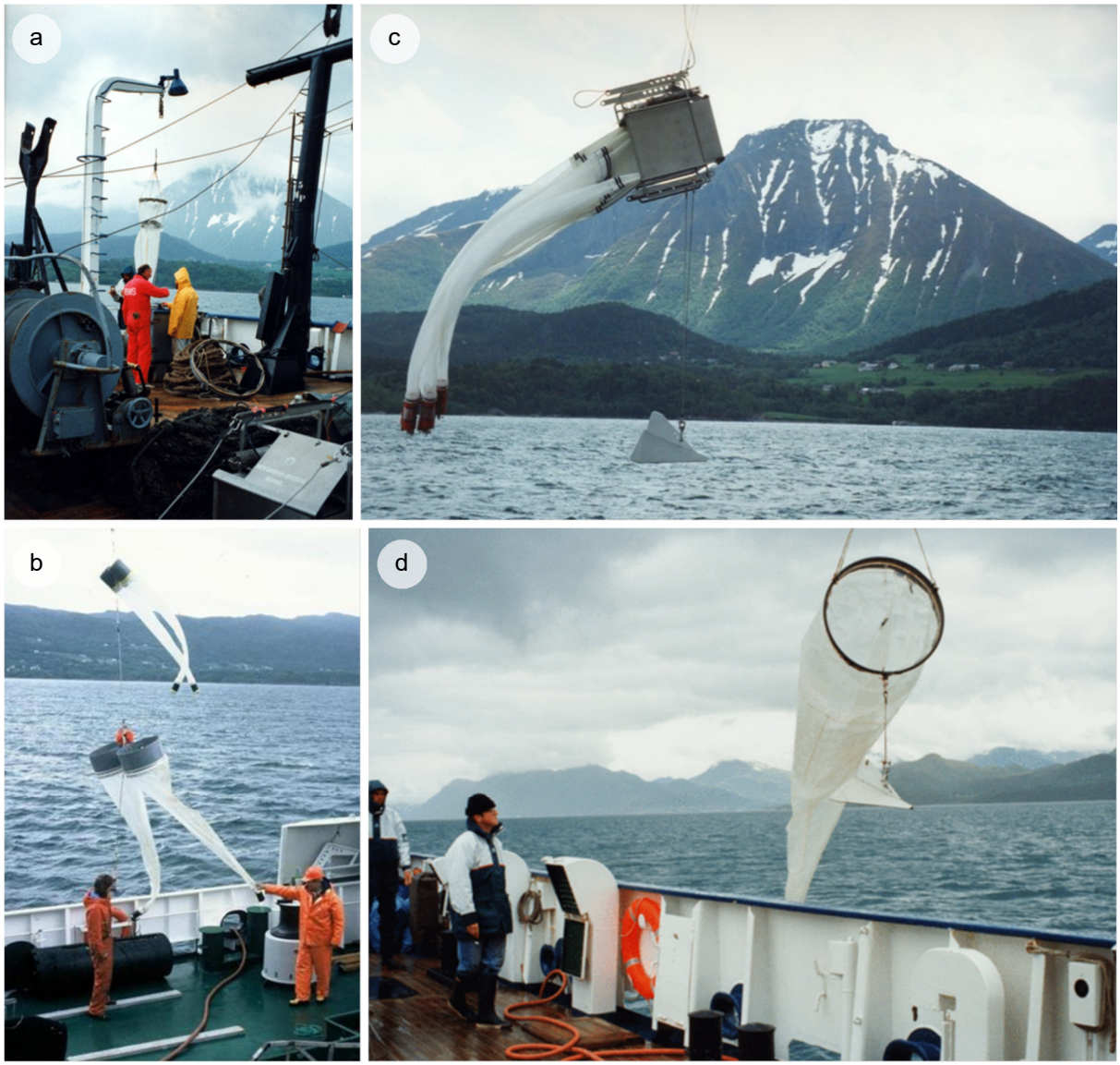

Figure 3.1: **a,** The WP-2 UNESCO Standard net being deployed aboard the R/V A. v. Humboldt; **b,** Twenty cm and 60 cm Bongo nets ready for deployment from the R/V Johan Hjort; **c,** The Multinet rigged for horizontal towing from aboard the R/V A. v. Humboldt; **d,** Deployment of a CalCOFI net from the R/V A. v. Humboldt. All photos stem from an ICES/GLOBEC Sea-going workshop for inter-calibration of plankton samplers at Storfjorden, Norway, June 1993 (ICES, 2002) (after Telesh et al., 2009).

After the net sample is taken out of the water, the net must be carefully rinsed *from the outside* with seawater. The sample, which is concentrated in the cod end of the net, must be transferred to a sample jar and preserved by buffered formalin with a final concentration of 4%. Labeling of the sample jar inside and outside by station number, date, time, and sampling depth interval is mandatory.

Species identification and counting are the basics of any zooplankton community analysis. These procedures are time-consuming and require considerable professional skills and experience. This fact often restricts the number of samples that can be analyzed with an acceptable effort within a reasonable time span. Attempts to overcome these difficulties by the automatic counting methods may help to solve the problem of under-sampling (Wiebe & Benfield, 2003). However, application of the automatic methods is limited to relatively uniform samples (e.g., laboratory cultures), certain size-class-specific analyses, or a coarse separation of organisms from higher taxonomic groups with significant differences in general body morphology. Coupling of such procedures with computerized image analysis may be helpful; however, it requires sophisticated technical equipment and special software.

Routinely, for monitoring purposes, counting is performed for the dominant organisms from easily identifiable taxonomic groups and their developmental stages. More taxonomic skills are required for the identification of certain species using the appropriate guidebooks. The species names should be used according to the *International Code of Zoological Nomenclature* (http://www.iczn.org). Information on the validity of names and actual taxonomic classification can be given, for example, following the Integrated Taxonomic Information System (http://www.itis.gov) and The European Register of Marine Species (http://www.marbef.org/data/erms.php).

The laboratory procedure of sorting mesozooplankton starts with removing the redundant formalin from the sample by its filtration through the mesh with size smaller than the mesh size of the sampling gear. (The filtrated preservative can be used again after the analysis for any further storage). The organisms are suspended in filtered tap water or distilled water for the analysis. The procedure should be carried out under a fume-hood. The sample is often so densely concentrated that it requires sub-sampling into aliquots. For example, 1/32 ± 1/8 of the sample were analyzed within the monitoring program of the Leibniz Institute for Baltic Sea Research, Warnemünde, in 2005 (Wasmund et al., 2006).

The volume of the total sample, measured in a graduated cylinder, is noted as the reference amount. The sample then is poured into a beaker to allow a thorough mixing until the organisms are distributed randomly before taking an aliquot. Repeated sub-sampling by the Stempel pipette (Hensen, 1887) produces a coefficient of variation of 7–9%, applying a bulb pipette 14–15%, and a Folsom splitter 5–18%. The variability between total counts amounts to 0.3–2.5% (Guelpen et al., 1982). The use of pipettes is 5 to 8 times faster than the splitter technique. Its limitation is inapplicability to sub-sample zooplankters which size is larger than the pipette's diameter. The Kott splitter (Kott, 1953) is more convenient in comparison to the Folsom splitter (Sell & Evans, 1982; Griffiths et al., 1984). The Kott splitter produces 8 sub-samples at the same time, while the Folsom device splits samples into halves and increases the error from one step to another (Behrends & Korshenko, pers. comm.).

For routine sorting of larger zooplankton, a dissecting stereomicroscope is used (Figure 3.2). It makes manipulation of the specimen during the identification procedure possible. For the smaller mesozooplankton, such as rotifers, cladocerans, copepods and their developmental stages, an inverted microscope accomplishes the same role. It allows routine survey with the 50× magnification and the analysis of details with a magnification factor of 80× to 125× as well. For more specific investigations of certain taxonomic features, like the examination of the fifth leg of copepods, a compound microscope with achromatic condenser and 10× to 70× objectives is the preferred instrument. For looking into the details of rotifer morphology, 100× oil immersion objective is needed.

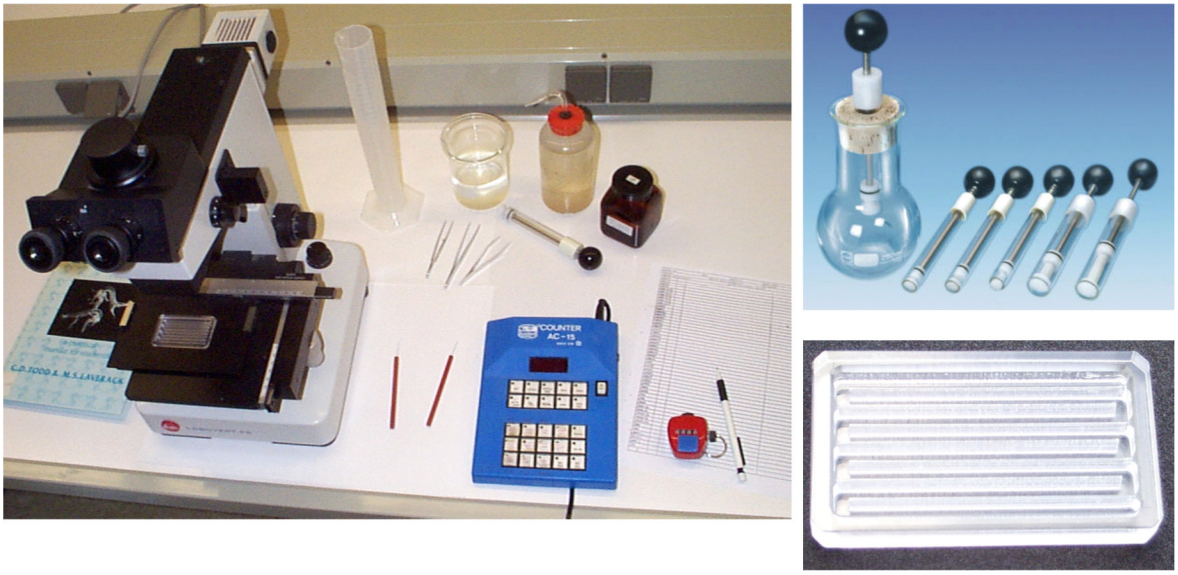

Figure 3.2: Working place for counting and identification of smaller mesozooplankton with an inverted microscope (Labovert, Leica Microsystems GmbH, Wetzlar, Germany) and accessory equipment like Stempel pipette (Hydrobios GmbH, Kiel, Germany), and Mini-Bogorov chamber (Postel et al., 2000, modified after Arndt, 1985).

For an inverted microscope, an open Plexiglas counting chamber of high transparency like the Mini-Bogorov chamber (modified after Arndt, 1985) is necessary. Closed types of counting chambers are preferably used in microzooplankton studies. The trays are provided with sections to allow a better orientation and to avoid a repeated counting of the same organism. One counting strip is fully covered with the 50× magnification. The Mini-Bogorov chamber (Figure 3.2) is easy to produce in a workshop. It has the following dimensions: the length, width, and height are 40, 70, and 8 mm, respectively. The counting paths are 6 mm deep, their width amounts to 3 mm, the section walls are 1 mm wide, and their height is 4.5 mm. The sides and walls are tapered sloping at top. The tray is made of clear plastic and needs to be polished to high quality (Postel et al., 2000). The table of the microscope has to be adapted to carry the tray (Figure 3.2). The Mini-Bogorov tray is filled with a known aliquot (e.g., 0.5 or 1 ml, which has to be considered for calculation of abundance) and finally made up to the top (10 ml) with filtered tap water or distilled water. The surface must be level to avoid any reflections. Therefore, the outer walls are 1.5 mm higher than those of the counting paths are.

Some organisms, for example, cladocerans, tend to float in the surface film. Addition of detergents or cetyl alcohol [$CH_3(CH_2)_{14}CH_2OH$] (Desmarias, 1997) reduces their surface tension and promotes sinking to bottom. This makes it easier to focus on all animals in the same way. Other sorting media are glycerol and propylene glycol, or lactic acid used for clearing tissues of small crustaceans (Omori & Ikeda, 1984). Contamination of a zooplankton sample by large quantities of phytoplankton makes the analysis more difficult. In this case, staining of animals by adding Eosin Y is a helpful tool. A few drops are enough for a 100 ml sample volume; several hours should be allowed for staining (Edmondson, 1971).

Lund et al. (1958), Cassie (1971) and others have considered the statistical aspects of counting errors, which allow identifying how many organisms have to be counted in order to obtain adequate estimation of abundance. The required accuracy of results depends on the purpose of the work. To detect differences between total

zooplankton abundance in space or time of 100%, an accuracy of 50% is adequate, and any time spent in making more accurate estimates is largely wasted. Generally, an error of ±20% is acceptable. If all organisms are randomly distributed, following the Poisson distribution, the accuracy of a sample and the precision of a single count depends only on the number of specimens counted. The 95% confidence limits ($C.L._{.95}$) are calculated from the number of counts (n) and the significance level of the Poisson distribution at the 5% probability error of 1.96:

$$C.L._{.95}[\%] = \pm 1.96 \cdot \left(\frac{100}{\sqrt{n}}\right)$$

In practice, one or more counting chambers (aliquots) with the same concentration should be analysed until 100 specimens of the most abundant taxonomic groups are counted in a sample (HELCOM, 2005).

The estimations of abundances of the remaining (less common) zooplankton groups are of lower precision. If the counting procedure is continued until 100 specimens of the other groups are reached, neglecting the more abundant groups, the different sub-sample sizes must be considered in the successive calculations. Finally, the remaining part of the total sample can be surveyed for rare species.

The number of individuals per unit volume of water is defined as abundance (N). Its calculation (e.g., as ind./m^3) needs to consider the number of counts (n), the fraction of the sample counted (k, i.e. the proportion of total volume to sub-sample volume), and the amount (volume) of water filtered by the sampling net (V, m^3):

$$N = \frac{n \cdot k}{V}$$

The abundance values for certain zooplankton species are further used for calculation of other structural (e.g. biomass) and functional (productivity, feeding rates, decomposition of organic matters, etc.) characteristics of populations of single species as well as entire zooplankton community. Information on methods for determining functional characteristics of zooplankton can be found elsewhere (see the List of references).

3.3 Biomass determination

The knowledge about body mass of zooplankton organisms is essential for the analyses of their productivity, energy balance calculations, and estimation of zooplankters' role in the trophic webs. Therefore, the calculation of biomass is a next step in zooplankton community analysis. For the adequate biomass calculation, the suitable individual body mass values or proper morphometric approaches are applied (for reviews see: Table 4.12 in Postel et al., 2000; Telesh & Heerkloss, 2002, 2004). Such biomass determination is zooplankton species-specific, in contrast to quantifying the biovolume or the other sum biomass parameters of the entire sample by volumetric or other procedures (for details see Postel et al., 2000). The zooplankton biomass values obtained with the help of the individual mass values or length/mass correlations are advantageous because these results cannot be falsified by phytoplankton and detritus that sometimes are very abundant in the zooplankton samples.

For zooplankton monitoring purposes in the Baltic Sea, the individual body mass values suggested by Hernroth (1985) are recommended. This compilation includes individual wet mass values of six common copepod and three cladoceran taxa, determined based on the body volume calculations using the morphometric approaches (Chojnacki & Jankowski, 1982; Chojnacki, 1983, 1986), and the successive conversion to wet mass of the organisms. The compilation was supplemented by literature data for rotifers, chaetognaths, appendicularians and some other copepods. Seasonal and regional differences were considered; therefore, the amount of data was sufficient for precise determination of the individual mass values, which is important as rough body mass calculations may cause significant errors when multiplied by large individual numbers. Therefore, usage of individual body mass values and length to mass ratios based on direct measurements is strongly recommended. There are some of those available from the Northern Baltic Sea (Kankaala & Johansson, 1986; Kankaala, 1987; Tanskanen, 1994); they are based on the kryo-conservation technique (Latja & Salonen, 1978; Salonen, 1979). For rotifers, direct species-specific estimations of carbon mass of 13 species are available for biomass calculation (Telesh et al., 1998).

The Baltic Sea as a brackish water system with a horizontal salinity gradient from south-west to north-east and a permanent vertical salinity stratification of the central basins is a unique pelagic ecosystem with limited distribution ranges of marine and freshwater species. The location of the Baltic Sea in the temperate climatic zone with oceanic impact in the south-western part and continental impact in the north-eastern areas affects the whole ecosystem through seasonality by causing a pronounced seasonal succession of plankton populations.