COMPARISON OF DIFFERENT METHODS OF BALTIC ZOOPLANKTON BIOMASS ESTIMATIONS

Witek, Z.[1], Breuel, G.[2], Wolska-Pys, M.[3], Gruszka, P.[4], Krajewska-Soltys, A.[1], Ejsymont, L.[5], Sujak, D.[5]

[1] Sea Fisheries Institute, ul. Kollataja 1, 81-332 Gdynia, Poland
[2] Institute of Baltic Sea Research, Seestr. 15, D-18119 Rostock-Warnemünde, FRG
[3] Institute of Environmental Protection, Branch of Gdansk, ul. Kollataja 1, 81-332 Gdynia, Poland
[4] Academy of Agriculture, ul. Krolewicza Kazimierza 4, 71-550 Szczecin, Poland
[5] Sea Fisheries Institute, Plankton Identification and Sorting Center, ul. Krolewicza Kazimierza 4, 71-550 Sczcecin, Poland

Abstract. Four different methods of zooplankton biomass measurement were compared during a BMB workshop in Gdynia Poland in Dec. 1992: Individual Body Volume (IBV) method, Standard Size Classes (SSC) method, Standard Weight (SW) method, and volumetric method. IBV values were used as reference values.
The volumetric method showed the greatest deviations from IBV values, reaching as much as 200%. SSC and SW methods showed deviations of 7 to 34% and 10 to 31%, respectively, in the case of total zooplankton biomass, and deviations of 11 to 42% and 22 to 76%, respectively, in the case of mean absolute deviations of individual taxons.
Due to the relative simplicity and short analysis time of the SW method, compared to IBV and SSC methods, this method is recommended for further application in Baltic Sea studies. However, this method needs revision of standard weights for some taxons. The SSC method may be recommended for those areas, communities or species for which there are no weight standards available. The use of the volumetric method should be terminated.

INTRODUCTION

Mesozooplankton is an important component of the Baltic Sea community and as such it was choosen as one of the parameters to observe long-term changes in the ecosystem, within the framework of the Baltic Monitoring Programme (HELCOM 1988). In this programme is recommended the standard weights method for the zooplankton biomass estimation (HELCOM 1988). This method requires microscopic analysis of the plankton composition and also gives the biomass of individual taxa (Hernroth 1985). Different laboratories use other methods: The method of displacement volume (Lillelund and Kinzer 1966) gives a very crude estimation of the total mesozooplankton biomass. Other methods are based on nomogrammes (Tschislenko 1968), individual body volume measurements (Chojnacki *et al.* 1980, 1982) or standard size classes (Witek 1986, Witek , Krajewska-Soltys 1989). Besides information on stocks they provide in parts also a basis for rough estimation of rates of some bioenergetical processes related to body weight (Suschtchenia 1972, Ikeda 1985).

Microscopical analyses of plankton are very difficult and time-consuming. In seeking for alter-native methods of plankton analyses different techniques have been developed. For example optical and electrical particle counters (Sheldon , Parsons 1967, Herman *et al.* 1993), flow-cytometers (Yentsch , Horan 1989), image analysis systems (Berman *et al.* 1984) and acoustic methods (Flagg , Smith 1989). Despite a variety of limitations (like inability of some techniques to distinguish between living and non-living particles; pure shape recognition etc.) progress has occurred in method development and much more is expected. Nevertheless, at present, as well as in the nearest future, only microscopic methods are acceptable for ecological studies and are widely used. Therefore there is a need to compare some of the most popular traditional methods, in order to be aware of their accuracy and, if possible, to improve them.

MATERIAL

Three samples of zooplankton from different regions, seasons and water layers were analysed (Table 1). All samples were taken with a WP 2 net (mesh size 100μm). In total 852 specimens were identified and measured.

METHODS

Four methods have been compared: Individual Body Volume (IBV) method, Standard Size Classes (SSC) method, Standard Weight (SW) method and volumetric method.

Table 1. Locations of zooplankton sampling and number of individuals measured.

Station	Region	Date	Water layer	n
Z	Gulf of Gdansk	April 1987	30 - 65 m	332
G2	Gdansk Deep	August 1987	0 - 15 m	144
113	Arkona Sea	October 1992	0 - 45 m	376

The IBV method

The methodology described in BMB-recommendation No.10 (Hernroth 1985) was used. For copepods only volumes of cephalothorax and abdomen were measured. Antennae and legs were assumed to contribute 3% to the total body volume. The volumes of nauplii, rotifers, *Evadne nordmanni, Bosmina coregoni maritima* and *Polychaeta* larvae were calculated as for ellipsoids, volumes of *Podon* spp. and *Appendicularia* were calculated as for two ellipsoids.

The SSC method

Organisms were classified into fixed size classes of the width 0.3 $\log_{10}V$ (where V is the body volume), on the basis of their length and length : width (or length : diameter) proportions, with the help of a purposely designed worksheet (Figure 1). In this worksheet lengths of bodies of different shapes but of the same fixed volume, corresponding to the boundaries between classes, are given. For the use with a particular microscope such a worksheet may be recalculated, so the dimensions may be expressed in divisions of eye-piece measuring plate, instead of µm (mm). Volume (wet weight) which is assigned to the individual size class is a geometric mean of lower and upper boundaries of the size class. For example, the mean volume (wet weight) in the size class from 1 mln µm³ to 2 mln µm³ (1 - 2 g) is

$$\sqrt{1 * 10^6 * 2 * 10^6} = 1.414 * 10^6 \ \mu m^3 \ (1.414 \mu g).$$

The width of the size classes (0.3 $\log_{10}$ V) is almost equal to 1 $\log_2$ V, which means that volume (wet weight) at upper class boundary is two times greater than at lower boundary as well as mean volume (wet weight) in class n + 1 is two times greater than in class n. Such a worksheet can be extanded to every size range and serve as a uniform basis for the whole community size structure studies.

The SW method

Standard weights proposed in the BMB-recommendation No. 10 (Hernroth 1985) were used. There were no standard weights for *Acartia tonsa*, copepod eggs and some meroplanktonic larvae.

The volumetric method

The method described by Lillelund and Kinzer (1966) was used. Sucking was stopped after one minute.

In order to investigate the effect of various methods on zooplankton metabolism estimation, respiration was calculated according to IBV, SSC and SW data on

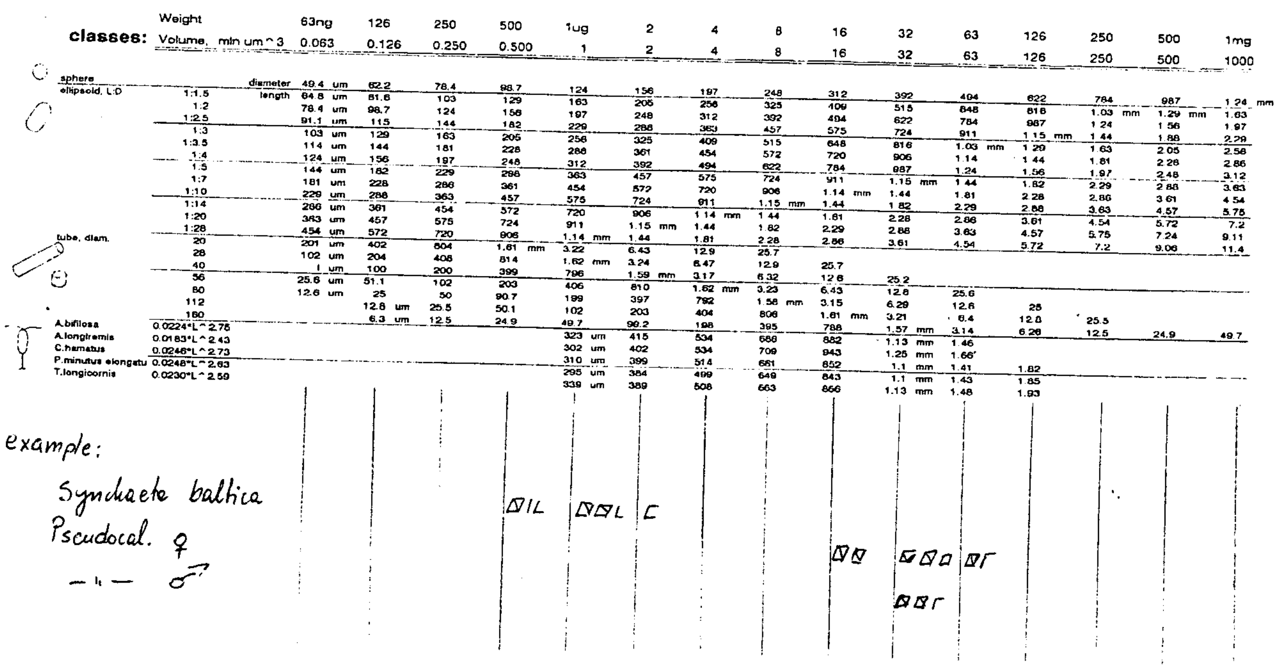

Figure 1. Worksheet for the Standard Size Classes method

zooplankton size composition. For calculations the relationship respiration - body weight - temperature of Ikeda (1985) was used. For all organisms a coefficient 0.06 µg C/µg wet weight was adopted for converting wet weight to carbon units. Calculations were done for the temperatures of 4, 17 and 10°C at stations Z, G2 and 113, respectively.

RESULTS

The results obtained with the IBV method were used as reference values. This method, although precise, is extremely time-consuming. The measurement of 500 specimen and the calculation of their body volumes requires one man week of work. Using the SSC method the same sample may be analysed within one working day and with the SW method - within several hours. The volumetric method needs only tens of minutes but of course this method does not give any information on the taxonomic composition of the sample.

With respect to total zooplankton biomass estimation, the SSC and SW methods showed similar accuracy. Deviations from IBV values were in the range of 7 to 34% in the case of SSC and 10 to 31% in the case of SW. The volumetric method consistantly produced higher values than the IBV method, with a deviation of 60 to almost 200% (Table 2).

Deviations were calculated for individual taxa if at least 20 measurements were done. In one sample (station 113) the SSC method always produced higher values (14 - 56%) of mean body weights than the IBV method (Figure 2). In the remaining two samples SSC means were more similar to IBV values and deviations did not exceed 25% (Figure 3). In 9 cases of 18, standard weights (SW), differed from IBV means for more than 25% and in 4 cases by more than 50%. The most deviant were standard weights for nauplii of *Temora longicornis*, nauplii and copepo-dites IV-V of *Pseudocalanus*

Table 2. Comparison of total mesozooplankton biomass as estimated with different methods. The percent differences from IBV values are given in brackets. A density of 1 g cm^{-3} is assumed.

Method	Stat. Z	Stat. G2	Stat. 113
Volumetric	1.013cm^{-3} (+63.4%)	1.359cm^{-3} (+62.0%)	1.840cm^{-3} (+199.2%)
IBV	0.620 g	0.839 g	0.615 g
SSC	0.676 g (+9.0%)	0.781 g (-6.9%)	0.825 g (+34.1%)
SW	0.430 g (-30.6%)	0.953 g (+13.6%)	0.675 g (+9.8%)

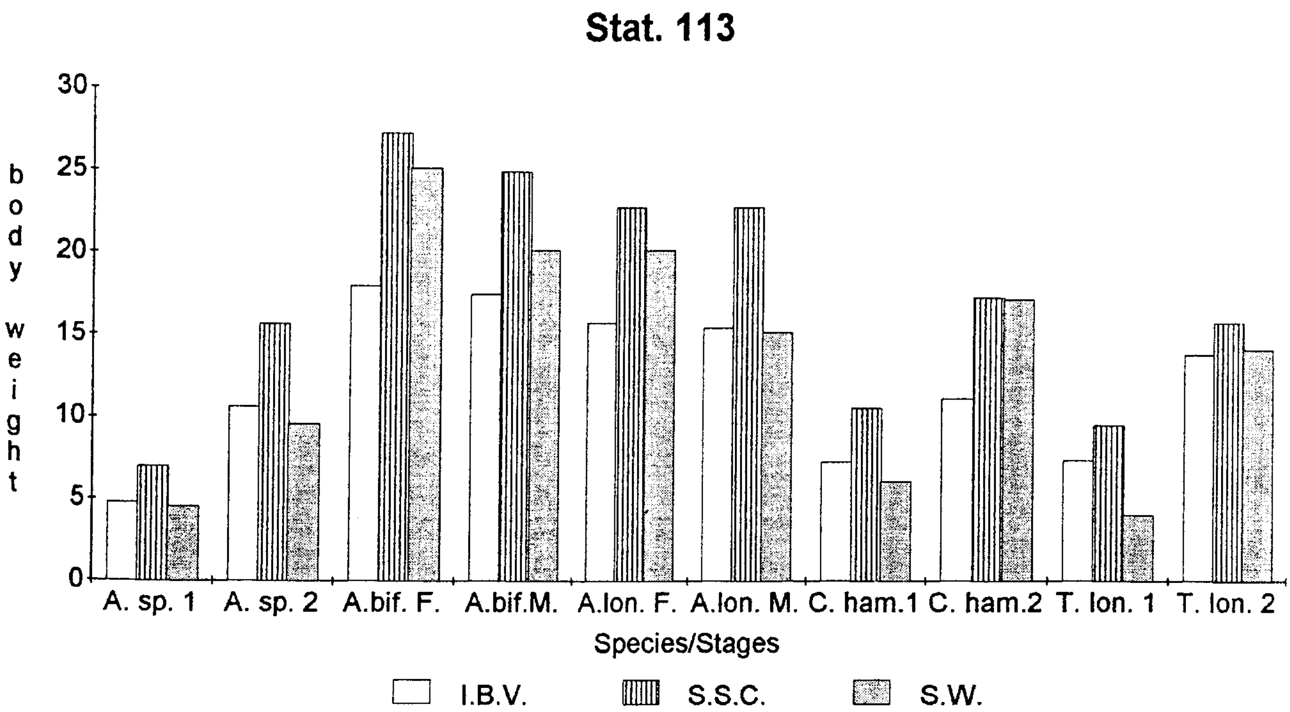

Figure 2. Mean body weight (µg) of individual taxa and stages, estimated with different methods in the Arkona Sea, October 1992 (1 - copepodit I - III, 2 - cop. IV - V, F - female, M - male, A. sp. - *Acartia* spp., A. bif. - *Acartia bifilosa*, A. lon. - *Acartia longiremis*, C. ham. - *Centropages hamatus*, T. lon. - *Temora longicornis*)

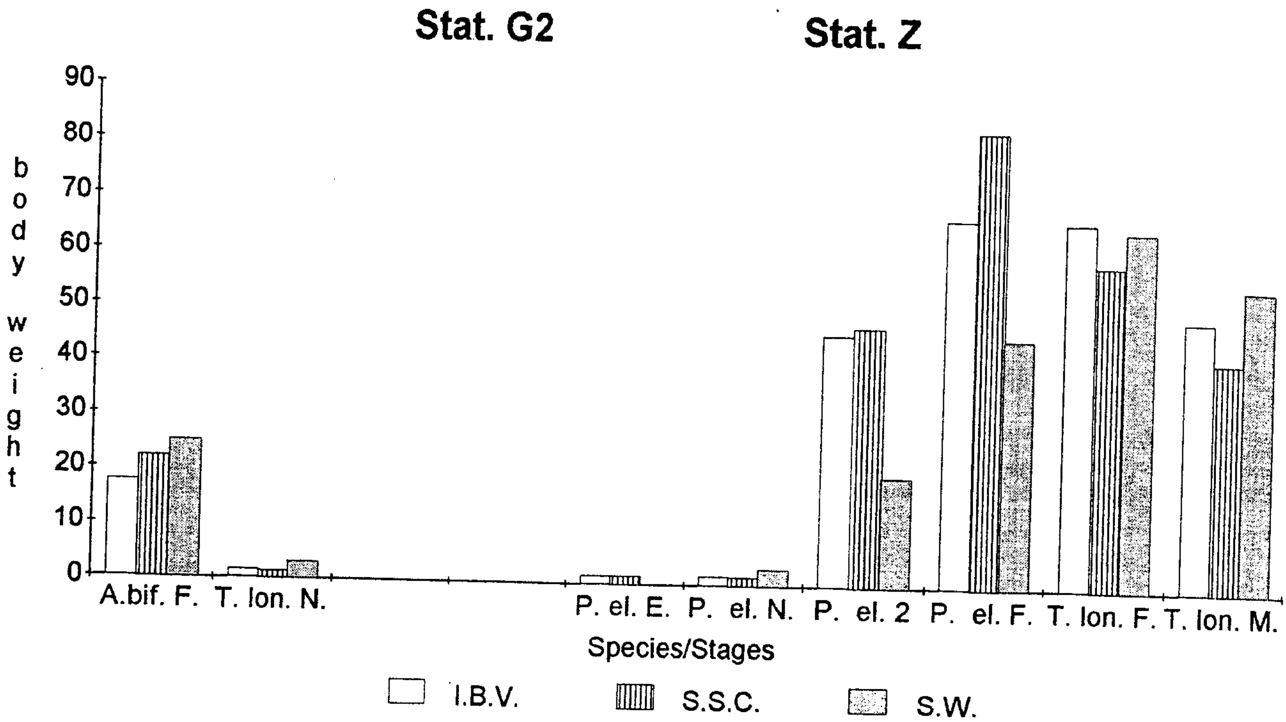

Figure 3. Mean body weight (μg) of different taxa and stages, estimated with different methods in the Gulf of Gdansk, April 1987 and the Gdansk Deep, August 1987 (E - eggs, N - nauplii, P. el. - *Pseudocalanus elongatus*, for other abbreviations see also legend of Figure 1)

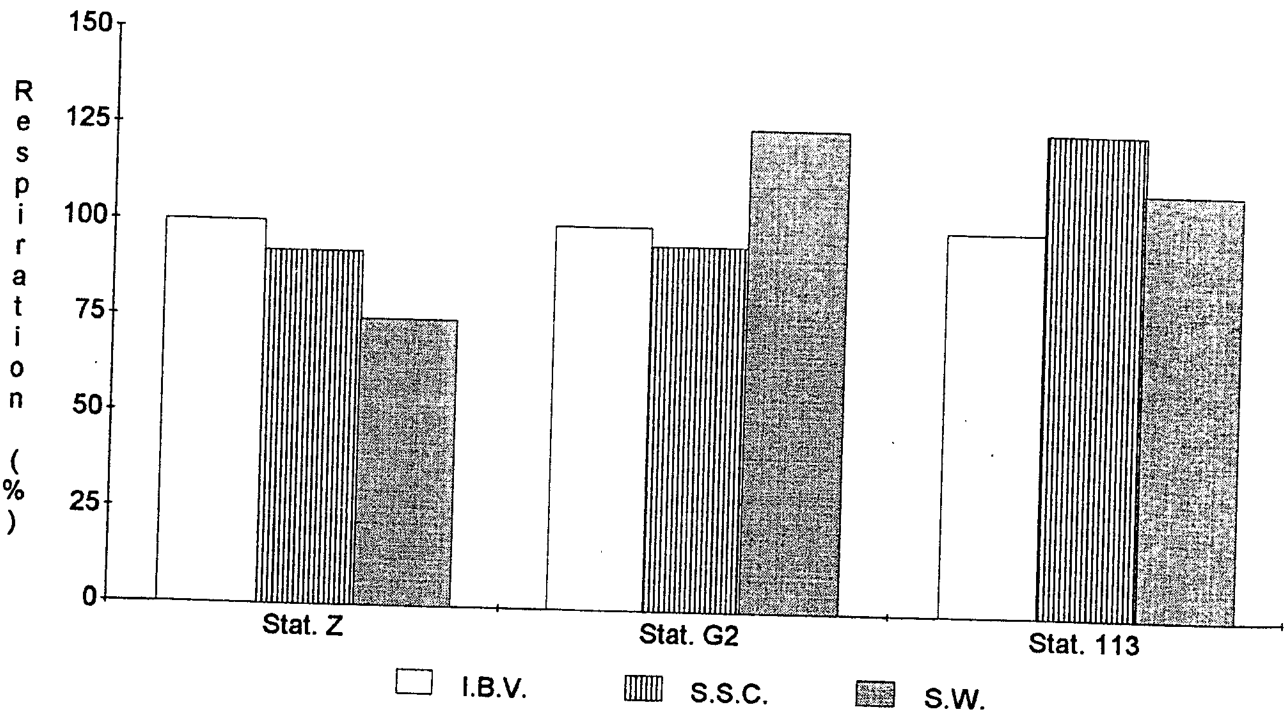

Figure 4. Comparison of relative mesozooplankton community respiration (%) calculated on the basis of specimen weights with different methods (IBV values are taken as 100%)

elongatus and copepodites IV-V of *Centropages hamatus* (Figure 2 and 3). Mean absolute deviations of individual taxon biomass using the SSC method were in the range of 11 to 42% while using the SW method - in the range of 22 to 76% (Table 3).

Table 3. Mean absolute deviation (%) of weights of individual taxons estimated with the SSC and SW methods from weights estimated with the IBV method.

Method	Stat. Z	Stat. G2	Stat. 113
SSC	11%	22%	42%
SW	40%	76%	22%

The calculations of mesozooplankton respiration based on zooplankton size structure showed similar maximum deviation of both SSC and SW methods, reaching 25%. However, in two of three samples the SSC method gave values that deviated less than 10% from the IBV values (Table 4, Figure 4).

in (1) dominance of one stage when a taxon comprises several developmental stages (e.g. nauplii I-VI; copepodites I-III and copepodites IV-V), (2) variability of sizes of the same stages but different generations, (3) greater regional variability than assumed by authors of recommendations (Hernroth 1985), (4) in the case of some taxons, insufficient number of measurements for calculating standard weight, and (5) natural variability of sizes, depending on health, food conditions etc..

Zooplankton taxonomic and size composition may be the basis for order-of-magnitude calculations of rates of different bioenergetic processes (Moloney and Field 1989, 1991) and zooplankton respiration serves here as an example. The proper reference material in this case should be of course the direct measurement of zooplankton respiration. Values obtained with SSC and SW methods, compared with the IBV method, showed that using standard weight estimations respiration may be ± 25% of the theoretical value, while with the SSC method, after some training, the deviation may be reduced to below 10%.

Table 4. Comparison of mesozooplankton respiration ($\mu l\ O_2 * m^{-3} * h^{-1}$) calculated for different stations and methods. The percent differences from IBV values are given in brackets.

Method	Stat. Z	Stat. G2	Stat. 113
IBV	27.06	92.99	32.95
SSC	24.96 (-7.8%)	183.55 (-4.9%)	1.42 (+25.7%)
SW	20.15 (-25.5%)	242.17 (+25.5%)	36.45 (+10.6%)

DISCUSSION

The comparison of methods showed that the total zooplankton biomass estimation, measured with Standard Size Classes (SSC) and Standard Weight (SW) methods, is in the range of about ± 30% the Individual Body Volume (IBV) estimations. When using the volumetric method the results may differ as much as three times the real biomass. The reason for such large discrepancies may be due to a great contribution of algae, medusae remnants, detritus or remaining water. Therefore, the results obtained with volumetric method should be treated with special caution, as they may even lead to false conclusions.

Mean weights of individual taxa obtained with the SSC method and standard weights differ more from IBV values than total biomasses which were integrated values, of both negative and positive deviations.

The reasons for SSC discrepancies may be (1) insufficient training in choosing the adequate figure patterns from the worksheet and (2) overestimation of the mean weight when the individuals in the largest size class are borderline cases with the lower size class, while in calculations the middle of each class is used as the mean weight in the class.

The reasons for standard weight deviations may lie

CONCLUSIONS

Taking into account the great and unstable deviations of the volumetric method, this method should not be further recommended. The Individual Body Volume method remains the most accurate. However, the long time required for measurements makes it inapplicable for most routine studies. This time may be reduced by computerised image analysis but is not shorter than the time required for the standard weight method.

Keeping in mind the relative simplicity of the Standard Weight method and the short time needed for an analysis, compared to IBV and SSC methods, we recommend this method for further application in the Baltic Sea studies. This method may give better results after completing and revising the standard weights of some taxons.

The Standard Size Classes method may be recommended for those areas, communities or species for which there are up to now no standard weights available and/or where the weight of individual taxons is expected to be unstable. This method does not depend on exact taxonomic identification of specimen, as required in the SW method. The SSC method may be adopted not only to mesozooplankton but also to any other group of organisms (phytoplankton, benthos, bacteria) as well as to the whole community.

REFERENCES

Berman, M., Green, I.R., Sherman, K. (1984). Applications of image analysis to the marine ecosystem studies. ICES C.M. L: 8, Biological Oceanography Committee

Chojnacki, J., Ciszewski, P., Witek, Z. (1980). The body weight of more important copepods in the southern Baltic. BMB WG 14 (mimeo)

Chojnacki, J., Janowski, M. (1982). Relationship between body volume and length of marine cladocera in the eastern sector of southern Baltic proper. BMB WG 14 (mimeo)

Flagg, C.N., Smith, S.L. (1989). On the use of the acoustic Doppler current profiler to measure zooplankton abundance. Deep Sea Res. 36 (3A): 455 - 474

HELCOM (1988). Guidelines for the Baltic Monitoring Programme for the third stage. Part D. Biological determinands. Baltic Sea Environment Proceedings 27D: 1 - 161

Herman, A.W., E.A. Cochrane, D.D. Sameoto (1993). Detection and abundance estimation of euphausiids using an optical plankton counter. Mar. Ecol. Prog. Ser. 94 (2): 165 - 173

Hernroth, L. (ed.) (1985). Recommendations on methods for marine biological studies in the Baltic Sea. Mesozooplankton biomass assessment. BMB Publ. No. 10: 1 - 32

Ikeda, T. (1985). Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Mar. Biol. 85 (1): 1 - 11

Lillelund, K. and Kinzer, J. (1966). Absetz- und Verdrängungsvolumen von Planktonproben. Untersuchungen zur Methodik. Intern. Rev. ges. Hydrobiol. 52: 757 - 774

Moloney, C.L. and Field, J.G. (1989). General allometric equations for rates of nutrient uptake, ingestion and respiration in plankton organisms. Limnol. Oceanogr. 34, 1290 - 1299

Moloney, C.L. and Field, J.G. (1991). The size-based dynamics of plankton food webs. I. A simulation model of carbon and nitrogen flows. Journal of Plankton Research, 13 (5): 1003 - 1038

Sheldon, R.W., Parsons, T.R. (1967). A practical manual on the use of the Coulter counter in marine science. Coulter Electronics, Toronto

Suschtchenia, L.M. (1972). Intensiwnost dychania rakoobrasnych. Kiew, Izdat. Naukowa Dumka (in Russian)

Tschislenko, L.L. (1968). Nomogramy dla opredelenia vesa wodnych organismow po rasmeram i forme tela. Moskwa, Izdat. Nauka (in Russian)

Witek, Z. (1986). Seasonal changes in the composition and size structure of plankton in the near-shore zone of the Gulf of Gdansk. Ophelia suppl. 4: 287 - 298

Witek, Z., Krajewska-Soltys, A. (1989). Some examples of the epipelagic plankton size struc-ture in high latitude oceans. Journal of Plankton Research. Vol. 11, No. 6: 1143 - 1155

Yentsch, C.M., Horan, P.K. (eds.) (1989). Cytometry in aquatic sciences. Cytometry Vol.10, No. 5