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Research Article

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Abstract

Intercomparison of nets commonly used for mesozooplankton sampling in the Black and Mediterranean seas was attempted within the SESAME (Southern European Seas: Assessing and Modelling Ecosystem Changes) project. Five nets were compared: three Juday nets equipped with 150 μm, 180 μm and 200 μm mesh size, a Nansen net (100 μm mesh size) and a WP2 (200 μm mesh size). Replicated samples were taken at one station in the western Black Sea offshore waters in April 2009. The samples were analyzed at species level (except for meroplankton), stages (for copepods) and body length was measured for all organisms. A decrease of total abundance values was observed with increasing mesh size, due to the significantly higher numbers of animals smaller than 1 mm in the samples obtained using fine mesh size rather than with coarser nets. Few comparisons were revealed significant as regards the abundance of 1-2 mm long animals, while no significance was detected for specimens larger than 2 mm. The above differences resulted in discrepancies between nets as regards species and stage composition. Biomass values did not differ significantly between nets, due to the important contribution of the large animals (*Calanus euxinus)* to total biomass. The smallest and the largest animals revealed high variability between replicates collected using the Nansen, Juday- 200 μm and WP2 nets. Correction factors were calculated for the conversion of abundance values between each pair of nets. The differences observed between nets regarding abundance and biomass, community taxonomic composition and size structure, as well as the estimated correction factors, provide useful information for the harmonization of data obtained using the above nets in the Black Sea.

Keywords: Seagrass, restoration, *Posidonia oceanica*, site selection, transplant, Mediterranean Sea. Intercomparison, mesozooplankton sampling, Black Sea, Medierranean Sea.

Introduction

A large number of sampling gears have been produced for the study of mesozooplankton (Wiebe & Benfield, 2003) and despite the efforts for sampling standardization (UNESCO, 1968) different gears are used by institutions and even by the same institution in the same study area (Ohman & Smith, 1995; Kane, 2009). In the last decade, many studies focused on the mesozooplankton variability related to climate change and even the detection of synchronies in the world ocean (Perry *et al.,* 2004); comparisons across different ocean areas and time periods depend on the harmonization or not of sampling methods (Skjodal *et al.,* 2013).

The SESAME (Southern European Seas: Assessing and Modelling Ecosystem changes) project aimed, among other things, at the detection of changes in the mesozooplankton communities of the Mediterranean and Black Seas, based on historical data collected by the partners, and at the assessment of their current status based on newly collected data at basin scale. Given that historical data were based on samples taken using different nets, those same nets were used during the SESAME cruises (spring and autumn 2008) in different areas of the Black and Mediterranean seas to obtain comparable data for the detection of interannual changes in each study area. Namely, the following nets were used: a) a Juday net with 150 μm mesh size (used by the Institute of Biology of Southern Seas, the National Institute for Marine Research and Development, and the Institute of Oceanology - Bulgarian Academy of Science in most cruises); b) a Juday net with 180 μm mesh size (used by the Shirshov Institute of Oceanology - Russian Academy of Science); c) a WP-2 net with 200 μm mesh size (used by the Hellenic Centre for Marine Research, the Instituto de Ciencias Marinas de Andalucia, the Institute of Marine Science-METU, the Osservatorio Geofisico Sperimentale, and the Stazione Zoologica "Anton Dohrn"); d) a Nansen net with 100 μm mesh size (used by the National Institute for Marine Research and Development in the spring 2008 SESAME cruise); e) a Juday net with 200 μm mesh size (used by the Institute of Oceanology - Bulgarian Academy of Science in the SESAME cruises).

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In order to allow for a better comparison of mesozooplankton communities from the Black Sea areas, an intercomparison of the different nets was considered necessary. For this purpose, a dedicated cruise was carried out in the Black Sea on 24-26 April 2009 where all the above nets were used to collect zooplankton samples. The results of this effort are presented herein and an attempt is made to estimate a correction factor, which could be used for the harmonization of data obtained using the aforementioned nets.

Methods

Sampling was performed between 11:30 and 17:30 (local time) on 25 April 2009 on board R/V Akademik at a station (43°01.00'N 29°28.00'E) positioned over 1945 m depth. Mesozooplankton samples were collected using five nets: A) a 70 cm mouth diameter Nansen conical net of 300 cm total length (thereafter named Nansen-100); the upper part of 100 cm length was made of canvas, the middle part of 100 cm length was made of 100 μm mesh size gauze and the lower part of 100 cm length was equipped with 55 μm mesh size gauze. B) Two biconical Juday nets with 36 cm mouth diameter and 70 cm long canvas cone expanding to 50 cm diameter; the upper 30 cm part of the second cone was made of canvas and the lower 80 cm filtering part was made of gauze. One Juday net was equipped with 150 μm mesh size gauze (thereafter named Juday-150) and the second one with 200 μm mesh size (thereafter named Juday-200). C) A biconical Juday net with 36 cm mouth diameter and 120 cm long canvas cone expanding to 50 cm diameter, followed by a 180 μm mesh size net 150 cm long (thereafter named Juday-180). D) A WP-2 57 cm mouth diameter cylindro-conical net with 200 μm mesh size (thereafter named WP2-200); the upper cylindrical part was 100 cm long and the conical part 166 cm long. Each net was towed vertically from 150 m depth to the surface at a speed of 0.5 m sec^{-1}. Three replicates were collected by each net according to a rotation system from Nansen-100 to Juday-200 (Table 1). A calibrated digital flowmeter (Model 23.091, KC Denmark) was mounted at midway between the centre and the net rim for the measurement of the filtered water volumes. The measured volumes were used for the estimation of taxa abundance and biomass. Filtered water volumes were also calculated based on the area of the net mouth and the length of the released wire (Sameoto *et al.,* 2000). The comparison between the measured and calculated volumes provided a rough idea of probable clogging of the nets, since large diatoms were highly abundant in the mesozooplankton samples. After each tow, the nets were rinsed very carefully. The samples were immediately fixed with buffered formaldehyde (4% final concentration of seawater-formaldehyde solution).

All samples were analyzed by the same scientist (D. Altukhov) to avoid subjective error. Each sample was thoroughly mixed and a Hensen stempel pipette (1ml) was used to obtain subsamples for the enumeration of small-sized animals (total length ≤ 1 mm). Small-sized animals were counted in five subsamples. The rest of the sample was divided by Folsom splitter and 1/8 or 1/4 aliquot was analyzed for the estimation of the abundance of large-sized animals. Animals were counted under dissecting stereomicroscope and their total length was measured with an ocular scale at x32 magnification. Overall, at least 800 individuals were counted per sample. Copepods (adults and copepodites) were identified at species level. Copepod nauplii and other taxa were identified at the lowest possible taxonomic level and total length of each specimen was measured. For copepodite stages, the taxonomic keys by Dolgopolskaya *et al.* (1969) and by Sazhina (1969) were used.

Table 1. Net parametres, sampling time and filtered volume per replicate and net. Measured volume is based on flowmeter counts and calculated volume is based on the mouth area and length of the net tow (150 m).

Type of net	Mesh size (μm)	Mouth area (m²)	Filtering area: mouth area ratio	Replicate	Time Start	Measured volume (m⁻³)	Calculated volume (m⁻³)
Nansen-100	100	0.385	3.28	A	11:30	16.63	57.73
				B	14:55	18.70	57.73
				C	16:20	10.97	57.73
Juday-150	150	0.1	7.77	A	11:57	12.06	15.26
				B	15:25	12.12	15.26
				C	16:50	12.54	15.26
Juday-180	180	0.1	14.26	A	12:10	12.33	15.26
				B	15:42	13.61	15.26
				C	17:02	13.77	15.26
Juday-200	200	0.1	7.77	A	12:20	15.17	15.26
				B	15:56	14.19	15.26
				C	17:19	15.75	15.26
WP2-200	200	0.255	4.14	A	11:45	37.03	38.25
				B	15:10	36.41	38.25
				C	16:35	36.41	38.25

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Biomass values, as wet weight, were estimated based on the number of individuals and the individual weight given per taxon and size class in Arashkevich *et al.* (2014). *Parasagitta setosa* and *Pleurobrachia pileus* biomass values were not included in the estimation of total biomass, because their high values "skewed" the size fractionation of biomass considerably.

Abundance and biomass values of total mesozooplankton and size fractions were tested for homogeneity by Levene's test (Milliken & Johnson, 1992). In case of homogeneity, differences among nets regarding the above parameters were tested by one-way ANOVA and were considered as significant at $p < 0.05$. The LSD test was post-hoc applied for comparison between nets. In the case of non homogeneity the Dunnett's test was used to test the significance of the differences among nets (Zar, 1996). The above tests were also performed for taxa abundance. Differences between measured and estimated volume of filtered water were tested by t-test and considered as significant at $p < 0.05$. Similarities between samples regarding species composition were investigated by performing hierarchical clustering; the Bray-Curtis similarity index was estimated on square root transformed abundance data and the group average linkage was applied.

An attempt was made to calculate a correction factor in order to convert the abundance estimates of net A and make them equivalent to net B values; the following ratio estimator was used:

$$R = \left(\sum_{i}^{n} = 1 y_i \right) / \left(\sum_{i}^{n} = 1 x_i \right)$$

where R is the ratio estimator, y_i and x_i are the abundance values obtained by nets A and B, respectively, for each replicate, *n* is the number of replicates, and the subscript *i* refers to the replicate (Stehle *et al.,* 2007). Factors were calculated for all pairs of nets (e.g. WP2-200 and Nansen-100, WP2-200 and Juday-150, WP2-200 and Juday-180, WP2-200 and Juday-200, etc.).

Results

Volumes of filtered sea water measured by flowmeter were generally lower than calculated volumes (Table 1). Measured volumes represented 79-105 % of calculated volumes for all Juday nets and WP2-200. In contrast, measured volumes filtered by the Nansen-100 net constituted only 19-32% of the calculated volumes, thus differing from the above pattern. Differences between measured and calculated volumes were significant for Nansen-100, Juday-150 and WP2-200.

Abundance and biomass

The Nansen-100 net collected by far the largest numbers of animals (mean and standard deviation 5898 ± 1757 ind. m⁻³) and total abundance values declined considerably with increasing mesh size (918 ± 156 ind. m⁻³ collected by WP2-200 net) (Table 2). The abundance estimated with the Juday-150, Juday-180, Juday-200 and WP2-200 nets represented 38%, 25%, 17% and 16% respectively of the abundance estimated with the Nansen-100 net. Consequently, total abundance differed significantly between nets, except between the Juday-200 and WP2-200 (Table 3). The WP2-200 and Juday-200 nets did not collect the same amount of mesozooplankters, despite having the same mesh size, and differences seem to vary with animal size, e.g. the WP2-200 seems to be more efficient than the Juday-200 for 0.7-2 mm specimens, while the opposite is true for specimens smaller than 0.7 mm. However, differences between the above two nets were not significant for any size fraction (Table 3). Abundance values of almost all size fractions revealed the same pattern of difference between nets as total abundance values. The numbers of 0.1-0.3 mm animals sampled using the Nansen-100 net was about 6 fold higher than the number collected by the Juday-150 net and differed by a factor of 20-32 between the Nansen-100 and the coarser mesh nets (Fig. 1). Abundance values did not differ significantly between the nets with 180 and 200 μm mesh size (Table 3). The abundance of the 0.3-0.5 mm animals were maxima in the Nansen-100 samples and differed by a factor of 5-8 between the Juday-150 and the 200 μm nets; differences were significant between all nets, except between the 200 μm mesh size nets. Differences between nets were lower with increasing animal size, but they are still significant for the 0.5-0.7 mm fraction. For the larger animals (0.7-1 mm and 1-2 mm), differences are significant only between some pairs of nets and not significant for the largest animals (>2 mm).

The contribution of each size fraction to total abundance varies between nets; the smallest fraction was by far dominant in the Nansen-100 (58% of total abudance)

Table 2. Total mesozooplankton abundance (ind. m⁻³) and biomass (mg m⁻³ of wet weight). A, B, C = replicates.

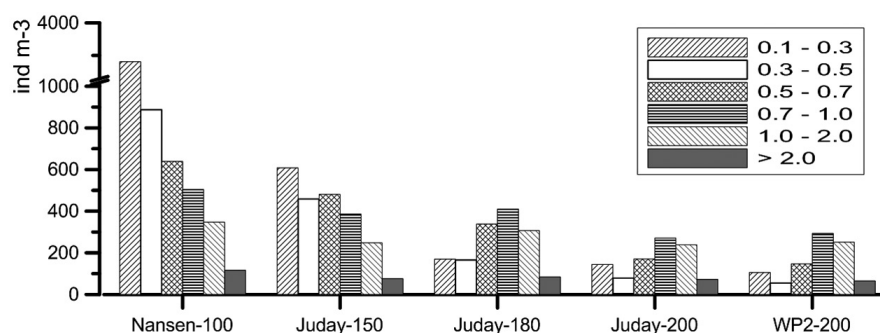|	Abundance					Biomass				
	A	B	C	Mean	STD	A	B	C	Mean	STD
Nansen-100	4963	4807	7924	5898	1757	56.9	69.3	116.4	80.9	31.4
Juday-150	2465	2302	2006	2258	233	42.8	52.1	61.2	52.1	9.2
Juday-180	1589	1418	1417	1475	99	51.4	57.7	71	60.0	10.0
Juday-200	906	1093	928	976	102	37.1	51.5	60.6	49.7	11.9
WP2-200	1066	934	754	918	156	37.9	50.3	43.6	43.9	6.2

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Fig. 1: Total mesozooplankton abundance per size class (mean values) collected by different nets.

samples and in the Juday-150 (27%) samples. The 0.7-1 mm fraction dominated in the Juday-180 (28%), Juday-200 (28%) and WP2-200 (32%) samples, while the Juday-180 seems to present a less skewed contribution of the size fractions than the other nets.

The variability of replicates was estimated through the coefficient of variation (standard deviation/mean x 100 %) (Fig. 2). The coefficient of variation of the abundance values was generally high for the samples collected by the Nansen-100 net (16-53%) and maximum variability was found

for the largest animals. The same fraction also presented the highest variability (34%) within the replicates collected by the Juday-150. The coefficient of variation was very low (5-12%) for the 0.5-2 mm fraction, which dominated in terms of abundance in the samples collected by the Juday-180 and Juday-200 nets. WP2-200 samples revealed the lowest variability for the largest fraction compared to the other nets. Similarly, the coefficient of variation was very low for total abundance values of WP2-200, while maximum variability was found for the Nansen-100 samples.

Table 3: Results of Analysis of Variance (and LSD) and Dunnett's test (in italics) performed on mesozooplankton abundance and biomass data. Significant p-values issued from ANOVA are in bold. Significant difference at p< 0.05 according to LSD are marked with an asterisk, ns=no significant difference. N-100 = Nansen-100, J-150 = Juday-150, J-180 = Juday-180, J-200 = Juday-200.

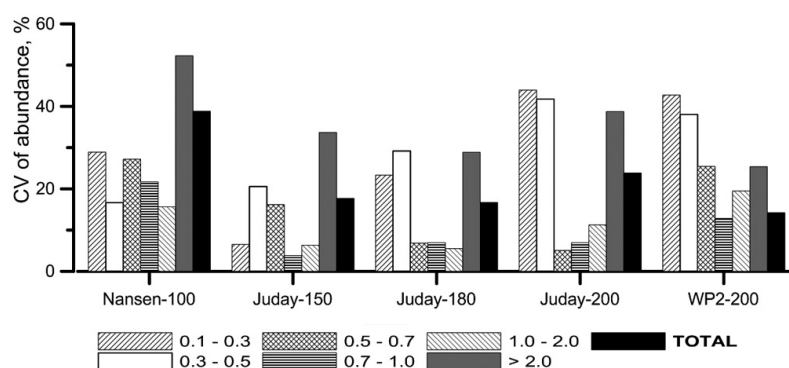|	Abundance						Biomass					
	Total	0.1-0.3	0.3-0.5	0.5-0.7	0.7-1	1-2	>2	Total	0.1-0.3	0.3-0.5	0.5-0.7	0.7-1	1-2	>2
Between nets	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.029**	0.161	0.100	**0.000**	**0.000**	**0.000**	*0.002*	**0.020**	0.447
N-100/ J-150	*	*	*	ns	*	*	ns	ns	*	*	ns	*	*	ns
N-100/ J-180	*	*	*	*	ns	ns	ns	ns	*	*	*	ns	ns	ns
N-100/J-200	*	*	*	*	*	*	ns	ns	*	*	*	*	*	ns
N-100/ WP2-200	*	*	*	*	*	*	ns	ns	*	*	*	*	*	ns
J-150/ J-180	*	*	*	*	ns	ns	ns	ns	*	*	ns	ns	ns	ns
J-150/J-200	*	*	*	*	*	ns	ns	ns	*	*	*	*	ns	ns
J-150/ WP2-200	*	*	*	*	*	ns	ns	ns	*	*	*	ns	ns	ns
J-180/ J-200	*	ns	*	*	*	*	ns	ns	ns	ns	*	*	*	ns
J-180/ WP2-200	*	ns	*	*	*	ns	ns	ns	*	*	*	ns	ns	ns
J-200/ WP2-200	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Fig. 2: Replicate variability (coefficient of variation, CV) for total abundance and abundance per size class.
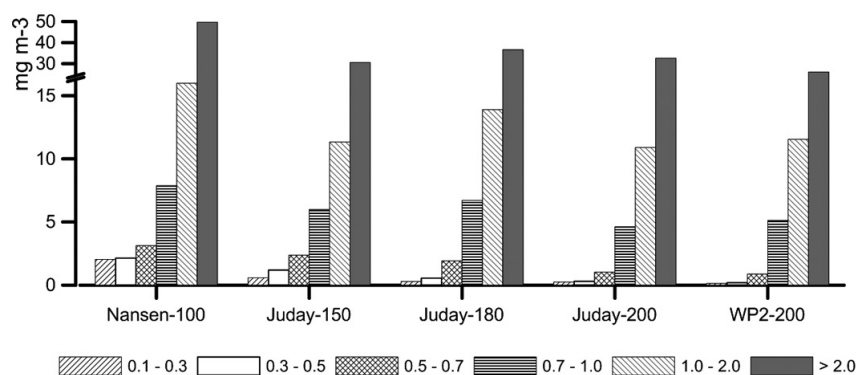
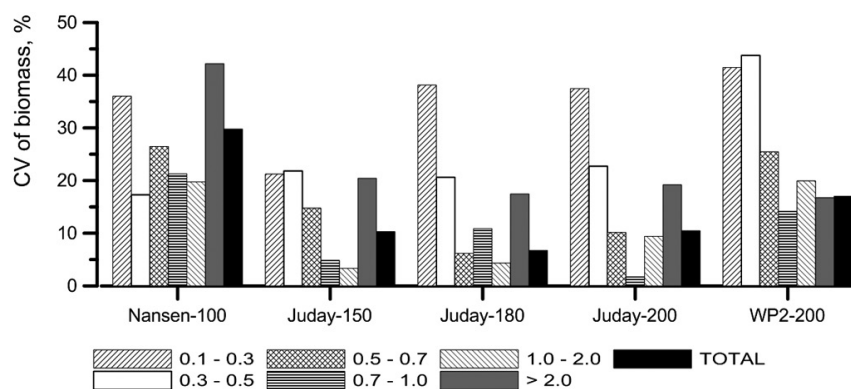**Fig. 3:** Biomass per size class (mean values) collected by different nets.

Fig. 4: Replicate variability (coefficient of variation, CV) for total biomass and biomass per size class.

Biomass values declined with increasing mesh size, though slightly higher values were obtained by the Juday-180 compared to the Juday-150 (Table 2). However, differences between nets were not significant (Table 3). In contrast to abundance, most of biomass was due to the >2mm animals for all nets (59-66 % of total biomass) (Fig. 3). The share of each fraction decreased in parallel with animal size and the biomass of the small animals was almost negligible in the samples collected by all nets. As regards biomass values per size fraction, differences between nets were significant for the smaller than 0.5 mm size fractions, except between nets with larger than 150 μm mesh size. The biomass values of the 0.5-0.7 mm size fraction did not differ significantly between the Nansen-100 and Juday-150 nets, between the Juday-150 and Juday-180 nets, as well as between the Juday-200 and WP2-200 nets. In most cases, the differences between nets were insignificant for size fractions above 0.7 mm.

The samples taken by the Nansen-100 net presented high coefficient of variation values for biomass, which ranged from 17% (for the 0.3-0.5 mm size class) to 42% (for the largest animals) (Fig. 4). Overall, low variability was revealed within the replicates of the Juday-150 net (3-22%). The coefficient of variation varied within almost the same range for the replicates collected by the Juday-180 and Juday-200 nets; maximum variability (48%) was measured between the biomass values of the

smallest sized animals and low (2-11%) for the 0.5-2 mm size classes. WP2-200 samples revealed high variability within replicates for all size fractions, especially for the small size classes (0.1-0.5 mm).

Community taxonomic composition

Copepods were dominant in the samples collected by all nets and their nauplii were very abundant in the Nansen-100 net samples (3197 ind. m^{-3}), (Fig. 5). Their abundance was lower in the samples collected by the Juday-150 net (572 ind. m^{-3}), and even lower in the Juday-180, Juday-200 and WP2-200 (51 ind. m^{-3}) samples (Fig. 5); differences between all pairs of nets were significant, except between the Juday-180 and Juday-200 (Table 4). *Pseudocalanus elongatus* was the first dominant species in terms of abundance; similar abundance values for the adults were encountered in the samples of all nets (about 200 ind. m^{-3}), while the abundance of copepodites CIV-CV was significantly lower in the 200 μm net samples compared to the other nets (Fig. 5, Table 4). The young copepodites (CI-CIII) were mostly collected by the Juday-150 and Nansen-100 nets, as confirmed also by the Dunnett's test. A small decrease in the abundance of *Paracalanus parvus* adults was observed in the 200 μm mesh size net samples (about 20 ind. m^{-3}), compared to the Juday-150, Juday-180 and Nansen-100 nets, but the differences were significant (Fig. 5, Table 4). The CIV-CV copepodites of *P.*

parvus were equally collected by the Nansen-100 and Juday-150 nets while their numbers decreased significantly in the coarser nets; the WP2-200 collected only 23% of the specimens collected by the Nansen-100 net. The numbers of CI-CIII copepodites obtained by the Juday-150 and Nansen-100 nets were 10 to 20 fold higher, respectively, than those collected by the coarser nets. The same decreasing pattern with increasing mesh size was found for the abundance of *Oithona similis* adults and copepodites and it was stronger for the young copepodites: WP2-200 collected only 2% of the young copepodites collected by the Nansen-100 (Fig. 5). Differences in abundance values were not significant mostly between the Nansen-100 and Juday-150 nets (Table 4).

No clear pattern and no significant differences were observed between nets regarding *Acartia clausi* adults and older copepodites (Fig. 6, Table 4). The numbers of young copepodites were almost double in the samples of the nets with smaller than 180 μm mesh size compared to the 200 μm mesh size nets. Interestingly, the Nansen-100 collected more specimens of *Calanus euxinus* (adults and copepodites) than the other nets, but differences were not statistically significant (Fig. 6, Table 4).

Among the other mesozooplankton groups, the appendicularian *Oikopleura dioica* was mostly collected by the Nansen-100 net (about 350 ind m^{-3}) (Fig. 6). Similar numbers of bivalve larvae were collected by the Nansen-100 and Juday-150 nets, while their abundance was lower in the samples obtained by the other nets, though not significantly. *Parasagitta setosa* was collected in significant higher numbers by the Nansen-100 net than by the nets with 180 to 200 μm mesh size. The ctenophore *Pleurobrachia pileus,* the cladoceran *Pleopis polyphemoides* and polychaete larvae were found in the samples of all nets but the abundance values were lower than 1 ind. m^{-3}. All the above species (9) and taxa were found in the samples collected by all nets.

Hierarchical clustering revealed rather high similarity between samples, since the first distinction of groups appeared at 73% similarity level: the first group includes samples collected by the Nansen-100 and Juday-150 nets and the second group is constituted of samples obtained by the Juday-180, Juday-200 and WP2-200 nets (Fig. 7). At higher similarity level, the Juday-180 samples were discriminated by the mixed group of the Juday-200 and WP2-200 samples. The highest similarity (91%) was measured between the first replicates of the above two nets, whereas the third replicate of the Nansen-100 net was quite dissimilar from the other two replicates.
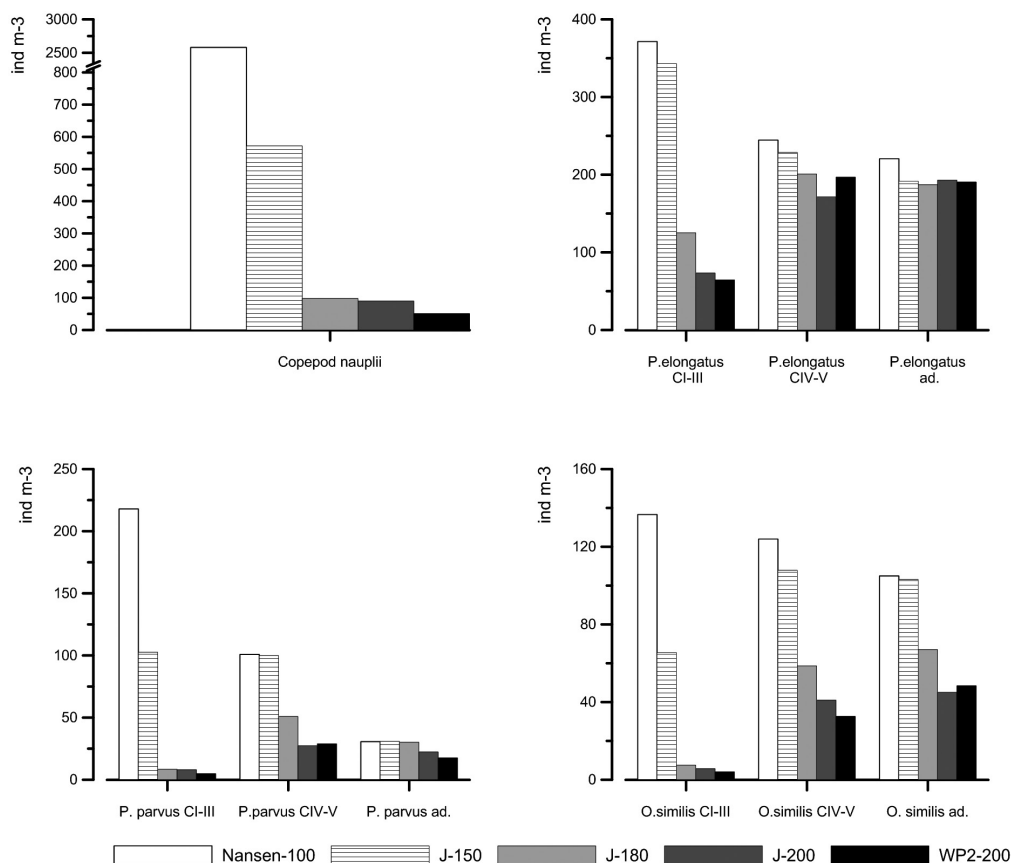

Fig. 5: Abundance (ind. m^{-3}) of copepod nauplii, and copepods *Pseudocalanus elongatus, Paracalanus parvus* and *Oithona similis* (per stage).

Table 4: Results of Analysis of Variance (and LSD) and Dunnett's test (in italics) performed on taxa abundance data. Significant difference at p< 0.05 are marked with an asterisk, ns=no significant difference. N-100 = Nansen-100, J-150 = Juday-150, J-180 = Juday-180, J-200 = Juday-200. A.clausi ad.= A.clausi adults.

Taxon	Size range	Between nets	N-100 J-150	N-100 J-180	N-100 J-200	N-100 WP2-200	J-150 J-180	J-150 J-200	J-150 WP2-200	J-180 J-200	J-180 WP2-200	J-200 WP2-200
A. clausi ad.	1.13-1.32	0.235	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A. clausi CIV-V	0.75-1.05	0.270	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A. clausi CI-III	0.35-0.66	**0.001**	ns	ns	*	*	ns	*	*	*	*	ns
P. parvus ad.	0.66-0.79	**0.037**	ns	ns	*	*	ns	ns	*	*	*	ns
P. parvus CIV-V	0.47-0.66	**0.000**	ns	*	*	*	ns	*	*	*	*	ns
P. parvus CI-III	0.3-0.45	**0.000**	*	*	*	*	*	*	*	ns	*	ns
P. elongatus ad.	0.95-1.22	0.107	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P. elongatus CIV-V	0.75-0.96	**0.010**	ns	ns	*	*	ns	*	ns	*	ns	ns
P. elongatus CI-III	0.41-0.69	***0.000***	ns	ns	*	*	ns	*	*	*	ns	ns
O. similis ad.	0.65-0.78	**0.001**	ns	ns	*	*	*	*	*	*	*	*
O. similis CIV-V	0.57-0.66	**0.000**	ns	*	*	*	ns	*	*	*	*	ns
O. similis CI-III	0.32-0.55	***0.000***	ns	ns	*	*	ns	*	*	ns	ns	*
C. euxinus ad.	3.08-3.3	0.717	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C. euxinus CIV-V	1.96-2.78	*0.107*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C. euxinus CI-III	0.84-1.59	0.334	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Copepoda nauplii	0.1-0.7	**0.000**	*	*	*	*	*	*	*	ns	*	*
O. dioica	0.1-0.5	**0.001**	*	*	*	ns	ns	ns	ns	ns	ns	ns
Bivalve larvae	0.1-0.5	0.073	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P. setosa		**0.014**	*	*	*	*	ns	ns	ns	ns	ns	ns

Ratio estimator

The values of the ratio estimator (Stehle *et al.,* 2007) for each pair of nets and regarding total abundance and abundance per size fraction are given in Table 5. The ratio varies according to the size fraction for the same pair of nets, and differences among size fraction ratios decrease in parallel with the decrease of difference between mesh sizes.

Discussion

The results of this study were based on three replicated samples taken by five nets that differed in shape and mesh size. According to Skjoldal *et al.* (2013), several factors contribute to variance and errors in the results of net intercomparison: the pattern of zooplankton distribution (random, even, patchy), net handling during and after sampling, calculation of filtered volume (calibration of flowmeter), sample transfer and processing from net to laboratory, splitting and subsampling, analyses for determination of biomass and species composition. The primary sources of error in sampling are escapement, avoidance and patchiness (Skjoldal *et al.,* 2013). Given the low number of replicated samples, a significant effort was made to avoid errors due to human interference. Sampling was performed by the same three scientists and the same calibrated flowmeter was used in all nets; processing, splitting, subsampling and analysis for species composition and biomass determination was carried out by the same scientist.

The observed decrease of total abundance and biomass values with increasing mesh size is in accordance with previous studies (Evans & Sell, 1985; Hernroth, 1987; Kršinić & Lučić, 1994; Calbet *et al.,* 2001; Makabe *et al.,* 2012; Skjoldal *et al.,* 2013). This decline was due to the significantly higher numbers of animals smaller than 1 mm in the samples obtained by the 100-180 μm mesh size nets. According to the ANOVA results, differences in the abundance values between each pair of nets with different mesh size were significant for 0.3-0.7 mm long animals. Most comparisons were revealed significant for the animals smaller than 0.3 mm and with 0.7-1 mm length. In contrast, differences in
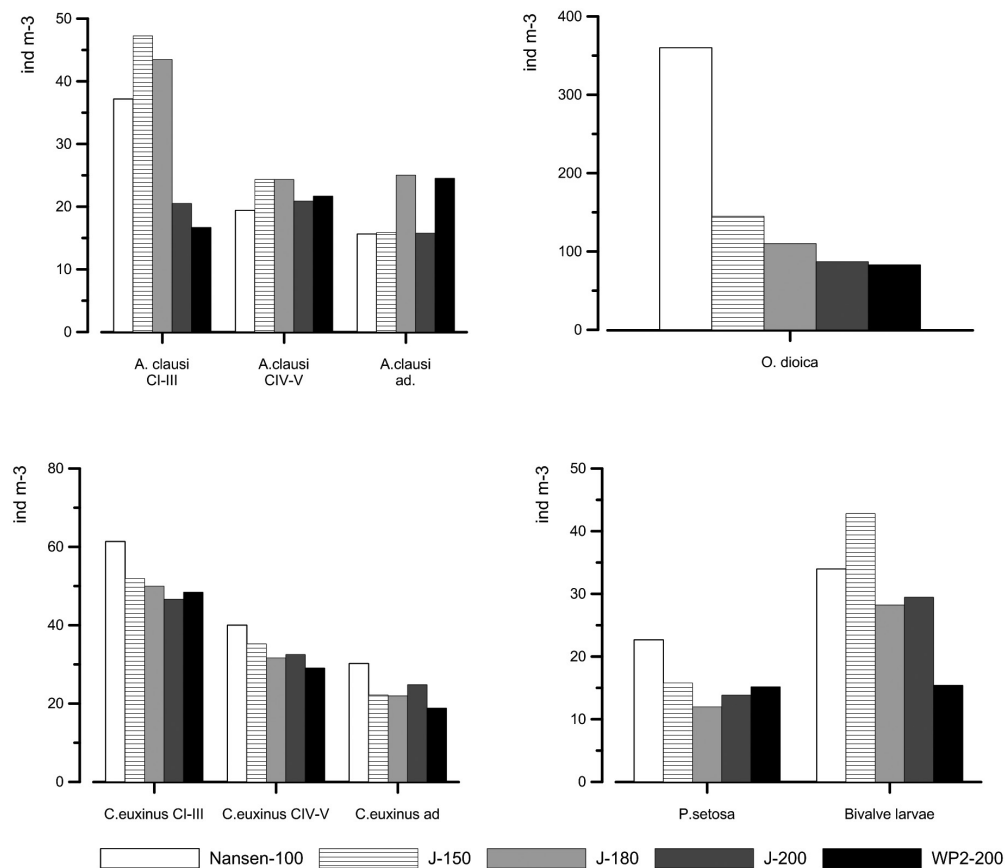
Fig. 6: Abundance (ind. m⁻³) of *Acartia clausi*, *Calanus euxinus* (per stage), *Oikopleura dioica*, *Parasagitta setosa* and Bivalvia larvae.

the abundance of 1-2 mm long animals were significant only between a few pairs of nets, while no significance was detected for specimens larger than 2 mm. The inefficiency of the WP2-200 and Juday-200 nets as regards the collection of the smallest fraction resulted in underestimation of total abundance by a factor of 6-6.4 compared to the Nansen-100. In the NW Mediterranean Sea, the comparison between a 53 μm and a 200 μm mesh size net revealed a difference in total mesozooplankton abundance by a factor of 4.4 (Calbet *et al.*, 2001). The abundance of nauplii collected by a 55 μm mesh size net in the Red Sea, was two orders of magnitude higher than that

obtained by a 150 μm mesh size net, while the abundance of copepodites and adults was 3-7 fold higher (Böttger-Schnack *et al.*, 2008). A Nansen net equipped with 60 μm mesh collected significantly higher numbers of copepod nauplii than a similar net with 160 μm mesh, while the latter was more efficient for the collection of adult copepods (Hernroth, 1987). According to the previous author, the difference was even more important in the collection of nauplii between a WP2 with 90 μm mesh and a similar net with 200 μm mesh; no significant difference was observed in their capacity to collect copepod adults and juveniles. Interestingly, the Juday-180 revealed a less
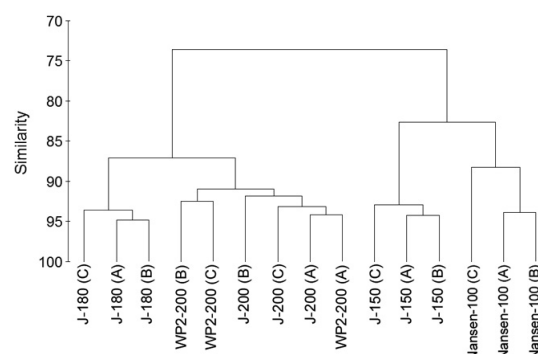

Fig. 7: Hierarchical clustering of the three replicated (A, B, C) samples collected by the five nets.

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skewed contribution of all size fractions and, therefore, it could be considered as more appropriate for representative collection of mesozooplankton than the other nets.

Very few previous studies have attempted a comparison between nets regarding community taxonomic composition (Cook & Hays, 2001; Rebstock, 2002; Makabe *et al.*, 2012; Skjoldal *et al.*, 2013). In addition, analysis of community size structure both in terms of abundance and biomass was restricted to three size fractions (Skjoldal *et al.*, 2013). Our results show a significant difference between the Juday-180 and Juday-200 nets as well as between the Juday-180 and WP2-200 nets for a wide range of sizes (0.3 to 1 mm). The difference between the Juday-180 and Juday-200 was also significant for the size range of 1-2 mm. The differentiation between the nets of this study regarding the collection of animals smaller than 1 mm, could account for the observed discrepancies between nets in species and stage composition. Indeed, the Nansen-100 and Juday-150 nets resulted in significanlty higher abundance values of *O. similis* (all stages), *P. elongatus* and *P. parvus* copepodites, *A. clausi* CI-CIII copepodites, *O. dioica* and bivalve larvae, compared with the coarser nets. The above differences in taxa composition apparently resulted in the distinction between the finer (Nansen-100 and Juday-150) and coarser (Juday-180, Juday-200, WP2-200) nets is reflected in hierarchical clustering. In contrast, no significant difference between nets was detected for larger-sized specimens, such as *P. elongatus* and *P. parvus* adults, *A. clausi* CIV-CVI and *C. euxinus* adults and copepodites. Differences in the collection of several taxa and/or size fractions by various mesh size nets have also been observed in previous studies. Skjoldal *et al.* (2013) pointed out the major influence of mesh size on community composition of samples. *Oithona helgolandica* (now *Oithona similis)* and early copepodites of *Calanus australis* and *Drepanopus forcipatus* were more efficiently collected by a 66 μm mesh net than a 150 μm mesh net (Antacli *et al.,* 2010). A 150 μm net is expected to catch substantially more in-

dividuals of 0.2 mm width (older copepodites of *Oithona, Acartia* and younger copepodites of *Pseudocalanus, Calanus*) than a 200 μm net (Skjoldal *et al.*, 2013). Even a small difference in mesh size can affect the abundance of some taxa, e.g. a 180 μm mesh size MOCNESS gave lower abundance values for cladocerans, appendicularians, echinoderms and polychaete larvae than a 200 μm mesh size WP2 or Multinet, though no clear difference was revealed for small forms (copepod nauplii, *Oithona* spp., *Oncaea* spp.) (Skjoldal *et al.*, 2013). According to this study, the abundance of the 0.3-1 mm sized animals (including young copepodites of *A. clausi*, adults and copepodites of *P. parvus,* older copepodites and adults of *O. similis*) differed significantly between the Juday-180 and each of the 200 μm nets; in contrast, no difference was revealed for the 0.1-0.3 mm sized animals (i.e. copepod nauplii, younger copepodites of *O. similis*). It seems that 180 μm and 200 μm gauze retain very small animals, while slightly larger animals are retained more by 180 μm rather than 200 μm gauze. It is noteworthy that the WP2-200 and Juday-200 samples revealed higher similarity regarding species composition and abundance than the Juday-200 and Juday-180 nets; apparently, pore size is the major factor for net efficiency and even a small difference in pore size (20 μm) plays a more important role than the shape (mouth opening, length, etc.) of the net.

Unlike abundance values, differences among nets were not significant for biomass values. This could be due to the strong contribution (more than 60%) to total biomass of the large animals fraction (>2 mm); namely, the older copepodites and the adults of *C. euxinus*, which were almost equally captured by the different nets. In contrast, Skjoldal *et al.* (2013) observed a decrease of biomass values (as dry weight) with increasing mesh size (from 55 to 400 μm). The above authors noticed that the 0.5-1 mm fraction was dominant in terms of biomass in the samples collected by all nets, while the biomass of larger animals was very low and did not increase considerably in the coarser nets. These samples included more than double

Table 5. Abundance ratio estimator (size fractions and total) per pair of nets. In bold are the ratio between nets that revealed significant differences in abundance values (see Table 3).

	0.1-0.3 mm	0.3-0.5 mm	0.5-0.7 mm	0.7-1 mm	1.0-2.0 mm	>2.0 mm	Total abundance
WP2-200 / Nansen-100	**0.031**	**0.062**	**0.230**	**0.581**	**0.723**	0.561	**0.156**
WP2-200 / Juday-150	**0.174**	**0.119**	**0.306**	**0.760**	1.013	0.864	**0.407**
WP2-200 / Juday-180	0.623	**0.331**	**0.435**	**0.715**	0.820	0.778	**0.622**
WP2-200/ Juday-200	0.733	0.694	0.863	1.083	1.052	0.905	0.941
Juday-200 / Nansen-100	**0.042**	**0.089**	**0.267**	**0.537**	**0.688**	0.620	**0.165**
Juday-200 / Juday-150	**0.237**	**0.172**	**0.354**	**0.702**	0.963	0.955	**0.432**
Juday-200 / Juday-180	0.849	**0.477**	**0.504**	**0.660**	**0.779**	0.859	**0.662**
Juday-180 / Nansen-100	**0.050**	**0.186**	**0.530**	0.813	0.883	0.722	**0.250**
Juday-180 / Juday-150	**0.279**	**0.361**	**0.704**	1.062	1.236	1.112	**0.653**
Juday-150 / Nansen-100	**0.179**	**0.517**	0.752	**0.765**	**0.714**	0.649	**0.383**

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the number of taxa than this study and were dominated by cladocerans, while the contribution of large taxa (e.g. *Calanus* adults), was very low. Consequently, the comparison between the 180 μm and 200 μm nets produced fairly similar biomass results with most of it in the <1 mm fraction (Skjoldal *et al.*, 2013). Apparently, dissimilarity in biomass values between samples collected by different mesh size nets depends largely on taxonomic and size composition of the community. Apart from the differences in community composition between areas, seasonal variability could affect the efficiency of nets within the same area. The comparison of abundance values per size fraction and taxon, for 80 μm and 200 μm nets, on a seasonal basis, revealed that the retention efficiency of the nets is seasonally dependent (Riccardi, 2010).

The collected mesozooplankton samples contained a large number of pennate diatoms, especially those obtained by the Nansen-100 net. Under these conditions, the nets are usually clogged. Indeed, the observed differences between the measured filtered water volume and the calculated volume suggest a clogging impact on the filtration efficiency of the nets, which was very important for the Nansen-100 and negligible for the Juday-200 and WP2-200 nets. Clogging of the Nansen-100 net appears to be very important since the measured filtered water volume was only 19-32% of the estimated one. Nets with mesh size lower than 100 μm were found to be clogged easily in conditions of high particle abundance in the water (Smith *et al.*, 1968; Evans & Sell, 1985; Hernroth, 1987). The strong clogging of the Nansen-100 net must have favoured the capture of large numbers of the smallest animals. Even the Juday-150 caught an important number of tiny animals (i.e. copepod nauplii, CI-III copepodites of *P. parvus* and *O. similis*). Usually, the 0.1-0.5 mm size fraction is underestimated by the Juday-150 net (Kovalev *et al.*, 1977; Vasilyeva *et al.*, 2009). However, some species with body length less than 0.5 mm might be captured by 150 μm mesh nets due to the presence of appendages (Saville, 1958). Hernorth (1987) observed that the biomass of total zooplankton collected by a 90 μm mesh WP2 net was 60% higher than that by a 200 μm mesh WP2 net when particles were abundant in the sea water; during periods of low particle abundance the corresponding value was 28% higher. Clogging becomes an increasing problem with finer mesh size nets. In spring, bloom situations with chain-forming diatoms could present a serious clogging problem even with a 200 μm net (Skjoldal *et al.*, 2013).

Mesh size is not the single factor affecting net collection capacity. The Nansen net was less efficient than the WP-2 net (with the same mesh size), and efficiency decreased under unfavourable conditions (high particle abundance (Hernroth, 1987). The cylindrical-conical shape of the WP2 net is superior to the conical shape of the Nansen net, as regards the prevention of clogging (Smith *et al.*, 1968; UNESCO, 1968). It is interesting to note that the Juday-200 and WP2-200 nets did not provide significantly different samples in terms of abundance, biomass and species composition, with the exception of copepod nauplii abundance.

Variability among replicates with respect to abundance values was generally more important for the Nansen-100 than for the other nets, especially for the smallest and the largest size fractions. The 40% decrease of filtered water volume in the third replicate of the Nansen-100 net contributed largely to the increased coefficient of variation. As mentioned above, clogging probably accounts for the high variability in the trapping of tiny animals and generally in the efficiency of the Nansen-100 net. Overall, the results obtained with the Nansen-100 net should be considered with caution. Generally, the abundance values of the 0.5-2 mm sized animals presented low variability, especially those obtained with the Juday nets, suggesting their suitability for the collection of this size class, which dominated in the samples. Skjoldal *et al.* (2013) observed that the variability of abundance values increased with decreasing taxa abundance. Very high coefficient of variation values (more than 100%) were found for the larger than 2 mm size fraction. The authors attributed this large variability values to the greater mobility of larger organisms as well as to the low number of organisms constituting this size fraction, which was also true in this study. We also observed great variability among replicates of the WP2-200 and Juday-200 nets as regards the abundance values of the smallest size fraction, whose contribution to total abundance was very low. It seems that the use of coarse mesh size nets for the collection of small animals does not provide consistent results. Overall, the smallest and the largest animals revealed higher variability in their abundance values than the medium-sized zooplankters. Patchiness is a major source of replicate tow variability (Wiebe & Holland, 1968) and small scale patches tend to be averaged out with larger sample sizes due to larger mouth area (Wiebe, 1971) as in the case of the WP-2 net, which showed the lowest variability of total mesozooplankton abundance.

The values obtained using the ratio estimator should be considered with caution, given the small amount of data and environmental conditions, i.e. phytoplankton bloom resulting in net clogging, which must have significantly influenced the results obtained by the Nansen-100 net. Moreover, different ratios could result from the analysis of data gathered in another season or month, since the efficiency of the nets was found to depend on the seasonality in species and size composition (Riccardi, 2010). However, our results could shed light on the magnitude of the difference between the nets used and, therefore, be useful in cases where data harmonization is required (i.e. studies dealing with analysis of historical data, validation of ecological models). Despite the usefulness of such correction factors, very limited attempts are available in the literature (Stehle *et al.*, 2007; Antacli

et al., 2010). It is notable that the estimator of this study produced some values that are comparable with those obtained by Antacli *et al.* (2010) for a 150 μm relative to a 66 μm net. Namely, for *O. similis* adults, the estimator by Antacli *et al.* (2010) is 0.618, quite close to the 0.765 that we calculated for the Juday-150 relative to the Nansen-100 net and the 0.7-1 mm sized fraction. For the small copepodites of *O. similis*, the estimator of Antacli *et al.* (2010) is 0.208 compared to 0.179 of this study.

This study constitutes a first attempt to compare the Nansen, Juday and WP2 nets, which were (and probably still are) used mostly in the Black Sea (see Kovalev *et al.,* 1977; Konsulov, 1990; Besiktepe & Unsal, 2000; Vinogradov *et al.,* 1992; Stefanova *et al.,* 2012; Arashkevich *et al.,* 2014) and the Baltic Sea (see Hernroth, 1987; Johansson *et al.,* 1993; Flinkman *et al.,* 1998; Ojaveer *et al.,* 1998; Kornilovs *et al.,* 2001; Mollmann *et al.,* 2005). The effort was based on tests performed for a variety of parameters (abundance, biomass-total and per size classes-, taxonomic composition), for nets with the same shape and mouth diameter, but with different mesh size (Juday with 150, 180 and 200 μm mesh size), and for nets with different shape, mouth diameter and length, but the same mesh size (Juday-200 vs WP2-200), as well as for nets with different shape and mesh size. The obtained results regarding total zooplankton abundance are mostly in accordance with similar studies, deriving from a greater sampling effort (more replicates or on an annual basis). Interestingly, differences in the taxonomic composition of the samples did not concern the number of species and other taxa, apparently due to the general low diversity of Black Sea mesozooplankton. However, a clear differentiation was revealed between nets as regards the contribution of taxa in the community, as well as in the size structure of the latter. Mesh size was found to be the major factor accounting for the dissimilarities observed even between animals with a 0.2 mm difference in length. Animal shape appears to be more crucial than somatic length *per se*. Functional diversity of the zooplankton community depends on taxonomic and size composition. Consequently, differences in the biomass and functioning of the zooplankton community could emerge from the use of different nets. The differences revealed between nets with respect to total abundance and biomass, and community composition and size structure, constitute evidence of the risk of erroneous conclusions based on data obtained by different nets. On the other hand, the observed similarities/dissimilarities and the estimated correction factor could be useful for the comparison and harmonization of data obtained by the nets of this study in the Black Sea during spring. A comparison could be attempted even if the samples were collected in areas other than that of the sampling station of this study, since mesozooplankton community composition was found to be homogenous in all Black sea areas during spring 2008 (Araskevich *et al.,* 2014). Evidently, further sampling studies in other seasons and areas of the Black Sea are necessary for more complete and accurate comparison of the nets and the improvement of the correction factor. The increasing interest in the analysis of historical data, in order to detect trends or regime shifts as well as the validation of biogeochemical models, requires data harmonization and underlines the great importance of methodological intercalibration. The necessity becomes stronger when models and historical analyses are attempted in several geographic areas.

Acknowledgements

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