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HOW TO EFFECTIVELY COLLECT ZOOPLANKTON IN ILLUMINATED CAGES FOR FISH REARING?

Andrzej Skrzypczak, Andrzej Springer, Anna Goździejewska, Grażyna Furgała-Selezniow

Department of Tourism, Recreation and Ecology
University of Warmia and Mazury

K e y w o r d s: lake cages, planktonic organisms, sample collection methods.

A b s t r a c t

The use of a reliable zooplankton sample collection method is important for the quantitative and qualitative assessment of the food source available for juvenile fish. The effectiveness of zooplankton collection in illuminated cages is related to its concentration in the subsurface layer of water around or under the light source. The aim of this study was to compare the effectiveness of three zooplankton sample collection methods in illuminated cages. The experiment was conducted in Lake Maróz, Poland, in illuminated net cages. The light source was an electric bulb (24 V, 60 W), located just above water's surface and switched on 2 hours before sample collection. The zooplankton samples were collected using a bottle sampler and conical tow-net (mesh 30 μm) hauled at two different speeds. In terms of qualitative and quantitative parameters, the optimal sample collection method was plankton net hauled at a slow vertical rate (0.05 m s^{-1}). Average results were obtained using a bottle sampler (5.0 dm^3 volume). Whereas, plankton net hauled vertically at a fast vertical rate (0.10 m s^{-1}) was the least effective method, due to the displacement of water outside of the net's inlet.

JAK EFEKTYWNIE POBIERAĆ ZOOPLANKTON W SADZACH OŚWIETLONYCH DO PODCHOWU RYB?

Andrzej Skrzypczak, Andrzej Springer, Anna Goździejewska, Grażyna Furgała-Selezniow

Katedra Turystyki, Rekreacji i Ekologii
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: młodociane stadia ryb, sadze jeziorowe, metody poboru prób.

Address: Andrzej Skrzypczak, University of Warmia and Mazury, ul. Michała Oczapowskiego 5, 10-719 Olsztyn, Poland, phone: + 48(89) 523 33 84, e-mail: andrzej.skrzypczak@uwm.edu.pl

Abstrakt

Zastosowanie miarodajnej metody poboru prób zooplanktonu w środowisku sadzów oświetlonych jest istotne w ocenie ilości oraz jakości pokarmu dostępnego dla ryb. Celem pracy było porównanie efektywności trzech wariantów metodycznych poboru prób zooplanktonu w środowisku sadzów oświetlonych. Eksperyment przeprowadzono w sadzach jeziorowych (jez. Maróz) oświetlonych od zmierzchu żarówką elektryczną (24 V, 60 W), umieszczoną tuż nad powierzchnią wody. Próby zooplanktonu pobierano czerpaczem butlowym oraz siatką planktonową techniką zaciągu pionowego w dwóch wariantach prędkości holu. W analizie porównawczej efektywności poboru prób zastosowano znormalizowany wskaźnik liczebności zooplanktonu. Referencyjnym wariantem metodycznym dla badań jakościowych i ilościowych zooplanktonu był powolny zaciąg pionowy siatką planktonową (prędkość holu 0.05 m s^{-1}, teoretyczne tempo filtracji 0.08 dm^3 dm^{-2} s^{-1}). Odzwierciedlał on największą liczebność zooplanktonu w każdej grupie taksonomicznej ($p \leq 0.001$ dla Copepoda i Rotifera oraz $p \leq 0.01$ dla Cladocera). Najmniejszą efektywność poboru prób, tj. liczebność zooplanktonu i wykrywalność taksonów, uzyskano po szybkim zaciągu pionowym siatką (prędkość holu 0.10 m s^{-1}, teoretyczne tempo filtracji 0.16 dm^3 dm^{-2} s^{-1}). Pośrednie wyniki uzyskano po zastosowaniu czerpacza butlowego o objętości 5.0 dm^3. Niska efektywność siatki planktonowej przy szybkim zaciągu spowodowana była zjawiskiem wypierania wody poza krawędź otworu wlotowego.

Introduction

Zooplankton sample collection for qualitative and quantitative analysis is most often performed in water ecology and aquatic ecosystem biodiversity research (SUTHERS and RISSIK 2009, WILLIAMSON and MCGOWAN 2010). The 1950's and 60's were a time of dynamic development in the research on the effectiveness and standardization of the zooplankton sample collection methods (BOGOROV 1959, MCGOWAN and FRAUNDORF 1966). However, over the last few decades the tools and methods for manual zooplankton sample collection did not change significantly (STARMACH 1955, DE BERNARDI 1984). Plankton nets of various design and parameters, calibrated bottle samplers and traps as well as pumps continue to be the basic tools of zooplankton researchers (DHARGALKAR and VERLECAR 2004, MCGAVIGAN 2012). Certain design features were modified for the purpose of zooplankton research in particular environments (KRŠINIĆ 1990, PAGGI et al. 2001). The developments in optical, electronic and digital technologies brought new methods of measuring zooplankton quantity and biomass (REMSEN et al. 2004, BROUGHTON and LOUGH 2006). Numerous studies were conducted to compare the quantitative and qualitative parameters of plankton animals based on samples collected in various environments using various tools and methods (SLUSS et al. 2011). Standardization of zooplankton sample collection methods is crucial in order to avoid false interpretation of results (MASSON 2004). Numerous flaws of the zooplankton sample collection methods have been identified. Once submerged, each tool produces turbulent flow near the inlet, which may influence the collected sample size and the reliability of obtained results (DE BERNARDI 1984,

DHARGALKAR and VERLECAR 2004). Simple conical nets have been used for many years with little modification in design. Their major source of error is that the filtration characteristics of conical nets usually are unknown. Filtration efficiency in No. 20 mesh cone nets ranges from 40% to 77% (GREENBERG et al. 1992).

Zooplankton research is also conducted in juvenile fish rearing in illuminated cages. This method of fish rearing was developed and applied in practice in Poland during the mid-1970's (MAMCARZ 1995a) and later adopted in other countries (CHAMPIGNEULLE and ROJAS-BELTRAN 1990). In such cage environment, the main (or only) foodsource for the fish is the naturally-occurred zooplankton. The method is based on the accumulation of zooplankton around lamp placed in a cage with fish (MAMCARZ 1995b).

The use of reliable zooplankton sample collection methods in illuminated cage environments is crucial for assessing the quality and quantity of the food source available to the fish or for the analysis of the effectiveness of attracting zooplankton to the cages of various design. Various research of zooplankton in illuminated cages indicates wide range of its count per 1 liter of volume – from single to tens of thousands of individuals (ŽILIUKIENĖ 2005, CECCUZZI et al. 2010, SICHROVSKY et al. 2013). However, straight comparison of these quantities would be inconclusive, as zooplankton sampling methodologies used in research mentioned above varied. Most popular methods included suction pumps (SKRZYPCZAK et al. 1998, SICHROVSKY et al. 2013), volume samplers (GRAVES and MORROW 1988, FRISCH and WOHLTMANN 2005), and plankton nets with different mesh size ranges from 30 μm to 50 μm (MAMCARZ 1995b, MARTYNOVA and GORDEEVA 2010, FURGALA-SELEZNIOW et al. 2014, SPRINGER and SKRZYPCZAK 2015). Methods of collecting zooplankton must take into account the size of planktonic organisms (eg. freshwater rotifers and immature microcrustacea is 20 μm and more) and the specificity of the environment (GREENBERG et al. 1992). Unfortunately, these methodologies are not standardized, and their effectiveness has not been tested in environment of illuminated cages for fish rearing.

The aim of this study was to compare the effectiveness of three zooplankton sample collection methods in illuminated cages. It should be expected that not all sampling methods are equally effective for objective assessment of food source density in fish rearing cages.

Materials and Methods

Sample collection

Experiment was conducted in Lake Maróz, Poland (N: 53°31.6'; E: 20°24.5'; eutrophic type, 332.5 ha, max. depth 41.0 m) in illuminated net cages 1.0 × 1.0 × 2.5 m, square mesh size 1.2 mm. The light source was an electric bulb (24 V, 60 W), located just above water's surface and switched on 2 hours before sample collection. The zooplankton samples were collected from three fishless cages, using three distinct methods, every three nights during May 2013, usually between 22:00–23:00. In two of the methods, the samples were collected by a plankton net (mesh size 30 ,m, round inlet with diameter 0.22 m, inlet's area 0.038 m^2, filtration area 0.24 m^2, volume 9.0 dm^3) hauled vertically from the bottom of the cages to the water's surface (2.0 m) using two different tow speeds. Each haul penetrated 76 dm^3 of a water column volume. In the S_1 sampling method, each tow had an average velocity of 0.05 m s^{-1} (total hauling time of about 40s) and the estimated filtration rate was about 1.9 dm^3 s^{-1}. In the S_2 method, the same plankton net was towed at an average velocity of 0.10 m s^{-1} (total towing time of about 20s), and the estimated filtration rate was about 3.8 dm^3 s^{-1}. In both methods the plankton net was towed manually and a standard analog stopwatch was used.

In the method R, the zooplankton samples were using a TON 2 bottle sampler (made by "MERA-BLONIE" Precision & Mechanical Plants, Gdansk – Poland, 1998) – 5.0 dm^3 volume, 0.14 m diameter of inlet and 0.33 m height. It has features of the Bernatowicz bottle and Friedinger bottle (DE BERNARDI 1984). It minimizes the generation of hydrodynamic whirlpools in front of the sampler as it is lowered and allows the immediate sealing of the inlet and outlet at the desired depth. This device was used to collect samples from the middle part of the water column (0.85–1.20 m under the surface, by lowering it on calibrated rope. Next, analogously to the first two methods (S_1 and S_2), the sample was filtered using a plankton net at mesh size 30 μm. That mesh nets effectively capture small-bodied and larger-bodied zooplankton (MACK et al. 2012).

The zooplankton samples collected using all three methods were condensed to the volume of 0.1 L, preserved in Lugol's solution and conserved in a 4% formaldehyde solution (RADWAN et al. 2004). The zooplankton identification was performed until the lowest possible taxonomic unit was identified (except juvenile stages of Copepoda) in accordance with methodology KIEFER and FRYER (1978). The quantitative analysis was performed using the Sedgewick-Rafter counting chamber and reported in the volume unit (ind. dm^{-3}).

Physical and chemical parameters of water were monitored during zooplankton sample collection in illuminated cages. A multi-parametric probe (YSI Professional Plus Quatro, 2011) was used to measure temperature, dissolved oxygen and pH whereas water transparency was measured using a Secchi disc.

Abundance index and statistical analysis

Separate qualitative and quantitative analyses of the zooplankton were performed for each sample day and for each sample collection method. The standardized abundance index (SAI_{tmi}) was calculated for each identified taxonomic unit. The normalized index was obtained as follows:

$$SAI_{tmi} = \frac{X_{tmi}}{X_{ti}\max}$$

where:

SAI_{tmi} – standarized abundance index for taxa *t* for the sample collection method *(m)* on the collection date *(i)*;

X_{tmi} – abundance of taxa *t* (ind. L^{-1}) for the sample collection method *(m)* on the collection date *(i)*;

$X_{ti}\max$ – maximum abundance of taxa *t* (ind. L^{-1}) on the collection date *(i)*;

The SAI_{tmi} value (range 0–1) was used to compare the effectiveness of zooplankton sample collection. Model $n = 1$ indicates that the particular sample collection method was the most effective and each day of its use resulted in the highest quantity of the particular taxon in the volume of water. The effectiveness of the sample collection methods was analyzed in terms of three taxonomic groups (Cladocera, Copepoda and Rotifera) and comparing the SAI_{tmi} values of all species in each of those groups. Non-parametric analysis of variance was applied to assess the general differences in the standardized abundance indices for each sample collection method (Statistica 10.0 for Windows, Statsoft; Tulsa, UK). The results were processed by ANOVA with the non-parametric Kruskal-Wallis test to determine the statistically significant differences ($P < 0.05$). Correlation analysis (r – Pearson) was used to assess the correlation between the effectiveness of zooplankton collection methods and the physical-chemical parameters of water.

Results

The analysis of sample collection effectiveness in illuminated cages, was conducted during a time of dynamic quantitative changes in the lake's zooplankton structure. The highest abundance of Cladocera in all three sample collection methods was obtained on May 19th: 5 taxa in S_1 and R methods and 4 taxa in the S_2 method (Figure 1). The dominant species was *Daphnia cucullata* and its abundance in the S_1, S_2 and R methods was 455 ind. dm^{-3}, 323 ind. dm^{-3} and 379 ind. dm^{-3}, respectively.

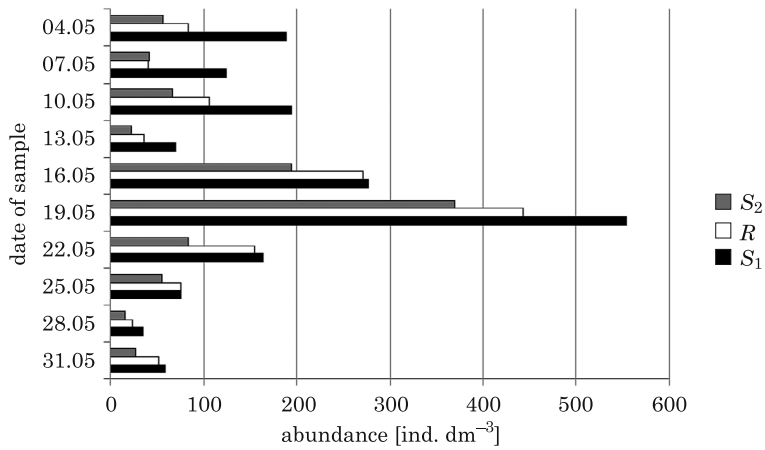

Fig. 1. Dynamics of the Cladocera abundance on the sample collection days using three sampling methods (S_1; S_2 and R – Materials and Methods)

The highest abundance of Copepoda was observed in the sample collected on May 4th, resulting from the dominance of two juvenile forms: nauplii and copepodites of *Cyclopoida* (Figure 2). All three sample collection methods yielded 10 Copepoda species and larvae forms. The Copepoda abundance was as follows: 908 ind. dm^{-3} (including 78.1% juvenile forms) in the S_1 method, 534 ind. dm^{-3} (including 85.2% juvenile forms) and 685 ind. dm^{-3} (including 74.4% juvenile forms) in the R sample collection method.

The highest abundance of Rotifera was confirmed in the sample collected on May 31st using methods S_1 and R (Figure 3). Each of these two methods yielded 8 taxa, whereas the method S_2 yielded 7 taxa. *Pompholyx sulcata* was the dominant species among the Rotifera and its abundance in the sample collection methods S_1, S_2 and R was 756 ind. dm^{-3}, 412 ind. dm^{-3} and 598 ind. dm^{-3}, respectively.

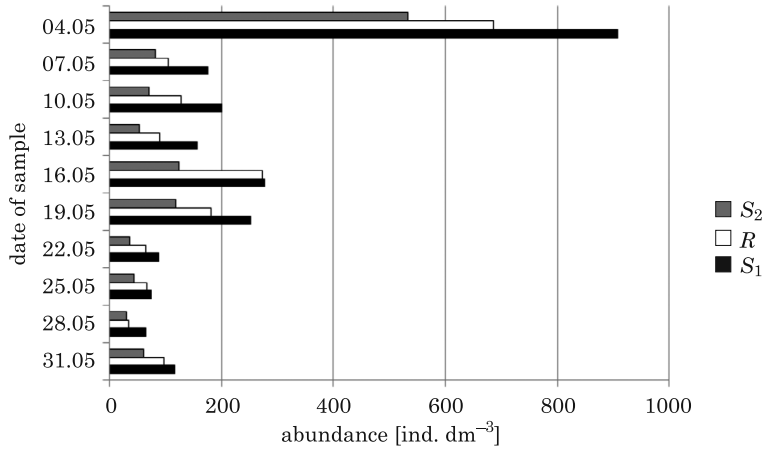

Fig. 2. Dynamics of the Copepoda abundance on the sample collection days using three sampling methods (S_1; S_2 and R – Materials and Methods)

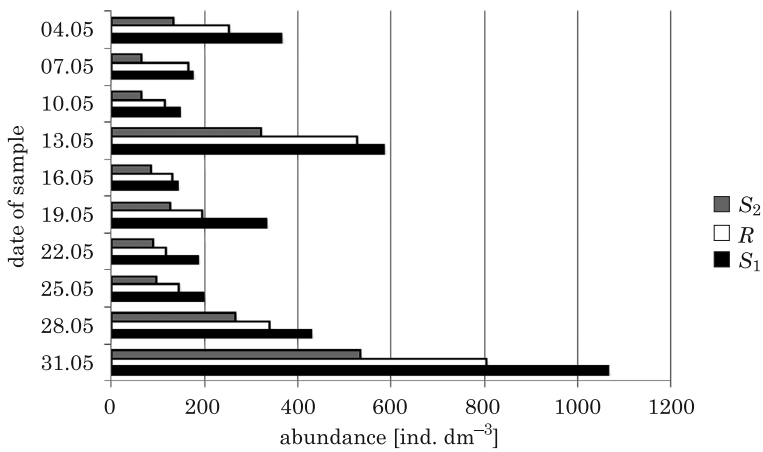

Fig. 3. Dynamics of the Rotifera abundance on the sample collection days using three sampling methods (S_1; S_2 and R – Materials and Methods)

The experiment was conducted in the water ranging from 12.9°C to 18.8°C (Figure 4*a*). The dissolved oxygen content oscillated from 11.6 mg dm^{-3} to 7.5 mg dm^{-3}, and the pH ranged from 7.3 to 8.3. The visibility of Secchi disc ranged from 1.9 m to 3.6 m. Independently of the sample collection method, statistically insignificant correlations were noted between the zooplankton abundance and the physical-chemical parameters of water ($P < 0.05$). Significant linear correlations were noted between the water temperature and its transparency and dissolved oxygen quantity. The visibility of the Secchi disc increased together with increasing water temperature ($r = 0.912$). Statistically

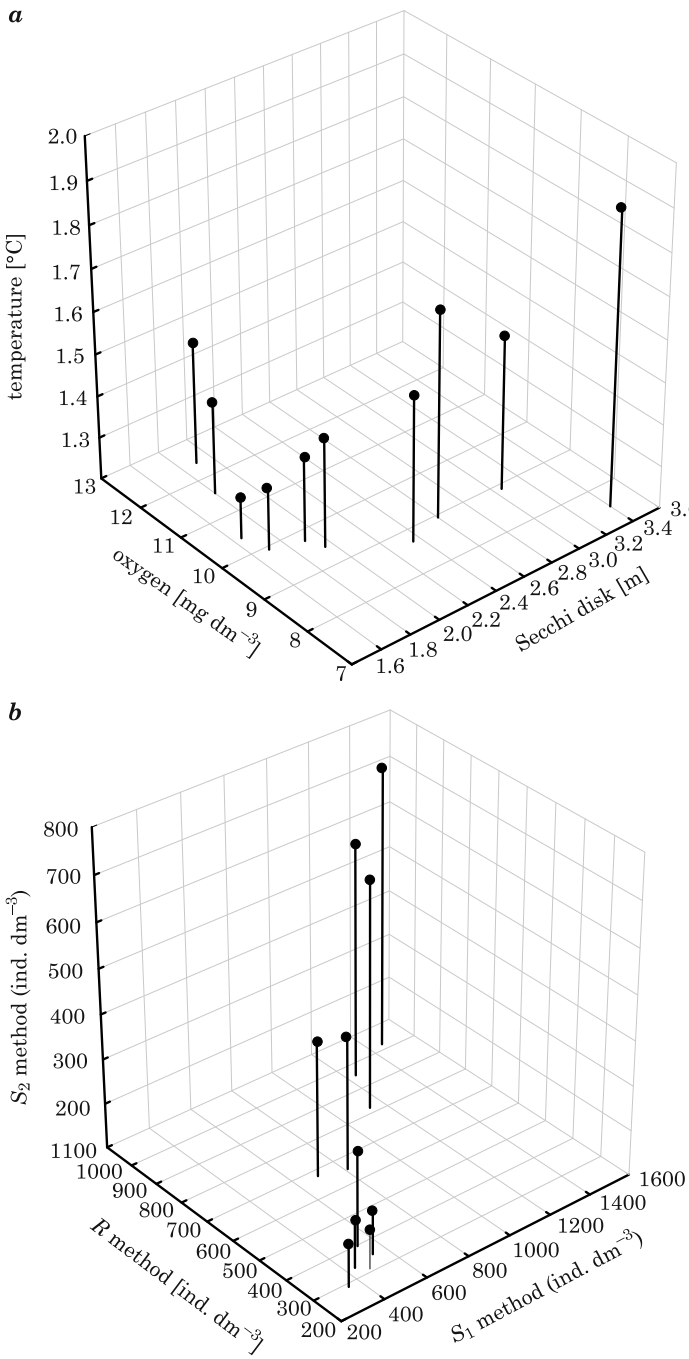

Fig. 4. Correlations between water temperature, dissolved oxygen and Secchi's visibility disc (*a*) and zooplankton quantities using three different sample collection methods (*b*)

significant linear correlations were also noted between the zooplankton quantities, which have been shown in all applied methods (Figure 4*b*). The strongest correlations were noted in the zooplankton abundance in samples collected using the R and S_2 methods ($r = 0.982$) as well as S_1 and S_2 methods ($r = 0.979$).

A total of 32 zooplankton taxa and forms have been identified on 10 sample collection days (Table 1). The most numerous were the Rotifera (13 species). Crustaceans were represented by 8 species of Cladocera and 11 Copepoda taxa (including 3 juvenile forms). The most frequently found zooplankton taxa in the samples were: 3 Rotifera species (*K. longispina, K. cochlearis and P. longiremis*), 5 species and forms of Copepoda (*E. graciloides, T. crassus*, nauplius forms, copepodit *Calanoida* and kopepodit *Cyclopoida*) and 1 Cladocera species (*D. cucullata*). The use of three different sample collection methods yielded variable detection of taxonomic groups and diversification of the average number of individuals. The comparative analysis revealed that the S_1 method was the most effective in that regard. On the contrary, the S_2 method was the least effective as it did not confirm the presence of Cladocera taxon on 9 occasions (twice: *Acroperus harpae, Eurycercus lamellatus Polyphemus pediculus;* once: *Bosmina coregoni, Diaphanosoma brachyurum, Leptodora kindtii)* and Copepoda on 5 occasions (twice: *Eucyclops macrurus*; once: *Trichocerca similis, Cryptocyclops bicolor, Mesocyclops leuckarti*). Whereas on two occasions the R method did not confirm the presence of Cladocera species (once: *Acroperus harpae, Leptodora kindtii*) and Copepoda taxa (once: *Cryptocyclops bicolor,* copepodit *Calanoida*). Only in the case of Rotifera species the S_1 and R methods had highest detection effectiveness. Samples collected using the S_2 method did not contain Rotifera taxa on three occasions (*Brachionus angularis, Euchlanis dilatata, Polyarthra longiremis).* The largest average abundance of organisms per volume was obtained using the S_1 method. Whereas the S_2 method indicated the least average density of Rotifera i Copepoda in the rearing cages. In case of 4 Cladocera species (*A. harpae, B. coregoni, D. brachyurum, P. pediculus),* the smallest average density was obtained using the bottle sampler method (R method).

The highest mean SAI_{tmi} values for the species in the Cladocera, Copepoda and Rotifera taxonomic groups were noted using the S_1 sample collection method: 0.98 (±0.04), 0.99 (±0.03) and 0.97 (±0.11). The highest maximal SAI_{tmi} values (1.0) were also noted in the S_1 method, ranging from 91.8% of Copepoda to 83.3% of Rotifera (Table 2). Whereas in the S_2 sample collection method, there was a minimal amount of maximal SAI_{tmi} only in the Rotifera group (2.4% of all analyzed cases). Using the sample collection method R, the maximal abundance of Cladocera, Copepoda and Rotifera species in 17.8%, 10.9% and 16.7% of all observations, respectively. The statistical analysis

Table 1

Structure of the zooplankton and the effectiveness of species identification in the three sample collection methods

Taxon	Average abundance (±SD) in the sample collection method (ind. dm^{-3})			Identification number in the sample collection method		
	S_1	S_2	R	S_1	S_2	R
Rotifera						
Ascomorpha ovalis	6.0 (±5.3)	2.7 (±1.2)	3.0 (±2.0)	3	3	3
Asplanchna priodonta	36.7 (±49.0)	21.2 (±24.7)	28.0 (±35.4)	6	6	6
Brachionus angularis	7.0 (±5.9)	2.4 (±1.9)	4.0 (±2.7)	5	4	5
Brachionus calyciflorus	28.0 (±24.0)	7.0 (±7.1)	20.0 (±22.6)	2	2	2
Conochilus unicornis	52.1 (±116.4)	31.6 (±73.0)	50.5 (±118.3)	8	8	8
Euchlanis dilatata	3.0 (±0.0)	1.0 (±0.0)	1.5 (±0.7)	2	1	2
Filinia longiseta	14.5 (±2.1)	3.6 (±0.9)	9.4 (±3.7)	2	2	2
Kellicottia longispina	14.1 (±10.7)	6.9 (±4.7)	13.2 (±11.7)	10	10	10
Keratella cochlearis	92.5 (±79.1)	40.2 (±33.5)	61.6 (±47.6)	10	10	10
Keratella quadrata	17.6 (±11.6)	7.9 (±6.0)	14.5 (±8.4)	8	8	8
Polyarthra longiremis	23.0 (±20.5)	8.9 (±6.8)	17.1 (±18.2)	10	9	10
Pompholyx sulcata	234.0 (±302.6)	129.4 (±167.1)	185.8 (±240.8)	5	5	5
Synchaeta sp.	10.3 (±4.9)	3.7 (±1.5)	7.7 (±3.8)	3	3	3
Copepoda						
Trichocerca similes	7.8 (±6.8)	3.8 (±1.7)	6.0 (±5.5)	5	4	5
Cryptocyclops bicolor	8.6 (±8.1)	1.6 (±0.5)	4.6 (±2.6)	7	6	6
Cyclops strenuous	12.0 (±5.0)	6.0 (±1.7)	6.3 (± 1.2)	3	3	3
Cyclops vicinus	25.0 (±17.3)	14.8 (±11.8)	17.3 (±14.0)	4	4	4
Eucyclops macrurus	3.0 (±1.4)	–	2.5 (±0.7)	2	–	2
Eudiaptomus graciloides	20.5 (±24.5)	9.8 (±16.5)	14.2 (±19.4)	10	10	10
Mesocyclops leuckarti	13.6 (±11.9)	8.3 (±5.7)	8.8 (±5.3)	5	4	5
Thermocyclops crassus	16.7 (±9.9)	5.6 (±3.4)	9.9 (±7.5)	10	10	10
Nauplius	88.3 (±121.4)	53.3 (±85.5)	78.2 (±114.4)	10	10	10
Kopepodit *Calanoida*	15.0 (±23.0)	3.9 (±4.6)	9.6 (±15.5)	10	10	9
Kopepodit *Cyclopoida*	58.5 (±87.2)	28.9 (±49.8)	42.3 (±57.0)	10	10	10
Cladocera						
Acroperus harpae	2.2 (±1.7)	3.0 (±0.0)	2.5 (±2.1)	3	1	2
Bosmina coregoni	19.8 (±16.1)	9.8 (±6.1)	8.9 (±7.6)	5	4	5
Bosmina longirostris	41.8 (±31.2)	14.3 (±11.3)	21.5 (±13.6)	8	8	8
Daphnia cucullata	115.1(±139.7)	70.8 (±104.0)	95.9 (±123.4)	10	10	10
Diaphanosoma brachyurum	7.1 (±2.6)	6.5 (±6.4)	5.0 (±3.0)	3	2	3
Eurycercus lamellatus	3.0 (±1.4)	–	1.5 (±0.7)	2	–	2
Leptodora kindtii	10.9 (±6.1)	5.8 (±5.1)	9.6 (±8.1)	9	8	8
Polyphemus pediculus	5.8 (±3.3)	3.0 (±2.8)	2.3 (±1.3)	4	2	4

Sample collection methods: S_1 – plankton net (slow vertical haul); S_2 – plankton net (fast vertical haul); R – bottle sampler

(ANOVA, Kruskal-Wallis test) of mean SAI_{tmi} values in the S_1 and S_2 collection methods did not reveal statistically significant differences between the taxonomic groups ($P > 0.05$). Such differences ($H = 6.48$; $P < 0.05$) were noted only in the sample collection method R, appearing only between the taxa Cladocera (mean SAI_{tmi} value = 0.59 (±0.27)) and Rotifera (mean SAI_{tmi} value = 0.71 (±0.21)).

Table 2

The characteristics of standardized abundance index of the zooplankton in the three sample collection methods

Methodical variant	Parameter	Unit of measure	Cladocera	Copepoda	Rotifera
S_1	SAI_{tmi} SAI_{tmi}max	$\bar{x}$ (±SD) %	0.98 (±0.04)^A 86.7	0.99 (±0.03)^A 91.8	0.97 (±0.11)^A 83.3
S_2	SAI_{tmi} SAI_{tmi}max	$\bar{x}$ (±SD) %	0.35 (±0.28)^A –	0.41 (±0.22)^A –	0.43 (±0.23)^A 2.4
R	SAI_{tmi} SAI_{tmi}max	$\bar{x}$ (±SD) %	0.59 (±0.27)^A 17.8	0.66 (±0.27)^{AB} 10.9	0.71 (±0.21)^B 16.7

Sample collection methods: S_1 – plankton net (slow vertical haul); S_2 – plankton net (fast vertical haul); R – bottle sampler; the mean of SAI_{tmi} with the same letter index are not statistically different ($P < 0.05$)

The analysis of the mean SAI_{tmi} values obtained using the three sample collection methods, reveals statistically significant differences in each taxonomic group (Figure 5). The highest statistical significance was noted among the Rotifera ($H = 154.8$; $P < 0.001$) and Copepoda ($H = 142.9$; $P < 0.001$) groups. In the Cladocera group, the mean SAI_{tmi} values were significantly different at the level of $P < 0.01$. Using the test statistics value $H = 80.6$, the lower statistical significance of the differences among the Cladocera group is a reflection of the relatively high standard deviation values for the sampling methods S_2 and R: 0.35 (±0.28) and 0.59 (±0.27), respectively. This phenomenon is directly related to the highest number of undetected species in the Cladocera group using the sample collection method S_2.

Discussion

The seasonal and cyclical abundance of zooplankton is typical for all types of waters in various climate zones. This phenomenon is directly related to numerous, previously described abiotic and biotic factors, particularly water temperature and eutrophy (WANG et al. 2007, SUTHERS and RISSIK 2009). Our study was based on two sample methods commonly used in open water plankton research (DHARGALKAR and VERLECAR 2004). Due to the peculiarity

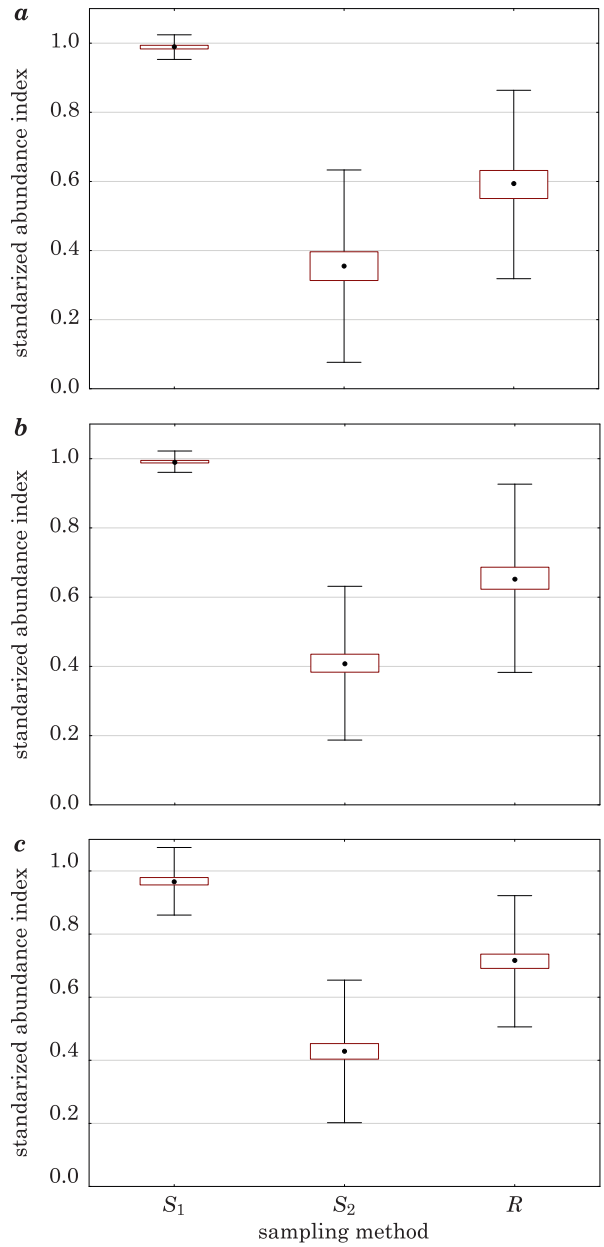

Fig. 5. Mean values of SAI_{tmi} with SE (frame) and SD (whiskers) for the species in taxonomic group Cladocera (*a*), Copepoda (*b*) and Rotifera (*c*) in the particular collection methods (S_1; S_2 and R – Materials and Methods). The means SAI_{tmi} values for Copepoda and Rotifera are significantly different at the level of $P < 0.001$, and for Cladocera at the level $P < 0.01$ (ANOVA, Kruskal-Wallis test)

of the illuminated surface cages (volume, zooplankton aggregation, free access, technological manipulation), the use of other tools and methods seems to be unjustified. The experiment was performed while maintaining the fish rearing technology standards in illuminated cages. The mesh size 1.2 mm of net cage has been used in koregonid rearing (MAMCARZ 1995a). This mesh size is not limited penetration of freshwater plankton inside the net cages (CECCUZZI et al. 2010, FURGALA-SELEZNIOW et al. 2014). In experimental cages were identified large planktonic organisms, eg. *Leptodora kindtii*.

The results obtained using the plankton net (S_1 and S_2 methods) demonstrate that despite travelling the same distance (water column), the amount of effectively filtered water was greater in the S_1 method. The expected filtration rate of the S_1 method was about 1.9 $dm^3\ s^{-1}$, while in the S_2 method was about 3.8 $dm^3\ s^{-1}$. The slower rate of real filtration in the S_2 method pushed the excess water outside and made hydrodynamic whirlpools. It is not possible to unequivocally determine that the net moved at an optimal speed in the S_1 method. A comparison of the zooplankton abundance on each of the sample collection days suggests that if theoretically, the net filtered 76 dm^3 of water during slow towing (S_1 method), then in the S_2 method it filtered about 30 dm^3. Our direct observations support this thesis. Using the S_2 method in an illuminated cage after dusk, it was observed that water was pushed upwards above the plankton net. Zooplankton forms (visible with the naked eye) were displaced outside the inlet together with the excess water. On the contrary, such escape of the zooplankton was not observed in the S_1 method.

Considering the hydromechanics theory, in an aquatic environment the filtration rate is directly related to the factors determining the extent of hydrodynamic resistance (LANDAU and LIFSZYC 2009). In practice, this means that with constant water density and plankton net porosity, the filtration rate will be inversely proportional to the hauling speed. According to HERON (1968), the effectiveness of this filtration is dependent on the net's material and mesh knots. It has also been observed that during sample collection using the plankton net, the effective filtration declines due to the mesh clogging by the filtered organisms (KNOECHEL and CAMPBELL 1992). Using the same hauling speed, the zooplankton collection can be more effective using a smaller diameter inlet or a larger filtration surface (TRANTER and SMITH 1968). Research on the optimal filtration and reduction of water turbulence in front of the plankton net was conducted also in flume tanks (MAHNKEN and JOSSI 1967). However in such controlled conditions it is not possible to account for the numerous factors, including those responsible for the reduced filtration rate during mesh clogging. As shown by research on zooplankton sample collection methods in various environments, many of the solutions designed and tested on models are not effective in the field (DE BERNARDI 1984, MASSON

2004, SEMENOVA 2011). In the riverine zooplankton research by SLUSS et al. (2011), three sample collection methods were used (alpha bottle sampler, manual bilge pump and Schindler trap) and the least abundant samples were collected using the Schindler trap. This tool was the least effective in collecting smaller zooplankton forms (*Bosmina* sp. and *Keratella* sp.).

Many previously published studies note the reduced effectiveness of zooplankton collection using a plankton net in comparison to other methods, particularly the calibrated traps (trap sampler). According to KANKAALA (2007), the plankton net's effectiveness was comparable to 75% of the trap's effectiveness, specifically in the case of Copepoda (41%), Cladocera (51%) and Rotifera (66%). This author does not recommend the use of plankton net in quantitative research due to the probability of larger crustaceans avoiding/escaping the net as well as the inability of the net to contain the small and soft-bodied Rotifera. Lesser effectiveness of the plankton net, as compared to the Ruttner sampler, was also demonstrated in research on the Vistula Lagoon zooplankton (GUTKOWSKA et. al. 2012).

The results we obtained using a plankton net hauled at a high speed and a bottle sampler might confirm the previously published conclusions (KANKAALA 2007, GUTKOWSKA et al. 2012). However, it must be noted that in many cases the unsatisfactory effectiveness of the plankton net results from the inability to match the hauling speed to the effective filtration rate. This is demonstrated in our results obtained using slow hauling speed as well as by the previously published report of high effectiveness of the plankton net in comparison to other sample collection methods (WIKTOR 1982). Based on the analysis of a wide range of study results, none of the zooplankton sample collection methods is universally-applicable. On the contrary, each of them is usually dedicated to specific environmental conditions (PAGGI et al. 2001). The effectiveness of sample collection and the reliability of the obtained results continues to be largely dependent on the accurate selection of methods and the researcher's manual abilities (LANGFORD 1953, MACK et al. 2012).

Conclusions

The results of this study allow an objective comparison of these three sample collection methods' effectiveness. Using each sample collection method in two identical settings allows a comparison using the normalized SAI. In addition, the environment of < 2 m deep cage illuminated at night (above surface 60W bulb) allows the analysis of relatively large samples and direct visual control of the vertical haul with minimal influence of water waving and movement on the zooplankton distribution.

In terms of the abundance and detection of taxa, the results of this study demonstrate that the reference method of zooplankton sample collection in illuminated cages is slow vertical haul using a plankton net. This was determined by the greatest abundance of each taxonomic group at the statistical level $P < 0.001$ for Copepoda and Rotifera and $P < 0{,}01$ for Cladocera. Average effectiveness was observed for the TON bottle sampler. The obtained results indicate that a 5 dm^3 water sample collected using the TON bottle sampler in the water column at the depth of 0.8–1.2 m allows a relatively precise qualitative assessment of the zooplankton in an illuminated cage. However, this method does not fully reflect the true abundance, confirming that the zooplankton is not uniformly distributed in an illuminated environment. The reduced density and underestimated abundance of the zooplankton in the samples results from the insufficient volume of the bottle sampler and the single sample collection point. The results might be more reliable if the samples were collected using the bottle sampler at different depths. Regardless, the water sampler produces turbulent flow during towing and alters the distribution of zooplankton congregated around the light source. This excludes the bottle sampler as an effective method. However, effectiveness of slow vertical haul is a reflection of the filtration efficiency. With a constant mesh size of conical tow-net, it will be dependent on planctonic organisms density and its species' structure, as well as of water temperature.

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