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Zoopsammon as a potential food base of fish inhabit the shallow littoral zone (*ex-situ* experiment)

Zoopsammon jako potencjalna baza pokarmowa ryb zasiedlających strefę płytkiego litoralu (eksperyment *ex situ*)

Summary. The objective of the study was to investigate the effect of different species of fish on the qualitative and quantitative structure of microorganisms (ciliates and rotifers) based on an experiment conducted in *ex situ* conditions. The effect of certain physical and chemical water parameters on the structure of the analysed groups of microorganisms was also analysed. The experiment was conducted in five experimental variants: the control variant (I) and variants involving juvenile perch (II), roach (III), bream (IV) and mixed species (V) (included specimens of roach, perch and bream). Both the qualitative and quantitative structure of microorganisms proved to be substantially impoverished in the experiment dominated by bream. The lowest pressure of fish on microbial communities was observed in the experimental variant dominated by perch.

Key words: fish, zoopsammon, food base

INTRODUCTION

The littoral zone of lake ecosystems plays an important role in the functioning of lakes. It is an important transitional zone (ecotone) participating in water purification. It also provides a habitat for a number of species of plants and animals [Tonn and Magnuson 1982, Benson and Magnuson 1992, Lewin *et al.* 2004]. The zone is also of importance part to ichthyofauna fauna. Its abundance and diversity in this zone is frequently substantially higher than in other parts of the lake [Werner *et al.* 1977, Keast 1985]. The majority of freshwater fish species, even considered as typically pelagic, often use the shallow part of the lake for a short period in the year, as spawning grounds or place juvenile development [Fischer and Eckmann 1997]. This is related to a higher supply of allochthonous matter, and therefore higher local availability of nutrients, consequently resulting in more intensive biological production [Naiman and De'camps

1997]. Juvenile fish often stay in the coastal zone, abundant in food in the summer months, for accelerated growth [Cerri and Fraser 1983]. The high structural complexity of the coastal zone also provides protection against potential predators [Werner *et al.* 1977, Crowder and Cooper 1982, Werner *et al.* 1983, Ross 1986, Greenberg 1991, Lobb and Orth 1991, Lewin *et al.* 2004].

Fish frequently inhabit combined pelagic, benthic and littoral macro-habitats due to their mobility and variable feeding tactics [Schindler and Scheuerell 2002]. The role of fish in the pelagic zone is relatively well known. The structure and factors affecting the fish population it in the littoral zone is investigated to a lower degree. All freshwater ecosystems are assumed to represent a similar trophic cascade, but the strength of the top-down effect of fish may vary depending on the trophic status of lake and is still a controversial issue [McQueen *et al.* 1986, DeMelo *et al.* 1992, Sarnelle 1992, Jeppesen *et al.* 2003, Carpenter *et al.* 2010, Zingel *et al.* 2012]. The role of selected aquatic microorganisms as a potential food base for adult or juvenile fish is also very little known [Watson and Davis 1989]. Only several authors have tried to estimate the importance of different species of protozoa in the diet of fish [Fukami *et al.* 1999, Nagano *et al*. 2000, Figueiredo *et al.* 2005]. Recent research shows that the majority of protozoa are cosmopolitan species. They can provide an easily accessible and rich food base for fish fauna. They can also be eaten away by small Metazoa (primary rotifers), also constituting one of the major dietary elements of fish [Kakareko 2002].

The objective of this study was to assess the potential impact some fish species juvenile (perch, roach, and bream) on the quantitative and qualitative composition of ciliates and small psammonic Metazoa in an experiment conducted in *ex situ* conditions. Changes in physical and chemical water properties and their impact on the qualitative and quantitative composition of zoopsammon, were also analysed.

MATERIAL AND METHODS

The impact of selected fish species on the structure of qualitative and quantitative microbial community was assessed based on a laboratory experiment on fish inhabiting the littoral zone. According to the literature the zone is inhabited by perch, bream, and roach [Rechulicz 2006, Kolejko 2010, Rechulicz *et al.* 2012].

Water from eutrophic Lake Sumin was used in the experiment. It is a polymictic lake representing the bream-zander type of fishing. The sand form the psammolittoral was collected in the higroarenal zone with no distrurbance of the microvertical arrangement. Its 5 cm layer was trensferred to five experimental aquaria with a volume of 30l each. The aquaria were filled with water and left for one day to for the biotic conditions to stabilise. The experiment was conducted in five experimental variants: the control variant (I) and variants involving juvenile specimens of perch (*Perca fluviatilis*) (II), roach (*Rutilus rutilus*) (III), bream (*Abramis brama*) (IV) and mixed species (V), including specimens of roach, perch, and bream (Fig. 1). The experiment also involved conducting physical and chemical water analysis (at the beginning and end of the experiment), and the analysis of qualitative and quantitative composition of psammonic organisms (every 4 days). The entire experiment was complete after 3 weeks.

Water temperature, conductivity, and pH were determined in situ using the multi-parameter sensor 556 MPS (Envag).Total organic carbon (TOC) was determined by means of a PASTEL UV. Total phosphorus was determined by means of PH-EN 1189 (spectrophotometric method with ammonium molybdate) and phosphates by means of norm PN-EN 1189. Ammonium and nitrate nitrogen contest were determined following the methodology by Hermanowicz *et al.* [1976].

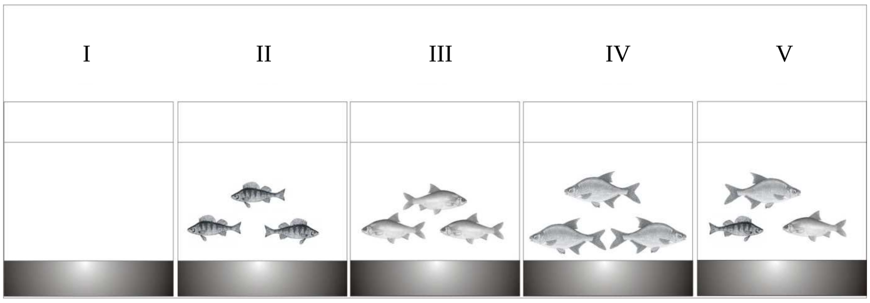

Fig. 1. Diagram of a laboratory experiment
(I – control, II – perch, III – roach, IV –bream, V – mixed)
Rys. 1. Schemat eksperymentu laboratoryjnego
(I – kontrola, II – okoń, III – płoć, IV – leszcz, V – obsada mieszana)

Samples were collected with a plastic sharp-edged tube with in diameter of 20 mm (psammonic samples with surface water). The samples were subject to qualitative and quantitative analysis by means of a microscope NIKON-Eclipse E200. Observation of living samples was applied for the taxonomic identification of ciliates. The quantitative determination was performed in samples fixed with Lugol's solution. Ciliate density was calculated per 1 cm^3 of the samples. The identification of species was based on Foissner and Berger [1996]. Ciliate biomass was estimated by means of multiplication of the numerical abundance by the mean cell volume calculated from direct volume measurements using appropriate geometric formulas. Ciliate biomass was calculated by means of multiplication of cell volumes by a correcting factor of 0.4 [Jerome *et al.* 1993]. Rotifers were identified in the same samples. Observation of living samples was applied for taxonomic identification. The identification of species was based on Radwan *et al.* [2004]. Rotifer density was calculated per 1 cm^3 of the samples. Biomass was estimated by multiplying the numerical abundance by mean volume [Ejsmont-Karabin 1998].

Statistical analyses of results were carried out using the STATISTICA 7.0 software. Pearson correlation coefficients were calculated between pairs of variables in order to determine the relationship between abundance of ciliates and rotifers and physical and chemical parameters. The significant differences between samplings were analyzed using t-Student test ($p \leq 0.1$).

RESULTS

The physical and chemical water parameters showed substantial differences in individual experimental variants. At the beginning of the experiment, the physical and chemical water parameters were at a comparable level in particular experimental variants. At the end of the experiment changes in chemical water parameters were substantial. This particularly concerns concentrations of nutrients and total organic carbon. Their concentrations increased in the course of the experiment. Slight changes occurred in water pH. The highest decrease in pH was observed in the aquarium with perch. Water conductivity increased in all of the experimental variants, except for the perch, where it remainded at the same level. The parameter showed a substantial decrease in the control variant (Table 1).

Table 1. Physical and chemical characteristic of water during of the laboratory experiments
Tabela 1.Właściwości fizyczno-chemiczne wody podczas eksperymentu laboratoryjnego

Wariant eksperymentu Variant of the experiment	S/E	Temperature Temperatura °C	pH	Conductivity Przewodność µS cm^{-1}	$N\text{-}NH_4$ mgN dm^{-3}	$N\text{-}NO_3$ mgN dm^{-3}	PO_4 $mgPO_4$ dm^{-3}	P_{tot} mgP dm^{-3}	TOC mgC dm^{-3}
Kontrola Control	S	23.42	8.41	247	0.1834	0.7349	0	0.0465	11.7
	E	22.7	8.38	208	0.1529	0.3415	0.0061	0.0051	10.8
I. Okoń Perch	S	23.15	8.38	250	0.1233	0.8311	0.0733	0.0264	11
	E	22.27	7.43	247	0.1843	1.7933	0.6891	1.0681	18.6
II. Płoć Roach	S	23.22	8.41	244	0.1626	0.7357	0	0.0205	12.5
	E	22.28	7.95	254	0.1664	6.4487	0.0737	0.2376	13.2
III. Leszcz Bream	S	23.22	8.33	247	0.1491	0.8478	0.017	0.0244	11.6
	E	22.36	8.02	265	0.186	1.9415	0.1705	0.3471	26.4
IV. Mieszana Mixed	S	23.33	8.39	247	0.1437	0.4945	0.0015	0.0064	11.9
	E	22.6	8.2	269	0.2283	1.1089	0.1509	0.1787	29

The species richness largely varied in particular experimental variants. The highest species richness was recorded in the variant with perch and roach. In other variants, it decreased (Fig. 2). The analysis of the abundance and biomass of microorganisms in the groups studied showed considerable differences between the experimental variants. The highest mean abundance and biomass of protozoa was recorded in the aquaria with perch (95 ind. cm^3, 13.73 µg cm^{-3}) and roach (44 ind. cm^{-3}, 7.58 µg cm^{-3}), and the lowest in the experimental variants with mixed species (23 ind. cm^{-3}, 2.48 µg cm^{-3}) and with bream cast (22 ind. cm^{-3}, 2.46 µg cm^{-3}). In experimental variants II-V, the abundance of the microorganisms was substantially lower than that in the control variant and compared to the values observed at the beginning of the experiment (Fig. 3). The statistical analysis showed substantial differences in the abundance of protozoa between the following variants: control variant vs. perch ($p < 0.1$ $F = 15.73$), control vs. bream ($p < 0.1$ $F = 23.33$), and control vs. mix ($p < 0.1$ $F = 16.01$).

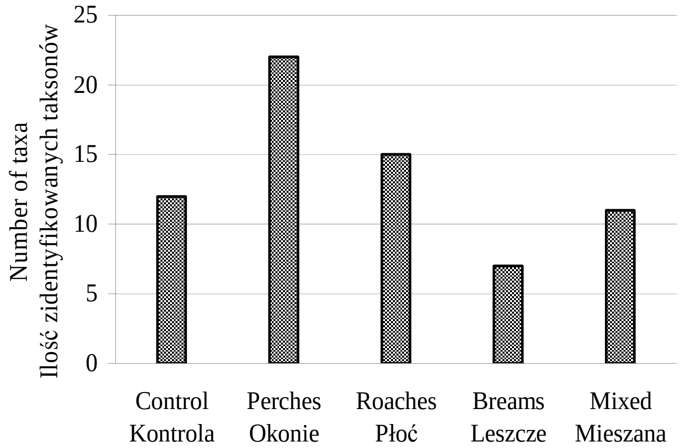

Fig. 2. Number of ciliate taxa in laboratory experiment
Rys. 2. Ilość zidentyfikowanych taksonów orzęsków w eksperymencie laboratoryjnym

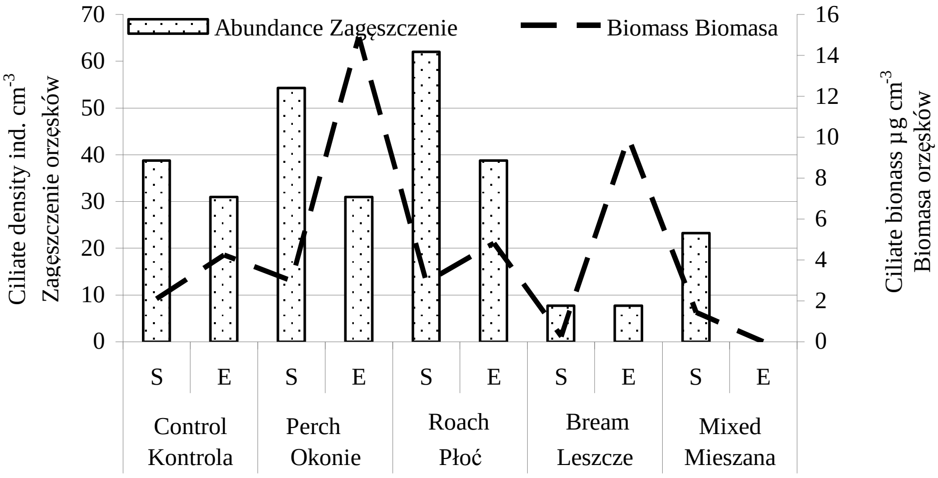

Fig. 3. Density and biomass of psammonic ciliates in laboratory experiment (S – start, E – end)
Ryc. 3. Zagęszczenie i biomasa orzęsków psammonowych w eksperymencie laboratoryjnym (S – początek doświadczenia, E – koniec doświadczenia)

The dominance structure showed no substantial differences between the experiment variants. The following taxa reached the highest percentage contribution in the total numbers: *Coleps* sp., *Aspidisca* sp., *Cyclidium* sp., *Cinetochilum* sp., and *Codonella cratera*. The control group was dominated by Scuticociliatida. In other variants, an increase in the percentage of Hypotrichidia and Prostomatidia was recorded (Fig 5). The most frequently identified taxa were those with the cell size not exceeding 50 µm. Taxa with larger dimensions were identified occasionally. An increase in their contribution was only observed in the control sample and the variant with perch.

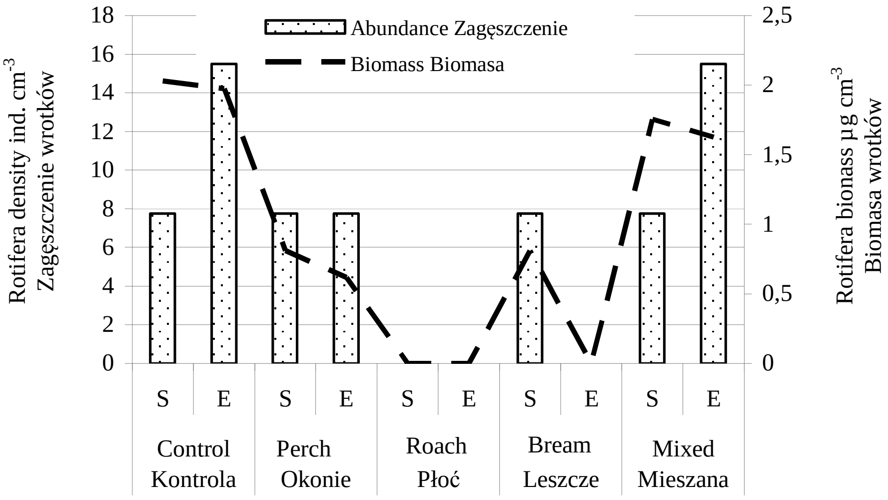

Fig. 4. Density and biomass of psammonic rotifers in laboratory experiment (S – Start, E – End)
Rys. 4. Zagęszczenie (A) i biomasa (B) wrotków psammonowych w eksperymencie laboratoryjnym (S – początek doświadczenia, E– koniec doświadczenia)

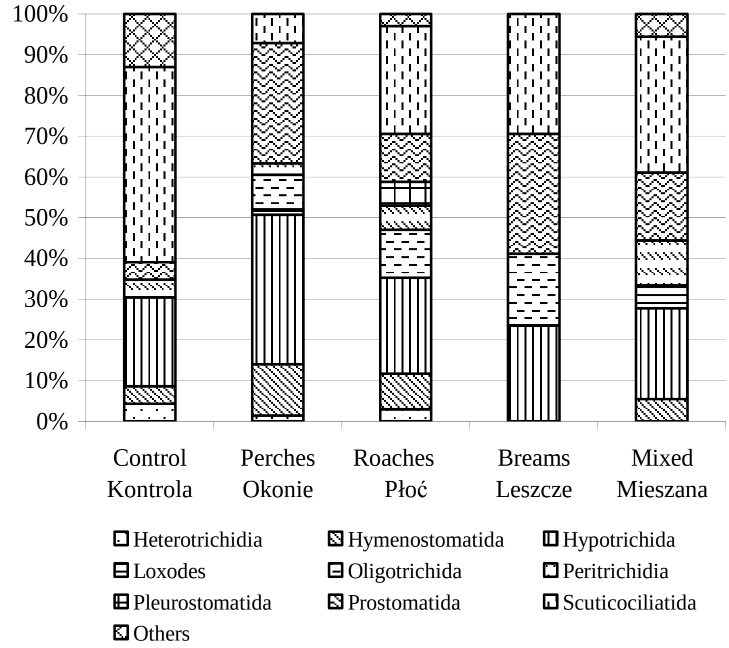

Fig. 5. Domination structure of psammonic Ciliata orders in laboratory experiment (% of total numbers)
Rys. 5. Struktura dominacji orzęsków psammonowych w eksperymencie laboratoryjnym (% ogólnej liczebności)

The analysis of the qualitative structure of rotifers showed substantial differences between particular experimental variants. The highest taxonomic diversity was observed in the sample with perch and mixed species. It decreased in the remining variants (Fig. 6). The highest mean abundance of rotifers (abundance and biomass) was found in the experiment dominated by perch (35 ind. cm^{-3}, 2.93 µg cm^{-3}), and the lowest and in case of the experiment with the dominance of roach (8 ind. cm^{-3}, 0.65 µg cm^{-3}) and bream (6 ind. cm^{-3}, 0.55 µg cm^{-3}) (Fig. 4). Substantial differences also occurred in the dominance structure in particular experimental aquaria. In the control sample and in experiment with bream were dominated Lecanidae (up to 80% of the total abundance). An increase in the percent contribution of *Lepadella* was observed in the aquaria with perch and roach (50%). The contributions of particular groups in the aquarium with mixed species were at a similar level (Fig. 6). The majority of the identified taxa had dimensions of 50–100 µm. Taxa with larger sizes were only identified in the control sample.

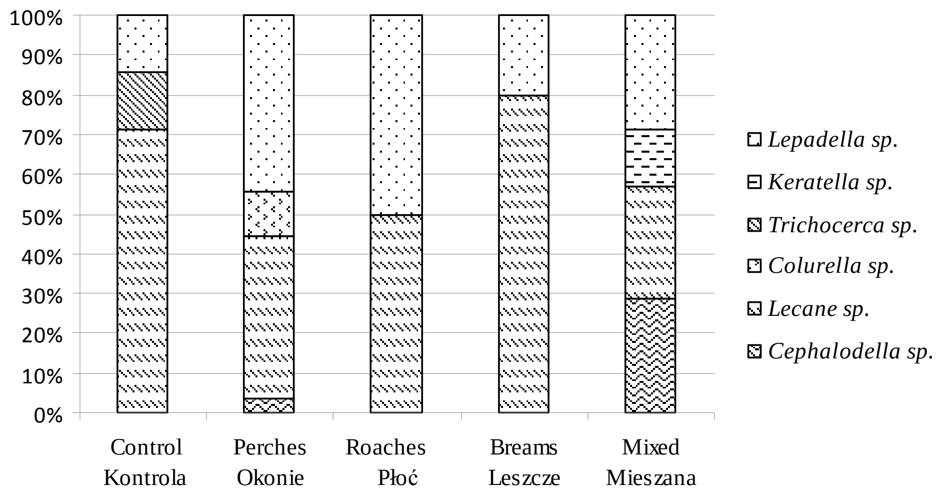

Fig. 6. Domination structure of psammonic Rotifera in laboratory experiment (% of total numbers)
Rys. 6. Struktura dominacji wrotków psammonowych w eksperymencie laboratoryjnym (% ogólnej liczebności)

The physical and chemical water properties showed various degrees of correlation with the density of the analysed zoopsammon groups in individual experimental variants. In the control sample, the relationship between physical and chemical water properties and the abundance of protozoa and rotifers was weak. In variants including fish, the abundance of protozoa and rotifers the most frequently correlated with the concentrations of nutrients in water and water pH. The strength of these relationships was the highest in the experimental variant with perch ($r = 0.61$ $p < 0.05$) and bream ($r = 0.58$ $p < 0.05$). The analysis of correlations in the protozoa-rotifers system (potential consumers) suggests the strongest relationship in the experimental variant with roach ($r = 0.67$ $p < 0.05$).

DISCUSSION

The experiment showed a significant effect of the species structure of fish fauna on the abundance and species composition of microorganisms inhabiting sand and surface water. The effect was of bidirectional character. The fish could control the abundance of protozoa by their direct consumption and the consumption of their potential predators (ie. rotifers). In the study by Zingel *et al.* [2012], the determined diet of juvenile fish included species of ciliates primarily belonging to Oligotrichida, Prostomatidia, and Scuticociliatida. The rotifer species the most frequently identified in the diet of fish were genera of belonging to *Keratella* and *Polyarthra* [Zingel *et al.* 2012]. The fish also substantially modified the environmental conditions. The water was enriched in nutrients. Changes in the physical and chemical properties strongly suggest a significant relationship between the presence of a some fish species and the content of nutrients in water. In the control sample, the content of nutrients decreased. This was probably related to the use of these substances by microorganisms to incorporate them into their biomass. This is in accordance with the short-term study by Weisse *et al.* [1990] who demonstrated that the availability of nutrient substrates is one of the mechanisms controlling the abundance of microorganisms. According to Pennak [1951], organisms living in sand are primarily dependent on organic detritus as the main source of food. As evidenced by Wickham *et al.* [2000], however, meiofauna, and particularly ostracods, can control the abundance of benthic protozoa. Similar patterns were also observed by Tarkowska-Kukuryk and Mieczan [2008]. The authors demonstrated that ciliates constitute from 11 to 79% of total biomass in the gastrointestinal tract of genus *Cricotopus*. In the scope of this study, however, no larvae of genus *Cricotopus* were observed. This could be related to their rapid consumption by fish, or the insufficiency of their potential food.

The study by Zingel *et al.* [2012] showed that ciliates constitute up to 60% of the total pool of carbon consumed by juvenile fish. Ciliates are organisms frequently reaching very high densities in lakes. They move slower than the majority of metazooplankton, and are easily caught by fish. Due to such qualities, they can provide a key source of food for juveniles fish [Zingel *et al.* 2012]. Studies by other authors have shown that ciliates are consumed in considerable amounts when occurring at high densities (up to 1500 individuals by one fish per hour) [Ohman *et al.* 1991]. Research by Hunt von Herbing and Gallager [2000] showed that larvae of cod (*Gadus morhua*) intensively consume protozoa, probably necessary for the survival of this species and the proper development of the early stages of life. Similar patterns can therefore also apply to other species of fish at early stages of development inhabiting the coastal zone of lakes.

In terms of size, zoopsammon was dominated by small species, with occasionally occurring taxa exceeding 100 μm. This is in accordance with the size-efficiency hypothesis by Brooks and Dodson [1965]. The hypothesis suggests an increase in the efficiency of the process of acquiring food along with body size, and explains the mechanism of this pattern. According to the thesis, when planktivorous fish are numerous, zooplankton is dominated by small forms, but in the absence of fish, large forms are dominant [Hall *et al.* 1976].

As evidenced above, the species composition of fish has a significant impact on the qualitative and quantitative structure of zoopsammon. Perch had the lowest impact involving the reduction of abundance of ciliates, whereas perch and roach on the abundance of rotifers. The highest decrease in the species richness and abundance of microorganisms was observed in the experiment dominated by bream. Changes in the physical and chemical water properties associated with differences in the species structure of fish could substantially affect the structure of the examined groups of microorganisms by determining the abundance of their potential food, e.g. bacteria or algae. The experiment results suggest that in the absence of protozoa in water, fish consume species inhabiting the bottom area, and can even search for food in the sand. This trend has been particularly observed in the case of juvenile bream as a typically benthivorous species.

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Streszczenie. Celem pracy było poznanie wpływu różnych gatunków ryb na strukturę jakościową i ilościową mikroorganizmów (orzęsków oraz wrotków) z wykorzystaniem eksperymentu przeprowadzonego w warunkach ex situ. Analizowano również wpływ wybranych czynników fizyczno-chemicznych wody na kształtowanie się struktury badanych grup mikroorganizmów. Doświadczenie przeprowadzono w pięciu wariantach eksperymentalnych: kontrola (I) oraz warianty z obsadą młodocianych osobników okonia (II), płoci (III), leszcza (IV) oraz obsada mieszana (V), tzn. w skład której wchodziły osobniki płoci, okonia oraz leszcza. Wykazano, że zarówno struktura jakościowa, jak i ilościowa mikroorganizmów uległa wyraźnemu zubożeniu w eksperymencie zdominowanym przez leszcze. Najmniejszą zaś presję ze strony ryb na zespoły mikroorganizmów stwierdzono w wariancie eksperymentalnym zdominowanym przez okonie.

Słowa kluczowe: ryby, zoopsammon, baza pokarmowa