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Dead Zooplankton Content in Saline Lakes of South Siberia (Republic of Khakassia, Russia) and Prospects for Its Assessment by Underwater Video Survey Method

Aleksandr P. Tolomeev[a, b*], **Olga P. Dubovskaya**[a, b], **Anton V. Drobotov**[a], **Anastasiya A. Lemesheva**[b] **and Angelica S. Perminova**[b]

[a]*Institute of Biophysics SB RAS*
Federal Research Center "Krasnoyarsk Science Center SB RAS"
Krasnoyarsk, Russian Federation
[b]*Siberian Federal University*
Krasnoyarsk, Russian Federation

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Abstract. We analyzed the proportions of dead individuals in the populations of crustacean zooplankton in six lakes located in the Minusinsk Basin in the Republic of Khakassia between 2021 and 2024. In three thermally stratified lakes, the proportions of dead zooplankton were estimated for the epi-, meta- and hypolimnion zones. The proportions of dead individuals determined by their abundance in the study lakes were relatively low, averaging 6.5 % for copepods, 2.6 % for cladocerans, and 5.2 % for the total crustacean zooplankton. A decrease in the number of dead individuals was observed in the hypolimnion, implying high rates of degradation of dead zooplankton organic matter in the epi- and metalimnion. For quantitative comparisons of degradation rates, a new coefficient, K_D, is proposed, calculated on the basis of profiles of vertical distributions of live and dead zooplankton. The use of underwater video imaging showed that the method was suitable for estimating natural mortality of zooplankton populations, but the underwater imaging system required technical modification. The results are generally consistent with the data obtained using the method of determining dead zooplankton by plankton net sampling and aniline blue staining. The accuracy of detection of dead zooplankters using underwater video imaging can be improved by increasing the volume of water scanned and employing machine learning techniques to distinguish between live and dead individuals.

* Corresponding author E-mail address: tolomeev@ibp.ru
ORCID: 0000-0002-9124-4566 (Tolomeev A.); 0009-0007-1722-4958 (Drobotov A.)

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Содержание мертвого зоопланктона в соленых озерах Юга Сибири (Республика Хакасия, Россия) и перспективы его оценки методом подводной видеосъемки

А.П. Толомеев[а, б], О.П. Дубовская[а, б], А.В. Дроботов[б], А.А. Лемешева[б], А.С. Перминова[б]

[а]Институт биофизики СО РАН
ФИЦ «Красноярский научный центр СО РАН»
Российская Федерация, Красноярск
[б]Сибирский федеральный университет
Российская Федерация, Красноярск

Аннотация. Проведен анализ доли мертвых особей в популяциях рачкового зоопланктона в шести озерах, расположенных в Минусинской котловине в Республике Хакасия в период с 2021 по 2024 гг. В трех озерах, имеющих температурную стратификацию, долю мертвого зоопланктона находили в зонах эпи-, мета- и гиполимниона. Доля мертвых особей, определенная по численности в исследуемых озерах, была сравнительно низкой и составила в среднем для копепод 6,5 %, для кладоцер 2,6 % и для всего рачкового зоопланктона 5,2 %. Показано снижение количества мертвых особей в гиполимнионе, которое предполагает интенсивную деградацию мертвой органики зоопланктона в эпи- и металимнионе. Для количественных сравнений интенсивности деградации предложен новый коэффициент K_D, рассчитываемый на основе профилей вертикальных распределений живого и мертвого зоопланктона. Использование подводной видеосъемки показало, что методика подходит для оценки естественной смертности зоопланктонных популяций, но требует технической модификации видеосистемы. В целом данные согласуются с методом определения мертвого зоопланктона с помощью лова планктонной сетью и его окраской анилиновым голубым. Точность определения мертвых зоопланктеров с помощью подводной видеосъемки может быть повышена за счет увеличения объема сканируемой воды и применения методов машинного обучения для классификации живых и мертвых особей.

Ключевые слова: зоопланктон, естественная смертность, подводные видеосистемы.

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Introduction

Zooplankton are one of the key components of aquatic ecosystems, providing a trophic link between primary producers and higher-level consumers (Wetzel, 2001). The non-predator mortality of zooplankton has traditionally been regarded as a trait that reflects the conditions under which zooplankton species develop. A high proportion of dead individuals usually indicates the occurrence of a stressful situation in a water body: changes in the physical and chemical properties of the environment, poor trophic conditions, the influence of toxic substances, the attack of viruses, etc. However, recently, the non-predator mortality of zooplankton has attracted the interest of researchers in the context of metabolic processes and carbon cycling in aquatic ecosystems. Zooplankton, after their death, become the substrate that sustains the functioning of the ‘microbial loop’ of the trophic system of the water body (Simon et al., 2002). This substrate is high-quality bioavailable organic matter that can trigger a chain of microbiological processes leading to what has been termed the ‘priming effect’ (Neubauer et al., 2021) – the stimulation of decomposition and recycling of persistent dissolved organic matter entering the water body mainly from terrestrial ecosystems (Guenet et al., 2014). As a result of the stimulation of micro-organisms by the organic matter of dead zooplankton, the local carbon cycle in the water body may be accelerated, leading to a shift in ecosystem metabolism from the accumulation and sequestration of organic matter to its decomposition and emission into the atmosphere in the form of CO_2.

Factors determining the balance of carbon sequestration and emission by aquatic ecosystems are the subject of extensive research (Li et al., 2024) related to the global carbon cycle and associated climate change. Studies show that salt lakes contribute more to CO_2 emissions than freshwater ecosystems (Duarte et al., 2008; Wen et al., 2016), due to the structuring effect of salinity on the functioning of the trophic system and the emergence of a complex layered structure in the vertical distribution of biological and chemical components (Gulati et al., 2017). However, both the role of dead zooplankton organic matter and the set of additional conditions necessary for the ‘priming effect’ to occur in salt lakes remain poorly understood. Therefore, monitoring the dynamics of dead zooplankton in lakes is a necessary step in accumulating factual data on its content and spatial distribution in the water column. The main method for identifying dead animals in samples is aniline blue staining (Dubovskaya, 2008; Bickel et al., 2009). This technique provides high accuracy in distinguishing between live and dead cladocerans and copepods and is an effective and relatively simple method for studying the natural mortality of zooplankton in water bodies. However, the manual processing of zooplankton samples is a labor-intensive process, requiring qualified specialists and significant time investment. Additionally, the number of samples processed manually is usually limited, making it difficult to achieve the necessary level of statistical reliability in the results. At the same time, in recent years, there have been attempts to automate zooplankton counting using laboratory and field video systems (Lombard et al., 2019; Tolomeev et al., 2024). Various machine learning methods are used for direct image analysis (Ciranni et al., 2024). In the present study, we employed a specially developed underwater flow-through imaging system (Tolomeev et al., 2024), designed to assess the content of live and dead zooplankton. The aim of the current research was to compare the data obtained using this system with the data acquired by the traditional method and to evaluate the prospects for using underwater video recordings in studying the natural mortality of zooplankton. In contrast to the staining method, the identification of live and dead zooplankton in video data is based on the swimming activity of zooplankters over a specified period of time (at least 40 seconds). Additionally, for the analysis of the degradation (destruction) rates of dead zooplankton in the water column, we intend to propose a new coefficient, K_D, calculated only from the vertical profiles of live and dead zooplankton distributions, which can be relatively easily obtained during field studies.

Methods

Zooplankton sampling

Six saline lakes in the Republic of Khakassia were used to study live and dead zooplankton in 2021, 2022, and 2024. Samples were collected from late June to mid-July during periods of stable thermal stratification. Samples were taken in the central parts of the lakes using a closing plankton net with an inlet diameter of 18 cm and a mesh size of 82 μm. In non-stratified lakes, sampling was conducted from the bottom to the surface, while in stratified lakes, the epilimnion, metalimnion, and hypolimnion zones were sampled separately. The boundaries of limnological zones were determined based on temperature gradients (Read et al., 2011): within the epilimnion and hypolimnion zones, the temperature gradient does not exceed a threshold of 1 °C per meter. Temperature and conductivity were measured using a CastAway-CTD probe (YSI, U.S.A.). A total of 91 zooplankton samples and 28 temperature and conductivity profiles were collected during the observation period. Zooplankton samples were delivered to the laboratory, where they were stained with aniline blue to distinguish between live and dead individuals, following established methods (Dubovskaya, 2008; Bickel et al., 2009). Stained samples were fixed with 10 % formalin. The samples were then scanned using an Epson Perfection V850 Pro scanner, and the abundances of live and dead zooplankton were determined from the scanned images. A detailed description of the zooplankton analysis method using scanned samples is provided in Gorsky et al. (2010) and Yolgina et al. (2022). Organisms in the images were counted manually using the ImageJ/Fiji software (Schindelin et al., 2012). The abundances of dominant cladoceran and copepod species were determined in the samples. In the copepod group, adults, copepodites, and nauplii were counted. Rotifers were not analyzed because of the difficulty of distinguishing between live and dead individuals using aniline blue staining. Data analysis expressed zooplankton concentration in both abundance (ind./m^3) and biomass (μg dry weight/L). Biomass is a more convenient parameter for analyzing the total content of combined taxonomic groups, whereas abundance is more relevant for comparing traditional zooplankton counting with video imaging methods. Dry weights of organisms were determined using size-weight relationships for each species (McCauley, 1984). The average size of individuals in each group was measured under a binocular microscope (32x magnification), with at least 50 specimens measured.

Underwater flow-through imaging system

In 2024, simultaneously with plankton net sampling, zooplankton were recorded using a specially designed underwater flow-through video system. The design of the system and procedure are described in Tolomeev et al. (2024). Briefly, the system uses action cameras equipped with macro lenses (1.5x magnification) to record zooplankton inside a submerged flow-through chamber. Water exchange in the chamber is facilitated by a connected hose and an external water pump. The hose is used to lower the underwater module into the water, with an electrical cable for LED illumination panels running alongside it. The pump operates in a cyclic mode controlled by programmable time relays. The pump is turned off during zooplankton video recording (40 seconds). After moving the submerged module to a new depth, the pump is briefly turned on (20 seconds) to refresh the water in the chamber before the next recording. In this study, 1 to 8 cycles were recorded at each depth. Zooplankton abundance in the videos was determined manually by counting actively swimming and immobile (dead) animals on the computer monitor. In the future, this procedure is planned to be automated using machine learning methods (Eerola et al., 2024). However, at this stage of research, initial data acquisition is conducted manually. These data will later be used for training and testing automated classification algorithms.

Dead zooplankton degradation index

The amount of dead zooplankton observed in the water column is determined by the balance of three main processes: the increase in dead animals due to natural mortality and their decrease due to sinking and degradation (decomposition). The latter process is associated with microbial decomposition of dead organisms, resulting in the breakdown of their bodies into parts that can no longer be identified in zooplankton samples.

To compare lakes in terms of dead zooplankton degradation levels, a simple index, K_D, is proposed. This index should reflect differences between dead zooplankton profiles formed in the presence or absence of degradation (D = 0) of sinking carcasses (Fig. 1). If degradation is absent, the concentration of dead zooplankton will increase linearly with depth. Under conditions of uniform vertical distribution of live zooplankton and equal

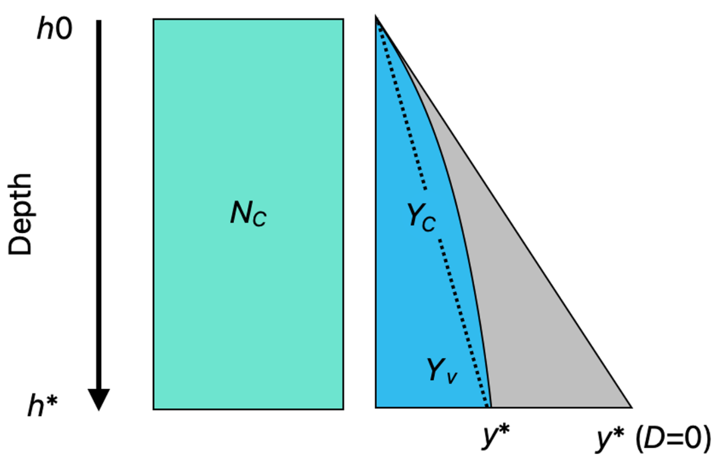

Fig. 1. A schematic representation of the processes of formation, sinking, and degradation of dead zooplankton in the water column. See notations in the text

mortality rates at all depths, the accumulation of dead zooplankton with depth will follow a linear relationship (detailed mathematical descriptions of the processes of formation, sinking, and degradation of dead zooplankton are provided in Dubovskaya et al., 2018, and Tolomeev et al., 2022). However, if degradation of sinking carcasses does occur in the water column, their concentration at lower depths may decrease, and they, potentially, may disappear entirely. This outcome will indicate that all dead zooplankton formed in the water column have been completely decomposed before reaching the bottom.

The K_D index is conveniently defined as a measure of the difference between the observed amount of dead zooplankton, Y_C, and its theoretical content, Y_V, in the water column (ind./m²) – the amount that would form in the complete absence of dead zooplankton degradation. The K_D index is expressed as:

$$K_D = \frac{Y_C - Y_V}{Y_C}, \tag{1}$$

where the observed amount of dead zooplankton down to depth h^* is determined as:

$$Y_C = \sum_{i=1}^{n} \frac{(h_{i-1} + h_i)}{2} \cdot y_i. \tag{2}$$

Here, n is the number of layers into which the water column of depth h^* is divided, h_i is the height of the i-th layer, and y_i is the observed concentration of dead individuals in that layer (ind./m³). The K_D index is dimensionless and varies from 0 to 1. Values close to 0 indicate that the vertical distribution of dead zooplankton matches the profile expected in the absence of degradation, while values approaching 1 indicate complete degradation.

In the case of ideal uniform distribution of live individuals in the water column, Y_V is determined by:

$$Y_V = \frac{h^* \cdot y^*}{2}, \tag{3}$$

Where y^* is the abundance of dead zooplankton at the lower boundary of the water column.

Since the vertical distribution of zooplankton in natural water bodies is rarely uniform, layers with higher zooplankton abundance will produce more dead individuals, affecting the vertical profile of dead zooplankton. In other words, the linear relationship between the number of dead individuals and depth will be disrupted. Therefore, under non-uniform vertical distribution of zooplankton, formula (3) cannot be used to calculate Y_V.

Meanwhile, the heterogeneity of live zooplankton distribution can be overcome by dividing the profile into narrow layers, within which the zooplankton distribution is considered uniform. The formula for Y_V, taking into account variations in live zooplankton content across layers, is:

$$Y_V = h^* \cdot y^* - \sum_{i=1}^{n} \frac{h_{i-1} + h_i}{2} \cdot p_i \cdot y^*, \tag{4}$$

where p_i is the relative proportion, defined as the proportion of live individuals N_i (ind./m³) in layer i relative to the total live zooplankton content N_C (ind./m²). The derivation of formula (4) is omitted as it is straightforward; just note that the trapezoidal method was used for integrating organism abundances in the water column in formulas (2) and (4). The proportions p_i are calculated as:

$$p_i = \frac{N_i}{N_C}, \tag{5}$$

and N_C is determined in the same way as Y_C (formula 2).

Certainly, K_D is an index that only approximates the degree of dead zooplankton degradation in the water column, as it is calculated using only two parameters – profiles of live and dead zooplankton distributions – under several assumptions. It is assumed that sinking and degradation rates of dead zooplankton do not significantly change with depth, and y^* is determined at the lower boundary of the distribution of zooplankton population. Additionally, K_D does not have a linear relationship with the absolute proportion of degraded dead zooplankton, K_{deg}, where $K_{deg} = (y^*_{D0} - y^*)/y^*_{D0}$ and y^*_{D0} is the theoretical concentration of dead zooplankton at depth h^* in the absence of degradation.

Nevertheless, the relationship between K_{deg} and K_D can be well described by an empirically fitted exponential function of the form

$$K_{deg} = a \cdot e^{b \cdot K_D} + c, \qquad (6)$$

based on simulations of dead zooplankton degradation and sinking processes (Tolomeev et al., 2022). If the reduction in live zooplankton abundance at depth h^* is 1 % of the maximum population abundance, the coefficients of the equation are: a = –1.21, b = –2.03, and c = 1.18 ($R^2 = 1$, $p < 0.0001$). In practice, absolute degradation, K_{deg}, is approximately 25 % higher than K_D values, if we do not include values at the boundaries.

Simulations also show that when calculating K_D at intermediate depths, where live zooplankton abundance is reduced to only 30 % of the maximum, real K_{deg} values will be approximately 7 % higher. Correct interpretation of K_D is only possible when calculated for zooplankton populations with stratified vertical distributions (epilimnion or metalimnion populations), provided that K_D is determined at the lower boundary of the distributions of these populations. Calculating K_D for uniformly distributed zooplankton or at depths of maximum biomass makes little sense, as the contribution of current depths to the total dead zooplankton biomass increases, leading to non-linear relationships between K_D and K_{deg} and limiting the upper bound of K_D to values <1. Thus, when correctly determined, K_D can be used to compare the rates of dead zooplankton degradation processes across different water bodies or seasonal changes within a single population in one water body.

Results

Physical characteristics of lakes and zooplankton composition

Zooplankton studies were conducted in six lakes with salinities ranging from 0.98 to 15.4 g/L, in the Republic of Khakassia (Table 1). Lakes Utichye 1, Krasnenkoye, and Chalaskol are shallow, non-stratified water bodies with low total dissolved salts (up to 4.3 g/L) and conductivity ranging from 1.55 to 7.63 mS/cm. During the study period, the water temperature in these lakes reached 21–23 °C. Deeper lakes – Shunet, Shira, and Vlasyevo – exhibited well-defined thermal stratification. Surface layer temperatures in these lakes were similar to water temperature in non-stratified lakes. The hypolimnion of Lakes Shunet and Vlasyevo was approximately 10 °C cooler than the epilimnion: 8–14 °C. In the deeper lake, Shira, hypolimnion temperatures dropped by more than 15 °C, reaching 2.5–3 °C at lower depths. Total salt content in the epilimnion, metalimnion, and hypolimnion was not measured, but changes can be inferred from closely related specific conductivity. Given the qualitatively similar salt composition of the study lakes (Zadereev et al., 2022), specific conductivity can serve as a direct proxy for total salinity. The most saline lake is Shunet (15.4 g/L, conductivity 15.3–42.7 mS/cm), although the epilimnion of Shunet in 2021 was less saline than that of Shira (15.3 and 16.17 mS/cm, respectively). The difference in salinity between the epilimnion and hypolimnion was 31 % in Lake Shunet and 12 % in Lake Shira. In

Table 1. Physico-chemical characteristics (mean value ± standard deviation) of lakes during the observation period in 2021, 2022, and 2024. In stratified lakes, the values are given for the epilimnion (EPI), metalimnion (META), and hypolimnion (HYPO)

Lake	Year	Depth Stratum	Layer (m)	Temperature (°C)	Conductivity (mS/cm)	Total Dissolved Salts* (g/L)
Shunet	2021	EPI	0–2	22.28 ± 0.57	15.3 ± 5.18	15.40
		META	2–4	18.51 ± 2.88	22.54 ± 6.59	
54.419047°, 90.228202°		HYPO	4–6.5	11.57 ± 4.33	31.47 ± 16.03	
	2022	EPI	0–2.5	20.68 ± 0.67	21.15 ± 0.33	
		META	2.5–3.5	18.33 ± 1.43	23.44 ± 1.41	
		HYPO	3.5–5	13.79 ± 1.67	26.56 ± 0.82	
	2024	EPI	0–2.5	19.05 ± 0.13	20.44 ± 0.07	
		META	2.5–3.5	16.24 ± 1.91	24.04 ± 2.28	
		HYPO	3.5–5	8.9 ± 1.7	42.72 ± 18.4	
Shira	2021	EPI	0–4	17.57 ± 0.74	16.17 ± 0.04	11.39
54.504697°, 90.201220°		META	4–7.5	12.65 ± 3.6	16.81 ± 0.56	
		HYPO	7.5–19.5	2.36 ± 1.32	19.1 ± 0.4	
	2022	EPI	0–6	19.56 ± 1.25	16.41 ± 0.13	
		META	6–10	12.57 ± 3.47	16.87 ± 0.35	
		HYPO	10–17	3.11 ± 1.25	18.36 ± 0.34	
	2024	EPI	0–4	19.74 ± 2.33	15.67 ± 0.28	
		META	4–9	10.26 ± 3.08	16.42 ± 0.55	
		HYPO	9–15	3.06 ± 1.27	18.12 ± 0.42	
Vlasyevo	2022	EPI	0–5	21.02 ± 0.88	4.83 ± 0.03	3.23
		META	5–9	14.52 ± 3.43	4.94 ± 0.1	
54.457138°, 90.383218°		HYPO	9–11	8.12 ± 0.81	5.23 ± 0.06	
	2024	EPI	0–5	19.53 ± 1.18	4.81 ± 0.01	
		META	5–6.5	16.22 ± 1.64	4.88 ± 0.04	
		HYPO	6.5–8	8.68 ± 2.74	5.35 ± 0.26	
Utichye 1 54.481329°, 90.414246°	2024		0–2	21.84 ± 1.31	7.63 ± 0.02	4.30
Krasnenkoye 54.445164°, 90.337008°	2024		0–0.7	22.92 ± 0.07	4.29 ± 0.05	1.60
Chalaskol 54.401568°, 90.213695°	2022		0–2	21.11 ± 1.01	1.55 ± 0.01	0.98

*- according to earlier studies (Zadereev et al., 2022)

the weakly saline lake, Vlasyevo (3.23 g/L), this difference was less than 7 %.

The dominant zooplankton species in Lakes Shunet and Shira was the copepod *Arctodiaptomus salinus* (Table 2). Cladocerans in the pelagic zones of these lakes were rare, occurring only as single specimens brought in from the littoral zone. In Lake Vlasyevo, *A. salinus* co-dominated with the cyclopoid *Megacyclops viridis* and the cladoceran *Daphnia* cf. *longispina*. The large cladoceran *Daphnia magna* dominated in the shallow, non-stratified

Table 2. Dominant species and groups of zooplankton of the study lakes. Occurrence, mean size ± standard error (minimum and maximum values), and dry weight characteristics are indicated

Species (groups)	Body length (mm)	Dry weight (µg)	Shunet	Shira	Vlasyevo	Utichye 1	Krasnenkoye	Chalaskol
Bosmina sp.	0.36 ± 0.05 (0.26–0.42)	1.1						x
Daphnia cf. *longispina*	0.91 ± 0.17 (0.6–1.38)	2.7			x			x
Daphnia magna	1.71 ± 0.31 (0.88–2.02)	35.5				x	x	
Arctodiaptomus salinus			x	x	x	x	x	x
Male	1.32 ± 0.2 (0.95–1.65)	13						
Female	1.46 ± 0.3 (1.12–2.02)	16.7						
Megacyclops viridis					x	x		x
Male	1.36 ± 0.08 (1.25–1.55)	14.4						
Female	1.88 ± 0.08 (1.77–2.02)	33.2						
Copepodites	0.81 ± 0.28 (0.35–1.62)	3.9	x	x	x	x	x	x
Nauplii	0.32 ± 0.08 (0.15–0.5)	1.3	x	x	x	x	x	x

lakes, Utichye 1 and Krasnenkoye. In Chalaskol, *Bosmina* sp. was abundant.

Analysis of live and dead zooplankton in the study lakes

The amounts of biomass of live and dead zooplankton in the lakes were analyzed separately for copepods and cladocerans (Figs. 2 and 3, Table 3). Table 5 also presents the percentage of zooplankton abundance, excluding copepod nauplii, for comparison with underwater video data. The biomass of live and dead zooplankton varied widely both between lakes and within the same lake across different years. In the stratified lakes, Shunet, Shira, and Vlasyevo, the biomass was mainly concentrated in the epilimnion. In Shunet and Vlasyevo, live zooplankton biomass in the epilimnion ranged from 164 to 762 µg DW/L across years, while in Shira, it was lower, ranging from 93 to 282 µg DW/L. In non-stratified lakes, the maximum live zooplankton biomass was observed in the hypereutrophic lake, Chalaskol – 12 mg DW/L. That lake also had the highest dead zooplankton biomass, reaching 212 µg DW/L. The relative proportion of dead zooplankton biomass in the epilimnion and metalimnion ranged from 3 to 9 %. In the hypolimnion, this proportion increased due to the sinking of dead animals from the upper layers (Fig. 4), reaching an average of 27 % (Lake Vlasyevo). Interestingly, in the hypolimnion, the proportion of dead cladocerans was higher than that of dead copepods, despite their similar proportions in the upper layers. The overall percentages of dead zooplankton by abundance and biomass are presented in Table 3. The proportion of dead zooplankton calculated by abundance is slightly higher than that calculated by biomass, likely due to higher mortality rates of copepod nauplii, which contribute more to abundance than to biomass. We also observed a general trend of an increase in dead zooplankton proportions in lakes with higher salinity (conductivity), except in the hypolimnion (Fig. 5). The hypolimnion cannot be used in this analysis because the proportion of dead zooplankton in this zone does not represent population mortality at this depth but rather the accumulation of sinking dead zooplankton

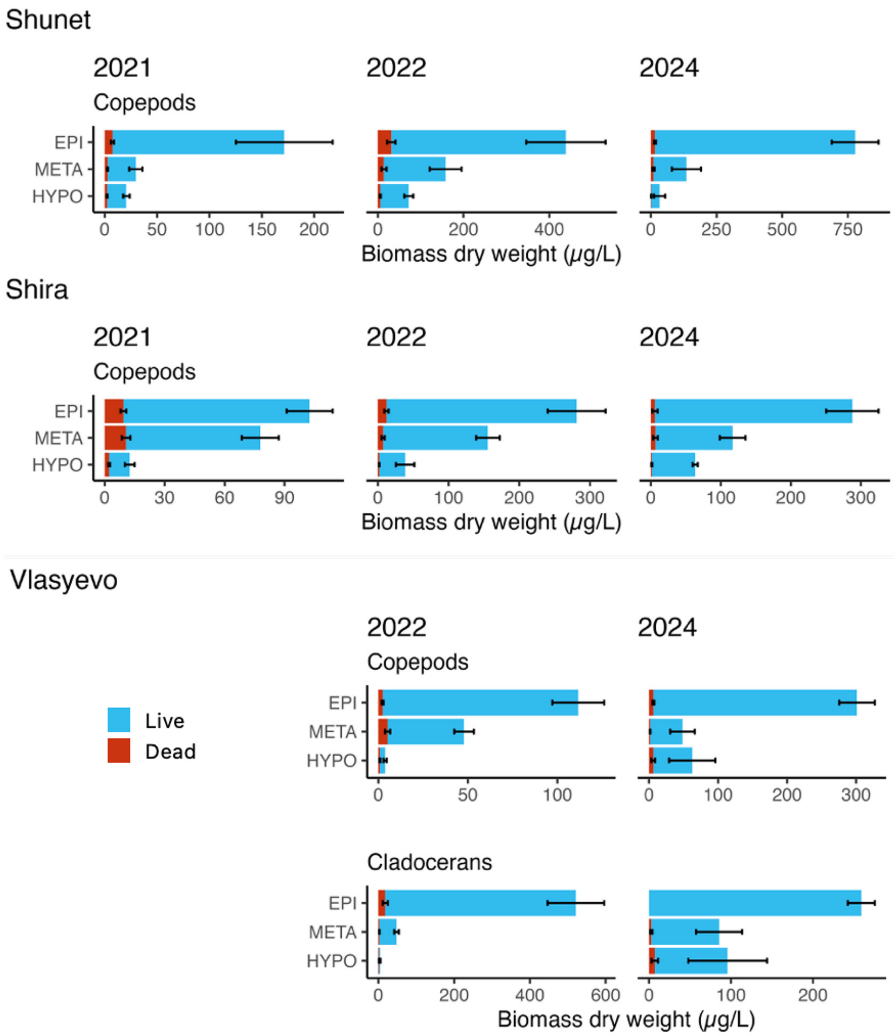

Fig. 2. Biomass contents of live and dead zooplankton in stratified lakes Shunet, Shira, and Vlasyevo during the observation periods in 2021, 2022, and 2024

from the upper layers. The linear regression is statistically significant (p = 0.00001 < 0.05), but the coefficient of determination, R^2 = 0.13, is extremely low, likely due to high data variability.

The degree of dead zooplankton degradation in the water column determined using the K_D index is presented in Table 4. The K_D index was calculated for two depth ranges: from the surface to the lower boundary of the metalimnion and from the surface to the lower boundary of the hypolimnion. The dead zooplankton degradation levels in Lakes Shunet and Shira were similar to each other. In the epilimnion and metalimnion, K_D averaged 0.67 and 0.56, respectively, increasing to 0.80 and 0.82 in the hypolimnion. Thus, more than half of the dead zooplankton formed in the epilimnion and metalimnion were already degraded. In the hypolimnion, degradation continued but at a slower rate, likely due to reduced decomposition rates at lower temperatures. Nevertheless, the greater portion of dead biomass was degraded in the water column.

In Lake Vlasyevo, the zooplankton community included both copepods and

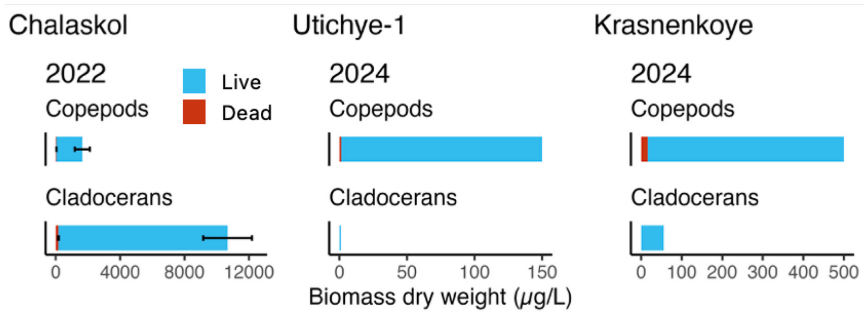

Fig. 3. Biomass contents of live and dead zooplankton in non-stratified Lakes Chalaskol, Utichye-1, and Krasnenkoye during the observation periods of 2022 and 2024

Table 3. Percentage of abundance (and biomass) of dead cladocerans and copepods in shallow and deep stratified lakes calculated per m^2. For cladocerans, the values calculated for abundance and biomass coincided. n is the number of observations for all the years

Lakes	n	% dead cladocerans	% dead copepods
Shallow lakes (Chalaskol, Krasnenkoye, Utichye)	11	2.0 ± 0.9	4.0 ± 0.9 (3.4 ± 0.8)
Deep stratified lakes (Shunent, Shira, Vlasyevo)	10	3.2 ± 1.0	7.4 ± 0.8 (5.7 ± 0.7)
Lakes in total	21	2.6 ± 0.6	6.5 ± 0.7 (5.1 ± 0.5)
Dead zooplankton in total	64	5.2 ± 0.6 (4.3 ± 0.4)	

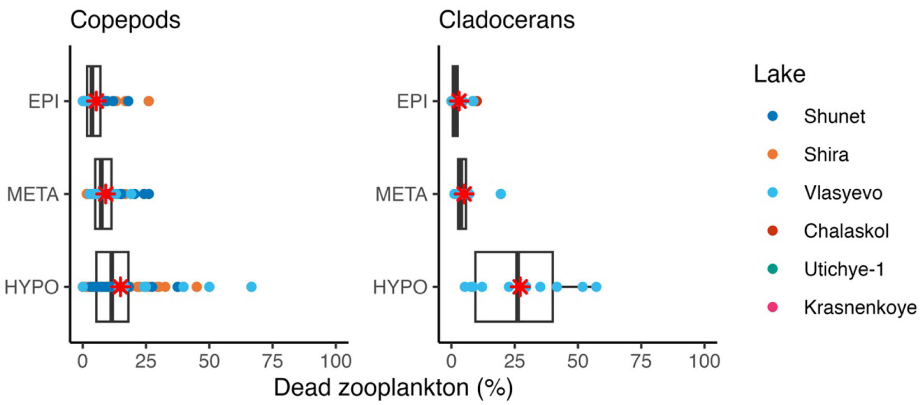

Fig. 4. Percentage abundance of dead copepods and cladocerans in the epilimnion (EPI), metalimnion (META), and hypolimnion (HYPO) in the study lakes. Asterisk shows the mean values

cladocerans, with comparable biomass levels (Fig. 2). However, the K_D index for these groups differed significantly. The degradation rate of dead copepods was lower by half than that of cladocerans, with degradation occurring in all lake zones. In the epilimnion and metalimnion, less than half of the dead animals were degraded, and in the hypolimnion, K_D increased by only 15 %, not reaching the levels observed in Lakes Shunet and Shira. In contrast, cladocerans were

Table 4. Degradation rates of dead zooplankton determined on the basis of K_D in the epilimnion-metalimnion (EPI–META) and epilimnion-metalimnion-hypolimnion (EPI–META-HYPO) depth strata of the study lakes. Y_C – observed content of dead individuals (x10³ ind. / m²), Y_V – estimated content of dead individuals (x10³ ind. / m²) in the absence of degradation

Lake	Depth stratum	Y_C	Y_V	K_D
Shunet	EPI – META	9.03±1.88	3.02±0.78	0.67
	EPI – META – HYPO	9.92±1.95	1.95±0.28	0.80
Shira	EPI – META	8.44±1.36	3.73±0.73	0.56
	EPI – META – HYPO	9.5±1.48	1.71±0.3	0.82
Valsyevo (Cladocerans)	EPI – META	11.5±4.09	1.9±0.46	0.83
	EPI – META – HYPO	11.92±4.16	2.19±0.46	0.82
Valsyevo (Copepods)	EPI – META	3.63±0.95	2.66±0.79	0.27
	EPI – META – HYPO	3.83±0.98	2.22±0.93	0.42

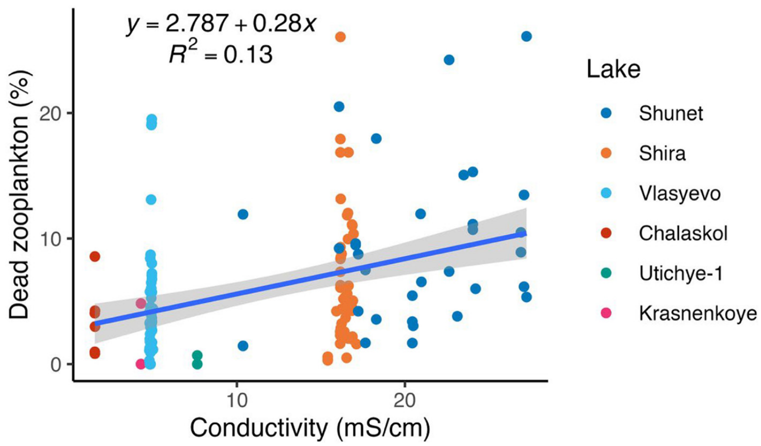

Fig. 5. Correlation of dead zooplankton content (% of total abundance) with specific conductivity (salinity equivalent, see Table 1) in the study lakes. The hypolimnion was excluded from the analyses. Linear regression is significant (0.00001 < 0.05)

almost completely degraded in the epilimnion and metalimnion (K_D = 0.83), with little degradation occurring in the hypolimnion.

Underwater video imaging

Results from the underwater video system are presented in Table 5 and Fig. 6. Table 5 shows the average abundances of live and dead zooplankton in the water column, compared with traditional plankton net sampling. Since only one zooplankton sample (concentrated from 40 L) per lake was taken from Lakes Utichye 1 and Krasnenkoye, comparison with video data was only possible for the percentage of dead zooplankton using a Z-test. Moreover, the number of cladocerans in samples from the central parts of the lakes was insufficient for testing, so they were excluded from the analysis.

Comparison of the two methods showed no statistically significant differences in most zooplankton groups. The exception was dead copepods in Lake Shunet. The underestimation of this group is likely due to their low concentration and the significantly smaller volume of water

Table 5. Comparison of the mean abundance of live ± standard error (ind./L) and dead ± standard error (ind./L and % of total abundance) zooplankton of the study lakes determined using a plankton net and underwater video survey. n – number of replicates. The total volume of water scanned is indicated. No significant differences were found between the two methods in Lakes Shunet, Shira, and Vlasyevo (p>0.05), except for the content of dead copepods in Lake Shunet (highlighted in bold). For the *Z*-test, the total number of animals in the samples is given (*italicized*)

Lake / Zooplankton group	Plankton net sampling			Underwater imaging			
	Live (ind./L)	Dead (ind./L)	n	Live (ind./L)	Dead (ind./L)	n	Volume (L) (pl. net/imaging)
				Shunet			
Copepods	59.1 ± 24.0	**3.0 ± 0.9 (5.1 %)**	9	62.6 ± 9.1	**0.6 ± 0.3 (1.0 %)**	25	127 / 8.7
				Shira			
Copepods	24.4 ± 13.1	0.9 ± 0.7 (3.7 %)	3	35.0 ± 2.6	0.7 ± 0.2 (2.0 %)	64	381 / 33.2
				Vlasyevo			
Copepods	24.9 ± 9.3	1.2 ± 0.3 (4.8 %)	9	6.5 ± 1.4	0.5 ± 0.3 (7.7 %)	12	203 / 4.2
Cladocerans	20.2 ± 4.7	0.5 ± 0.2 (2.5 %)	9	20.8 ± 2.7	4 ± 0.9 (19.2 %)	12	
				Utichye 1			
Copepods *Z-test*	6.1 *145*	0.04 *1 (0.7 %)*	1	21.4 ± 2.1 *342*	0.2 ± 0.2 *3 (0.9 %)*	20	40 / 4.2
Cladocerans	0.2	0 (0 %)	1	3.8 ± 1.1	0.5 ± 0.3 (13.2 %)	20	
				Krasnenkoye			
Copepods *Z-test*	82.2 *493*	4.2 *25 (5.1 %)*	1	5.2 ± 0.5 *84*	0 *0 (0 %)*	48	40 / 5.6
Cladocerans	7.8	0 (0 %)	1	0.2 ± 0.1	0 (0 %)	48	

scanned by the video system compared to plankton net sampling. On the other hand, the absence of statistical differences does not always indicate similarity of the methods but may result from high variability in the measured values obtained by each method. This variability often reflects the heterogeneous spatial distribution of zooplankton (Gilyarov, 1987), influenced by local aggregations, migrations, and physicochemical factors. For example, during net sampling, the standard error of mean values was around 40 %. However, for some groups, both methods yielded similar results. Differences in the abundances of live copepods in Lake Shunet and live cladocerans in Lake Vlasyevo were less than 6 %, while differences in live and dead copepods in Lake Shira did not exceed 30 %. Additionally, the percentages of dead copepods in Lake Utichye 1 determined by the two methods were similar (0.7 % and 0.9 %, respectively). In other cases, dissimilarities were greater, reaching 87 %.

Clearly, the accuracy of determining the abundance of organisms in each group depends on both the total abundance of the group and the volume of water scanned (or sampled). Plankton nets filter an order of magnitude more water than the video system can scan (Table 5), and, thus, the data obtained by the traditional method can be considered statistically more reliable. However, underwater video imaging is a way to

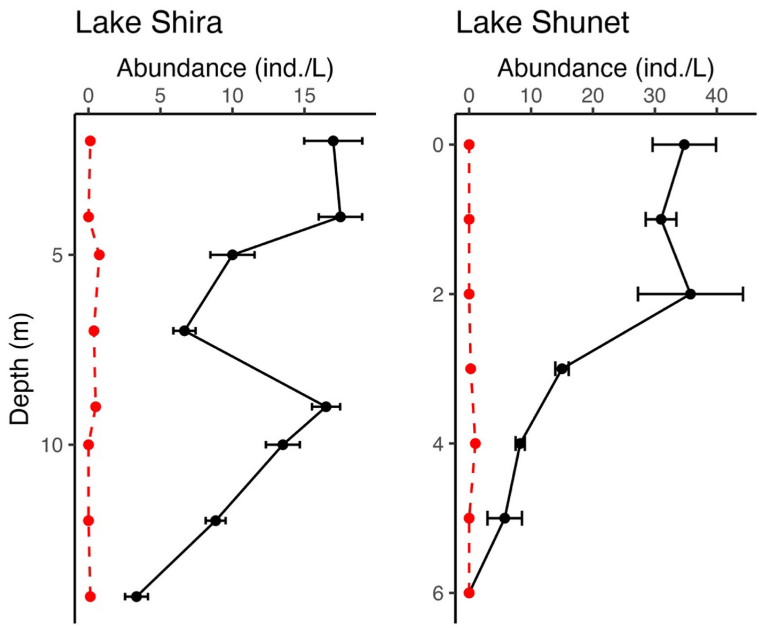

Fig. 6. Vertical distributions of live (solid line) and dead (dashed line) copepods in Lakes Shunet and Shira obtained by underwater video imaging

conduct a detailed study of vertical zooplankton profiles at selected depth resolutions. Figure 6 shows the vertical profiles of live and dead copepods in Lakes Shira and Shunet determined by underwater video imaging, which are fully consistent with long-term observations from previous studies (Barkhatov et al., 2023). In Lake Shira, live copepods form two abundance maxima – in the epilimnion and hypolimnion – while in Lake Shunet, most of the *A. salinus* population is concentrated in the epilimnion. Determining dead copepod profiles in the lakes from video data was not possible because of their very low concentrations. However, single dead individuals were observed in video frames in the metalimnion of Lake Shira and the hypolimnion of Lake Shunet, which was generally consistent with the data provided by plankton net samples (the maximum dead copepod abundance in Lake Shira in 2024 was found in the metalimnion, see Fig. 2). Obviously, to obtain statistically reliable dead zooplankton profiles, the volume of water scanned by the video system needs to be increased considerably, to several dozen liters. Just adding more recording cycles at each depth is not an effective solution, as it would substantially increase processing time. An optimal solution could be to pre-concentrate zooplankton in the flow-through chamber before video recording. Such a modification could be achieved by using an automatic closing filter mesh to concentrate zooplankton while pumping the water for each recording cycle.

Discussion

The average percentage of dead zooplankton (copepods and cladocerans) across all study lakes over the three-year period was 5.2 % by abundance and 4.3 % by biomass, consistent with values reported for brackish and freshwater systems in similar studies (Tang et al., 2014). The maximum values of 13–19 % (based on video measurements) were recorded for cladocerans in Lakes Utichye 1 and Vlasyevo, but they also fell within the range typically observed for dead zooplankton (7.4 ± 2.1–47.6 ± 5.1). The proportion of dead zooplankton naturally increased in the lower layers, due to both the accumulation of sinking dead animals from the upper layers and the reduced concentration of live individuals in the hypolimnion. On the whole, the observed concentrations of dead zooplankton present a balance between two opposing processes. On the one hand, dead individuals accumulate in the water column as a result of natural population mortality (proportional to population size), but on the other hand, they are removed through sinking and degradation. Traditionally (Gilyarov, 1987), an increase in the proportion of dead individuals is interpreted as a sign of declining population size due to adverse environmental factors (e.g., poor trophic conditions, presence of toxic substances).

The proportion of dead zooplankton in the study lakes was low and relatively stable, indicating that populations were not significantly affected by external stressors during the observation period, from late June to early July. On the other hand, the low content of dead zooplankton may reflect rapid degradation processes in the water column. This hypothesis is supported by the high K_D values calculated for zooplankton in stratified lakes. That is, dead zooplankton mostly degrade in the water column before reaching the hypolimnion. The exception was copepods in Lake Vlasyevo, which had a low degradation coefficient (K_D = 0.4). Since low degradation rates and the percentage of dead zooplankton are negatively related, the percentage of dead copepods in Lake Vlasyevo was indeed slightly higher (4.8–7.7 %) than in Lakes Shunet and Shira (1.0–5.1 %), where K_D = 0.8.

An increase in the relative proportion of dead zooplankton may also result from slower sinking rates. It is likely that in more saline lakes, dead zooplankton sinking rates are lower than in freshwater systems due to the reduced density difference between zooplankton bodies and the surrounding water. The presence of steep density gradients caused by thermal stratification may also contribute to slower sinking rates. This is supported by the positive correlation between the proportion of dead zooplankton and the conductivity (salinity equivalent) of the study lakes in the epilimnion and metalimnion. Thus, although the total amounts of dead zooplankton in Lakes Shunet and Shira were comparable to or even lower than in less saline lakes, their proportions in the upper layers were greater. Therefore, the dataset obtained in the current study provides insights into the major processes and factors influencing the relative content of dead zooplankton.

Underwater video imaging can become an effective method for studying natural zooplankton mortality. The algorithm for calculating specific mortality rates is based on determining the concentration of live and dead zooplankton in the water column, as well as estimating sinking rates of dead animals using sediment traps (Gladyshev et al., 2003; Dubovskaya et al., 2003). Recent studies have shown that accurate determination of natural mortality should be underpinned not only by the data on the total content of live and dead zooplankton but also by detailed vertical distribution profiles (Tolomeev et al., 2022). These profiles are necessary for determining specific degradation rates of dead animals in the water column, which are then used to calculate specific mortality rates. Furthermore, the accuracy of models describing the processes of formation, sinking, and degradation of dead zooplankton can be improved by incorporating depth-dependent sinking and degradation parameters. Underwater video imaging could address some of the challenges associated with profiling live and dead zooplankton components and determining particle sinking rates below the thermocline (Simoncelli et al., 2019). Currently, the prototype of the flow-through video system performs well in profiling live zooplankton. For statistically reliable profiling of dead zooplankton, which have significantly lower abundance, the system needs to be modified to enable pre-concentration of zooplankton before video recording. Such modifications are feasible, as there are examples of underwater devices that concentrate zooplankton before video detection, such as the LOKI system (Schulz et al., 2010). However, in contrast to our system, zooplankton concentration in LOKI occurs passively, during device descent or ascent, without prolonged recording of zooplankton inside the scanning chamber. Thus, the next step in improving the procedure will be to modify the video system in order to study low-concentration objects (present as single individuals per liter).

Conclusion

A study of the content of dead zooplankton in six saline lakes in southern Siberia, including stratified and non-stratified lakes, performed over three years (2021, 2023, and 2024) demonstrated that they constituted a relatively low proportion (5.2 %) of the total zooplankton abundance. This low content is most likely associated with the rapid degradation of dead zooplankton in the water column, resulting in insignificant amounts of observed dead zooplankton. High degradation rates are supported by the high K_D coefficient, calculated based on vertical distribution profiles of live and dead zooplankton. Underwater video imaging was found to be adaptable to comprehensive examination of vertical zooplankton distributions and distinguishing between live and dead individuals. This method was evaluated against the conventional approach to studying natural zooplankton mortality using aniline blue staining, revealing its potential as an alternative technique. The concentration of live zooplankton was sufficiently high for analysis using the developed video system. However, dead zooplankton had low concentrations in the study lakes and rarely entered the flow-through chamber, making it difficult to objectively study their vertical distribution across layers. Nevertheless, the video system allowed an integrated estimation of dead zooplankton abundance throughout the water column. Clearly, technical improvements to the video system, enabling pre-concentration of zooplankton before recording, will make it possible to determine abundances of zooplankton groups at low concentrations (less than one individual per liter). For profiling live and dead zooplankton, machine learning methods should be employed to automate classification, processing, and analysis of video data.

References

Barkhatov Y.V., Tolomeev A.P., Drobotov A.V., Zadereev E.S. (2023) The response of zooplankton abundance in saline meromictic Lake Shira to a change in circulation regime. *Journal of Oceanology and Limnology*, 41(4): 1321–1330

Bickel S.L., Tang K.W., Grossart H.-P. (2009) Use of aniline blue to distinguish live and dead crustacean zooplankton composition in freshwaters. *Freshwater Biology*, 54(5): 971–981

Ciranni M., Murino V., Odone F., Pastore V.P. (2024) Computer vision and deep learning meet plankton: Milestones and future directions. *Image and Vision Computing*, 143: 104934

Duarte C.M., Prairie Y.T., Montes C., Cole J.J., Striegl R., Melack J., Downing J.A. (2008) CO_2 emissions from saline lakes: A global estimate of a surprisingly large flux. *Journal of Geophysical Research: Biogeosciences*, 113(G4)

Dubovskaya O. P. (2008) Evaluation of abundance of dead crustacean zooplankton in a water body using staining of the samples by aniline blue technique: methodological aspects. *Journal of Siberian Federal University Biology*, 1(2): 145–161 (in Russian)

Dubovskaya O.P., Gladyshev M.I., Gubanov V.G., Makhutova O.N. (2003) Study of non-consumptive mortality of crustacean zooplankton in a Siberian reservoir using staining for live/dead sorting and sediment traps. *Hydrobiologia*, 504(1–3): 223–227

Dubovskaya O. P., Tolomeev A. P., Kirillin G., Buseva Z., Tang K. W., Gladyshev M. I. (2018) Effects of water column processes on the use of sediment traps to measure zooplankton non-predatory mortality: a mathematical and empirical assessment. *Journal of Plankton Research*, 40(1): 91–106

Eerola T., Batrakhanov D., Barazandeh N. V., Kraft K., Haraguchi L., Lensu L., Suikkanen S., Seppälä J., Tamminen T., Kälviäinen H. (2024) Survey of automatic plankton image recognition: challenges, existing solutions and future perspectives. *Artificial Intelligence Review*, 57(5): 114

Gilyarov A.M. (1987) *Population dynamics of freshwater planktonic crustaceans*. Moscow, Nauka, 191 p. (in Russian)

Gladyshev M.I., Dubovskaya O.P., Gubanov V.G., Makhutova O.N. (2003) Evaluation of non-predatory mortality of two *Daphnia* species in a Siberian reservoir. *Journal of Plankton Research*, 25(8): 999–1003

Gorsky G., Ohman M.D., Picheral M., Gasparini S., Stemmann L., Romagnan J.-B., Cawood A., Pesant S., Garcia-Comas C., Prejger F. (2010) Digital zooplankton image analysis using the ZooScan integrated system. *Journal of Plankton Research*, 32(3): 285–303

Guenet B., Danger M., Harrault L., Allard B., Jauset-Alcala M., Bardoux G., Benest D., Abbadie L., Lacroix G. (2014) Fast mineralization of land-born C in inland waters: first experimental evidences of aquatic priming effect. *Hydrobiologia*, 721(1): 35–44

Gulati R.D., Zadereev E.S., Degermendzhi A.G. (Eds.) (2017) *Ecology of meromictic lakes. Ecological studies, Vol 228*. Springer, Cham

Li X., Yu R., Wang J., Sun H., Liu X., Ren X., Zhuang S., Guo Z., Lu X. (2024) Greenhouse gas emissions from Daihai Lake, China: Should eutrophication and salinity promote carbon emission dynamics? *Journal of Environmental Sciences*, 135: 407–423

Lombard F., Boss E., Waite A. M., Vogt M., Uitz J., Stemmann L., Sosik H. M., Schulz J., Romagnan J.-B., Picheral M., Pearlman J., Ohman M.D., Niehoff B., Möller K.O., Miloslavich P., Lara-Lpez A., Kudela R., Lopes R.M., Kiko R., Karp-Boss L., Jaffe J.S., Iversen M.H., Irisson J.-O., Fennel K., Hauss H., Guidi L., Gorsky G., Giering S.L. C., Gaube P., Gallager S., Dubelaar G., Cowen R.K., Carlotti F., Briseño-Avena C., Berline L., Benoit-Bird K., Bax N., Batten S., Ayata S.D., Artigas L.F., Appeltans W. (2019) Globally consistent quantitative observations of planktonic ecosystems. *Frontiers in Marine Science*, 6: 196

McCauley E. (1984) The estimation of the abundance and biomass of zooplankton in samples. *A manual on methods for the assessment of secondary productivity in freshwaters*. Downing J.A., Rigler F.H. (Eds.) Blackwell Science Publishers, London, p. 228–265

Neubauer D., Kolmakova O., Woodhouse J., Taube R., Mangelsdorf K., Gladyshev M., Premke K., Grossart H.-P. (2021) Zooplankton carcasses stimulate microbial turnover of allochthonous particulate organic matter. *The ISME Journal*, 15(6): 1735–1750

Read J.S., Hamilton D.P., Jones I.D., Muraoka K., Winslow L.A., Kroiss R., Wu C.H., Gaiser E. (2011) Derivation of lake mixing and stratification indices from high-resolution lake buoy data. *Environmental Modelling & Software*, 26(11): 1325–1336

Schindelin J., Arganda-Carreras I., Frise E., Kaynig V., Longair M., Pietzsch T., Preibisch S., Rueden C., Saalfeld S., Schmid B., Tinevez J.-Y., White D.J., Hartenstein V., Eliceiri K., Tomancak P., Cardona A. (2012) Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7): 676–682

Schulz J., Barz K., Ayon P., Lüdtke A., Zielinski O., Mengedoht D., Hirche H.-J. (2010) Imaging of plankton specimens with the lightframe on-sight keyspecies investigation (LOKI) system. *Journal of the European Optical Society – Rapid Publications*, 5: 10017s

Simon M., Grossart H.-P., Schweitzer B., Ploug H. (2002) Microbial ecology of organic aggregates in aquatic ecosystems. *Aquatic Microbial Ecology*, 28(2): 175–211

Simoncelli S., Kirillin G., Tolomeev A.P., Grossart H.-P. (2019) A low-cost underwater particle tracking velocimetry system for measuring in situ particle flux and sedimentation rate in low-turbulence environments. *Limnology and Oceanography: Methods*, 17(12): 665–681

Tang K.W., Gladyshev M.I., Dubovskaya O.P., Kirillin G., Grossart H.-P. (2014) Zooplankton carcasses and non-predatory mortality in freshwater and inland sea environments. *Journal of Plankton Research*, 36(3): 597–612

Tolomeev A.P., Dubovskaya O.P., Kirillin G., Buseva Z., Kolmakova O.V., Grossart H.-P., Tang K.W., Gladyshev M.I. (2022) Degradation of dead cladoceran zooplankton and their contribution to organic carbon cycling in stratified lakes: field observation and model prediction. *Journal of Plankton Research*, 44(3): 386–400

Tolomeev A.P., Zadereev E.S., Drobotov A.V., Yaskelyaynen D.D. (2024) Underwater video imaging systems to study zooplankton abundance and diversity: challenges and opportunities. *Journal of Siberian Federal University. Biology*, 17(4): 420–437

Wen Z., Song K., Zhao Y., Jin X. (2016) Carbon dioxide and methane supersaturation in lakes of semi-humid/semi-arid region, Northeastern China. *Atmospheric Environment*, 138: 65–73

Wetzel R.G. (2001) *Limnology: lake and river ecosystems. 3rd Edition*. Academic Press, San Diego, 1006 p.

Yolgina O.E., Tolomeev A.P., Dubovskaya O.P. (2022) Computer processing and analysis of scanned zooplankton samples: guidelines. *Journal of Siberian Federal University. Biology*, 15(1): 5–30 (in Russian)

Zadereev E., Drobotov A., Anishchenko O., Kolmakova A., Lopatina T., Oskina N., Tolomeev A. (2022) The structuring effects of salinity and nutrient status on zooplankton communities and trophic structure in Siberian lakes. *Water*, 14(9): 1468