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AUTOMATED QUANTIFICATION OF *DAPHNIA SP*. POPULATION IN WATER RESERVOIRS USING DIGITAL IMAGE PROCESSING

T. Klochko[1], K. Nosov[2], I. Hnoievyi[3], O. Hryhoriev[3], Yu. Bespalov[2], I. Babaryka[3]
[1]National Aerospace University named after M.E. Zhukovsky "KHAI", Kharkiv, Ukraine
[2]V. N. Karazin Kharkiv National University, Kharkiv, Ukraine
[3]State Biotechnological University, Kharkiv, Ukraine
E-mail: t.klochko@khai.edu

Annotation. We propose a scientific and technical methodology for creating a preliminary procedure to remotely detect the presence of a substantial population of zooplankton filter feeders within a specific region of a reservoir. This detection is achieved through computer processing of digital photographs taken in that area. It is about a method involving the phototactic attraction of zooplankton using white light. The proposed approach entails computing correlations between the RGB model components of digital photographs taken in a phytobenthos area before and after zooplankton attraction using light. Subsequently, the systemic colorimetric parameters can be employed in the procedure for remotely detecting a substantial population of zooplankton filter feeders within a specific reservoir section. Specifically, we seek colorimetric parameters that mirror the developmental patterns outlined by Margalef's model of succession. This investigation involves comparative analysis of correlation tables for RGB model components in digital photos taken before and after zooplankton attraction using white light. Our study focuses on Daphnia pulex. The study utilized digital photos obtained from aquarium experiments conducted under conditions simulating drone-based photography at a height of 1-2 meters above the water's surface.

The results are novel from the perspective of aquatic ecosystem ecology, as they provide a formalized description of changes in the systemic colorimetric parameters of Margalef's model of succession. These changes are attributed to the influence of coloration on these systemic parameters within this specific waterplane ecosystem of zooplankton filter feeders. The applied significance of these results lies in identifying systemic colorimetric parameters that can be utilized in remote registration procedures to detect the environmental and biological presence of zooplankton filter feeders in specific areas of a reservoir. This is significant for water purification from bacterial suspensions and for establishing a natural food source for juvenile fish.

Key words: *remote registration of zooplankton, zooplankton filter feeders, digital photos, image processing, Margalef's succession model, drones.*

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ПРОЦЕДУРА ОБРОБКИ ЦИФРОВОГО ЗОБРАЖЕННЯ ДЛЯ ДИСТАНЦІЙНОЇ РЕЄСТРАЦІЇ КІЛЬКОСТІ *DAPHNIA SP.* У ВОДОЙМІ

Т.А. Клочко[1], К.В. Носов[2], І.В. Гноєвий[3], О.Я. Григор'єв[3], Ю.Г. Беспалов[2], І.Г. Бабарика[3]

[1]Національний аерокосмічний університет ім. М.Є. Жуковського «ХАІ», м.Харків, Україна

[2]Харківський національний університет ім. В.Н. Каразіна, м. Харків, Україна

[3] Державний біотехнологічний університет, м. Харків, Україна

E-mail: t.klochko@khai.edu

Анотація. Наведено науково-технічний підхід до розробки ескізного варіанту процедури дистанційної реєстрації наявності на певній ділянці водоймища значної кількості фільтраторів зоопланктону шляхом комп'ютерної обробки цифрового фото цієї ділянки. Йдеться про процедуру, що включає приманювання зоопланктону білим світлом. Поданий підхід передбачає побудову шляхом комп'ютерного аналізу відповідних цифрових фото, таблиць кореляцій значень компонентів RGB-моделі цифрових фото ділянки фітобентосу до і після приманювання зоопланктону світловим променем. Після цього здійснюється знаходження системних колориметричних параметрів, які можуть бути використані в процедурі дистанційної реєстрації наявності на певній ділянці водоймища значної кількості фільтраторів зоопланктону. Йдеться про знаходження системних колориметричних параметрів, що відображають закономірності розвитку маргалефової моделі сукцесії. Шляхом порівняльного аналізу таблиць кореляцій значень компонентів RGB-моделі цифрових фото, зроблених до і після приманювання білим світлом зоопланктонного організму – *Daphnia pulex*. Робота виконана на матеріалі цифрових фоторезультатів акваріумних експериментів в умовах, що імітують зйомку з дрону, що завис на висоті 1-2 м над поверхнею води.

Результати є новими з точки зору екології водних екосистем, оскільки вони забезпечують формалізований опис змін у системних колориметричних параметрах моделі видового різноманіття Маргалефа. Ці зміни пояснюються впливом забарвлення на системні параметри в специфічній водній екосистемі природних фільтраторів, яким є зоопланктон. Прикладне значення цих результатів полягає у визначенні системних колориметричних параметрів, які можуть бути використані в процедурах дистанційної реєстрації для виявлення екологічної та біологічної присутності зоопланктону в певних областях водойми. Це важливо для очищення води від бактеріальних суспензій і для створення природного джерела їжі для молоді риб.

***Ключові слова**: дистанційна реєстрація зоопланктону, фільтратори зоопланктону, цифрові фото, обробка зображень, Маргалефова модель сукцесії, дрони.*

Introduction. Global climate change presents numerous acute challenges, particularly environmental issues that frequently give rise to biosecurity risks (Lenssen et al., 2019). These challenges highlight the need to develop advanced, technology-driven methods for monitoring and enhancing the natural self-purification processes in reservoirs and watercourses. In conflict zones, the significance of these issues escalates due to the destruction of water treatment facilities.

A crucial contributor to the natural self-purification of reservoirs is the vital role played by zooplankton filter feeders. Notably, it has been observed for quite some time that they can significantly reduce bacterial populations in ponds, often achieving a reduction of approximately 99% (Golubkovskaja, 1978). Bacterial pathogens responsible for infectious diseases can infiltrate water bodies, especially when untreated domestic water mixes with sewage from destroyed systems. Such pollution poses significant biosecurity risks, affecting large swaths of terrain, including remote and inaccessible regions. Consequently, it becomes essential to explore remote (aerospace) technologies for monitoring the status of zooplankton filter feeders in water bodies situated within these challenging areas. The vital activity of zooplankton filter feeders plays a crucial role in water purification by reducing bacterial populations. This, in turn, mitigates the risk of dangerous infectious diseases, including epidemics caused by these bacteria. Additionally, remote monitoring methods for zooplankton status are relevant to food security. Zooplankton significantly contribute to the nutrition of fry across all fish species, maintaining this role throughout the lives of many commercially important fish.

Scientific and technical prerequisites have long been established for the development and implementation of remote methods to monitor the status and living conditions of zooplankton (Levashov et al., 2004; Picheral et al., 2010).

Currently, we can predominantly discuss the utilization of aerospace methods to monitor the status and vital activity conditions of marine zooplankton (Olenin et al, 2021). This focus arises in the context of World Ocean productivity challenges, particularly concerning the problematic reproduction conditions for commercial fish populations. The issue of fish reproduction has long been a matter of concern (Pauly and Zeller, 2016). In this study, we emphasize the significance of remote sensing procedures for assessing the state of zooplankton filter feeders in continental freshwater reservoirs, including small bodies of water that could serve as sources for the spread of dangerous infectious disease pathogens. This method becomes crucial when domestic wastewater containing bacterial pathogens enters these small reservoirs, especially in cases where zooplankton filter feeders are absent. Their presence is essential for effectively purifying water from bacterial suspensions. Let’s discuss methods that can be implemented using widely available and cost-effective approaches. One such example involves processing digital photos of reservoirs, specifically those captured by drones. Ideally, we focus on equipment included in the standard delivery package of common and affordable drone models. These images provide valuable data for monitoring zooplankton filter feeders and assessing water quality.

In the paper, we investigate the feasibility of remotely detecting a substantial population of zooplankton filter feeders within a specific reservoir area. This detection relies on computer processing of digital photographs taken in that region, with the added factor of attracting zooplankton individuals to the area using light. Our research focuses on the genus *Daphnia*, which plays a significant role in releasing bacterial suspension from water due to the vital activity of zooplankton filter feeders. Additionally, these organisms contribute to the natural food supply for freshwater fish fry. In your aquarium experiment, you investigated how attracting *Daphnia sp.* individuals with light in a specific area affected the system colorimetric parameters (SCPs) of digital photos taken in that region.

Review of literary sources and statement of the problem. In research, we study how the presence of zooplankton filter feeders in specific water surface areas affects SCPs of digital photos. These SCPs reflect the colorimetric relationships outlined by Margalef’s model of succession (MMS) (Margalef, 1967). Bespalov et al. (2017) demonstrated that the colorimetric parameters linked to these relationships – whose dynamics align with those of MMS – can be extracted through computer processing of the RGB model components from digital photos of phytobenthos plant communities. (Against a background of these plant communities, it’s common to observe accumulations of *Daphnia sp.*)

Adaptive strategies related to the protective coloring of fish, particularly in terms of their biological significance and color-based mechanisms, were thoroughly studied (Grigoriev et al., 2021; Vysotska et al., 2022). Drawing from these findings, they propose approaches for developing methods to remotely sense fish against the backdrop of phytobenthos. These methods leverage computer processing of digital photos.

The mathematical modeling of systemic aspects of animal coloration has seen significant advancements in biological research (Newport et al., 2017; Green, 2021). Researchers have explored various aspects related to adaptation strategies of coloration during ontogenesis and phylogenesis. Notably, this field traces its origins back to the pioneering work of Alan Turing

(Turing, 1952). His contributions laid the foundation for understanding color patterns and their significance in the natural world.

The study of animal coloration continues to captivate researchers across various contexts (Endler and Mappes, 2017). In this study, we explore several aspects related to the coloration of fish – specifically, hydrophones. These hydrophones play a crucial role in aquatic ecology, closely intertwined with the zooplankton ecosystem. Additionally, climatological aspects are addressed in a prior work (Panayotova and Horth, 2018). Consequently, we can make the following observations. In the case of *Daphnia sp.*, we can anticipate that water temperature and oxygen content influence the red color component. This influence arises from the presence of hemoglobin in the body of this zooplankton representative. Consequently, specific adaptations are necessary for the remote monitoring procedure of zooplankton filter feeders in the context of phytobenthos. Given the destruction of thermal power plants within the combat zone in Ukraine, it is crucial to remain mindful of this issue. The destruction of thermal power plants frequently disrupts the hydrobiological regime of cooling ponds, resulting in alterations to water temperature and oxygen content. Vissio et al. (2021) delve into the neurohumoral aspects underlying fish coloration formation and function. When considering *Daphnia sp*., it becomes essential to account for the potential impact of transitioning from parthenogenesis to reproductive processes, including the emergence of males within the population, on coloration. Such a transition becomes feasible when temperature conditions in the population's habitat deteriorate.

The aspects related to breeding color play a crucial role in facilitating the detection of sexual partners. These aspects exhibit significant expression, which can be remotely recorded. For instance, the mating coloring patterns of salmon exemplify this phenomenon (Kudo et al., 2012; Maselko and Connor, 2016). Moreover, these aspects have been studied extensively using remote (aviation) technologies during natural spawning migrations (Groves et al., 2016).

In the context of protective coloring in animals, certain aspects significantly impact observer detection. These aspects relate to scenarios where prey must avoid detection by predators or vice versa. Specifically, dismembering coloration, which plays a crucial role in these interactions, has been thoroughly analyzed in (Duarte et al., 2017). In the case of animals with relatively stable protective coloration over time, the diversity of dissecting (camouflage) coloration assumes a crucial role. In the context of protective coloration, it is essential that at least one spot of such coloring seamlessly blends with the background across all points in space and time during the development of MMS (motion-mediated selection). This blending effect serves to disrupt the holistic visual perception of the animal's silhouette. Bespalov et al. (2013) have demonstrated that the absence of diversity in protective coloration among animals can be offset by its uniformity. In the current context, we can discuss the application of the principle of optimal diversity, which posits that the diversity within a biological system may be constrained by the scarcity of specific resources (Bukvareva, 2013). In the context of permanent camouflage coloration in animals, the angular size of the silhouette serves as a critical resource. This silhouette accommodates a finite number of spots with varying colors. However, for *Daphnia sp*., the nature of camouflage coloring is dynamic due to the continuous movement of micro-crossbars within the gastrointestinal tract and the presence of hemoglobin in the gaps of the circulatory system. Within the scope of this study, it is pertinent to focus on variations in SCPs that emerge as a consequence of the substantial presence of *Daphnia sp*. individuals against the backdrop of the phytocenosis.

Significantly, the population size of *Daphnia sp*. holds relevance for public health beyond their role as filter feeders, which contributes to purifying water by removing bacterial suspensions. In a related context, it is pertinent to mention *Gambusia sp*. Indeed, the contribution of these small fishes in combating malaria is well-documented. Malaria poses significant biosecurity risks in numerous tropical and subtropical regions worldwide (World Health Organization, 2019; World Health Organization, 2021). Given the significant role of *Gambusia sp*., the study of their physiology constitutes a crucial aspect of ichthyological research (Hou et al., 2019; Huang et al., 2019). Furthermore, ongoing efforts focus on developing remote sensing methods for detecting these fish in reservoirs (Vysotska et al., 2022). To complement these methods, it is essential to incorporate remote sensing techniques for monitoring the state of their natural food base. Notably, during the early stages of ontogenesis, *Daphnia sp*. plays a significant role in this ecological context.

When considering variations in the spectral SCPs as potential markers for the presence of *Daphnia sp*. clusters within the phytocenosis backdrop, several key points should be highlighted. These SCPs offer valuable insights into the ecological dynamics of these zooplankton populations. When considering a phytocenosis governed by the principles of MMS, it becomes prudent to explore the correlations between colorimetric parameters associated with photosynthetic productivity, stability, and pigment diversity. These parameters can serve as valuable SCPs for understanding the ecological dynamics within such ecosystems. Let's delve into the implications of these premises regarding colorimetric parameters and their significance in the context of *Daphnia sp*. This premise should be augmented by considering the impact of plant pigments within the digestive system of translucent *Daphnia sp*. individuals and the role of hemoglobin in their circulatory system lacunae on the values of these SCPs. The nature of this influence will be characterized by specific correlations between the values of the components in the RGB model of a digital photograph.

Drawing from the aforementioned information, we can formulate the following goals and objectives for this study.

The objective of this study is to devise a precise scientific and technical methodology for constructing a preliminary version of a procedure aimed at remotely detecting the presence of a substantial population of zooplankton filter feeders within a designated reservoir area. This detection process relies on computer analysis of digital photographs captured in that area, with a specific focus on attracting zooplankton using white light.

To address this objective, we need to tackle the following challenges:

- computing tables of correlations for the RGB components by analyzing the corresponding digital photos. This model represents digital photos of a phytobenthos area before zooplankton attraction using white light;

- computing tables of correlations for the RGB components by analyzing the corresponding digital photos. This model represents digital photos of a section of phytobenthos after attracting zooplankton using light;

- identifying SCPs that can be employed in the procedure for remotely detecting a substantial population of zooplankton filter feeders within a designated reservoir section. This involves a comparative analysis of correlation tables based on the RGB model components of digital photos taken both before and after attracting zooplankton using white light.

Material and methods of research. We investigated the diagnostic potential of statistically significant ($p > 0.05$) correlations of SCPs identified in this study under controlled aquarium conditions. In two experimental variants, zooplankton attraction was achieved through radiation from a white LED. In two control variants, this attraction was not implemented. The diagnostic utility of the identified SCPs is relevant to the procedure for remotely detecting a substantial population of zooplankton filter feeders within a specific area of the reservoir. During the aquarium experiments, we captured digital photos of 2x2 cm areas located directly adjacent to the point where zooplankton attraction occurred in the experimental variants using white LED radiation. These photos were taken under conditions simulating the scenario, utilizing onboard drone equipment hovering at a height of 1-2 m above the water surface.

In the study, computer processing of the acquired photos was performed using a Python-based software package which enables the determination of RGB model component values for each pixel in the image, which can then be entered into a table. Based on the table's data, correlation tables (Pearson correlation) that capture the relationships between colorimetric parameters in digital photos of the aforementioned aquarium sections obtained from both experimental and control variants were computed.

Results and their discussion. In the study, the following four variants of aquarium experiments were conducted:

- control variant: we consider a phytocenosis that simulates a biofilm of microscopic algae on the river bottom. This variant excludes zooplankton individuals and involves attraction using white light (RBNL);
- experimental variant: we consider a phytocenosis that simulates a biofilm of microscopic algae on the river bottom. This variant excludes zooplankton individuals and involves the implementation of white light (RBL);
- control variant: we consider a phytocenosis that simulates a biofilm of microscopic algae on the pond bottom. This variant includes the presence of *Daphnia pulex* individuals, averaging 70-100 specimens per liter, but excludes their attraction using white light (PBNL);
- experimental variant: we consider a phytocenosis that simulates a biofilm of microscopic algae on the pond bottom. This variant includes the presence of *Daphnia pulex* individuals, averaging 70-100 specimens per liter, and involves attracting them using white light (PBL) to enhance their population size.

Based on the work conducted, we have computed correlation tables that capture the colorimetric relationships within the aforementioned aquarium sections. These colorimetric parameters provide insights into specific aspects of the MMS and their interaction with *Daphnia pulex* accumulations.

The expression R/(R+G+B) represents a colorimetric parameter that reflects the presence of yellow and red-orange phytopigments in MMS. Additionally, it correlates with the coloration of *Daphnia pulex*. The abundance of these phytopigments in the simulated MMS system is linked to the aging and demise of photo-producer cells that can no longer engage in active photosynthesis. The expression G/(R+G+B) serves as a colorimetric parameter that indicates the presence of green chlorophyll phytopigments within MMS. This parameter also correlates with the coloration of *Daphnia pulex*. Specifically, the abundance of these phytopigments in MMS is directly linked to the photo producers' capacity for active photosynthesis. The expression (G+R)/(R+G+B) represents a colorimetric parameter that encompasses the total contribution of green, yellow, and red-orange phytopigments within MMS. Additionally, it correlates with the coloration of *Daphnia pulex*. This parameter provides insights into the presence of various cell types, including young, living, actively photosynthesizing cells, as well as older and deceased cells. The expression R/G corresponds to a colorimetric parameter known as the "yellow-green index." This parameter provides insights into the diversity and stability of pigments within the simulated MMS system.

In the context of this study, the correlation relationships among these colorimetric parameters are quantified using Pearson correlation coefficients. These coefficients hold diagnostic potential for the procedure aimed at remotely detecting a substantial population of zooplankton filter feeders within a specific section of a reservoir. SCPs obtained from the four aquarium experiment variants are included in Table 1-4.

Table 1

Pearson correlation coefficients between the colorimetric parameters of the phytobenthos microalgal film in the RBNL aquarium experiment. The coefficients, along with their statistical significance indicators, are provided at the intersections of rows and columns in the table

Colorimetric parameters	(R+G)/(R+G+B)	G/(R+G+B)	R/(R+G+B)	R/G
(R+G)/(R+G+B)		0.42 (p<0.05)	0.95 (p<0.05)	0.76 (p<0.05)
G/(R+G+B)	0.42 (p>0.05)		0.12 (p<0.05)	-0.27 (p<0.05)
R/(R+G+B)	0.95 (p<0.05)	0.12 (p<0.05)		0.92 (p<0.05)
R/G	0.76 (p<0.05)	-0.27 (p<0.05)	0.92(p<0.05)	

Table 2

Pearson correlation coefficients between the colorimetric parameters of the micro-algal film of phytobenthos, obtained in the RBL aquarium experiment

Colorimetric parameters	(R+G)/(R+G+B)	G/(R+G+B)	R/(R+G+B)	R/G
(R+G)/(R+G+B)		0.41 (p<0.05)	0.97 (p<0.05)	0.77 (p<0.05)
G/(R+G+B)	0.41 (p>0.05)		0.14 (p<0.05)	-0.27 (p<0.05)
R/(R+G+B)	0.97 (p<0.05)	0.14 (p<0.05)		0.92 (p<0.05)
R/G	0.77 (p<0.05)	-0.27 (p<0.05)	0.92(p<0.05)	

The data in Table 1 and Table 2 exhibit minimal differences, which are statistically insignificant. Illumination with white LED light increases the brightness of an object across the red, green, and blue regions of the spectrum, but to an equal extent for all of these regions. Consequently, substantial alterations in the correlations among the aforementioned colorimetric parameters are not anticipated. The exceedingly minor discrepancies manifest during the digital photo registration process. Such discrepancies may arise due to the stochastic nature of photo registration processes at the individual pixel level. Notably, zooplankton were absent in the aquarium experiment variants RBNL and RBL. Hence, as anticipated, the zooplankton's response to white LED radiation did not yield a discernible effect.

In the aquarium experiment variants PBNL and PBL, areas without attraction to white LED radiation exhibited *Daphnia pulex* concentrations ranging from 70 to 100 specimens per liter. In areas where attraction to white LED radiation occurred, these concentrations increased by 3-4 times. This change was accompanied by statistically significant differences in the values of SPCs. These differences are as follows

- in the PBNL variant, there is a statistically significant ($p < 0.05$) positive correlation of the values of the parameters R/G and (R+G)/(R+G+B);
- in the PBL variant, there is a statistically significant ($p < 0.05$) negative correlation of the values of the parameters R/G and (R+G)/(R+G+B);
- in the PBNL variant, there is a statistically significant ($p < 0.05$) positive correlation of the values of the parameters R/G and (R+G)/(R+G+B);
- in the PBL variant, there is a statistically significant ($p < 0.05$) negative correlation of the values of the parameters R/G and (R+G)/(R+G+B).

Table 3

Pearson correlation coefficients between the colorimetric parameters of the micro-algal film of phytobenthos, obtained in the PBNL aquarium experiment

Colorimetric parameters	(R+G)/(R+G+B)	G/(R+G+B)	R/(R+G+B)	R/G
(R+G)/(R+G+B)		0.26 (p<0.05)	0.50 (p<0.05)	0.18 (p<0.05)
G/(R+G+B)	0.26 (p>0.05)		-0.70 (p<0.05)	-0.90 (p<0.05)
R/(R+G+B)	0.50 (p<0.05)	-0.70 (p<0.05)		0.94 (p<0.05)
R/G	0.18 (p<0.05)	-0.90 (p<0.05)	0.94(p<0.05)	

Table 4

Pearson correlation coefficients between the colorimetric parameters of the micro-algal film of phytobenthos in the PBL aquarium experiment

Colorimetric parameters	(R+G)/(R+G+B)	G/(R+G+B)	R/(R+G+B)	R/G
(R+G)/(R+G+B)		0.99 (p<0.05)	0.94 (p<0.05)	-0.87 (p<0.05)
G/(R+G+B)	0.99 (p>0.05)		0.88 (p<0.05)	-0.93 (p<0.05)
R/(R+G+B)	0.94 (p<0.05)	0.88(p<0.05)		-0.65 (p<0.05)
R/G	-0.87(p<0.05)	-0.93 (p<0.05)	-0.65 (p<0.05)	

Comparing the data in Table 3 and Table 4 enables us to identify SCPs that exhibits statistically significant changes ($p < 0.05$) when zooplankton are attracted with white LED radiation. These SCPs can serve as a marker for the presence of significant zooplankton concentrations in the water. It is about correlations of the values of the parameters R/G and R/(R+G+B) and correlations of the values of the parameters R/G and R/(R+G+B).

When discussing the results of this study, it is essential to consider the following factors.

Indicators of substantial *Daphnia pulex* presence in water have been identified. Notably, *Daphnia pulex* plays a crucial role in the life of mid-latitude zooplankton filter feeders by efficiently removing bacterial suspensions, including those caused by pathogens capable of triggering epidemics. Similarly, other representatives of the genus *Daphnia*, sharing biological similarities with *Daphnia pulex*, along with other *Cladocera*, also fulfill this role. The color nature of their translucent bodies may influence SPCs captured in digital photos of water surfaces when a substantial concentration of these hydrobionts is present. This increase occurs due to their attraction to white LED radiation. We are examining the impact of hemoglobin's SCPs within the circulatory system lacunae of these aquatic organisms on the observed changes. Additionally, we consider the influence of phytopigments from microalgae present in the digestive system of zooplankton filter feeders. These SCPs serve as the aforementioned markers. Their values reflect the correlations between the colorimetric parameters of MMS and the alterations induced by the attraction of zooplankton filter feeders to white LED radiation. Considering the objectives of your work, it is crucial that SCPs are derived through a procedure involving remote computer processing of digital water surface photographs. This approach ensures efficient data acquisition and analysis. In practice, this method can be implemented using drone platforms equipped with standard hardware included in the delivery package of commonly available and cost-effective drone models.

Indeed, it is appropriate to conclude that the study's objectives have been met, and the overarching goal has been successfully accomplished.

Conclusions.

The authors acknowledge that their findings are preliminary. While these results may not directly enable remote registration of substantial zooplankton filter feeder populations in water, they do provide a basis for an innovative approach to developing registration procedures. This approach takes into account the specific conditions under which such registration occurs. Indeed, expanding the technological repertoire for automatic and automated environmental monitoring systems is of paramount importance. This urgency is underscored by the biosecurity threats arising from global climate change. Considering the creation of new science-intensive technologies for fundamental hydrobiological research, it is reasonable to conclude that the obtained results hold both practical and theoretical significance.

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