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Underwater Video Imaging Systems to Study Zooplankton Abundance and Diversity: Challenges and Opportunities

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Abstract. Underwater zooplankton video imaging systems have been reviewed in the context of their development, methods, and applications, with a more detailed focus on systems that have appeared in the last five years. Three notable trends are observed: 1) reduction in the size and price of systems; 2) transition from expensive equipment and licensed software to free hardware and software; 3) expanding the capabilities of systems to be applicable both in the laboratory and in the field. Extraction of ecological and biological information from underwater videos is greatly facilitated by a variety of machine learning techniques; however, their accuracy is highly dependent on the quality of the training datasets. A new optic-fluorescent flow-through system for zooplankton assessment (ZooFluoBox) is presented to expand the range of the parameters measured by underwater imaging instruments and enhance the control of turbulent and light conditions during video recording. Fluorescence detection allows distinguishing between eating and non-eating zooplankters: diapausing or dead and live ones. The system is designed to profile zooplankton abundance and depth distribution, mostly in inland waters, as its depth limit is 60 m and object size limit is 5 mm.

Keywords: underwater imaging, fluorescence, stereoscopic system, zooplankton.

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Системы подводной видеосъемки для изучения численности и разнообразия зоопланктона: проблемы и возможности

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Аннотация. Системы подводной видеосъемки зоопланктона рассмотрены в контексте их развития, методов и областей применения. Отдельное внимание уделено системам, появившимся в последние пять лет. Наблюдаются три заметные тенденции: 1) уменьшение размеров и стоимости систем; 2) переход от дорогостоящего оборудования и лицензионного программного обеспечения к бесплатному оборудованию и программному обеспечению; 3) расширение возможностей систем для применения как в лабораторных, так и в полевых условиях. Извлечение экологической и биологической информации из подводных видеозаписей значительно облегчают различные методы машинного обучения, однако их точность сильно зависит от качества обучающих наборов данных. Представлена новая оптико-флуоресцентная проточная система для оценки зоопланктона (ZooFluoBox), позволяющая расширить спектр измеряемых параметров подводных приборов и обеспечить контроль турбулентных и световых условий при видеосъемке. Детекция флуоресценции позволяет различать питающихся и не питающихся (диапаузирующих или мертвых) зоопланктеров. Система предназначена для изучения зоопланктона, преимущественно во внутренних водоемах, поскольку ее возможности ограничены глубинами до 60 м и размерами объектов до 5 мм.

Ключевые слова: подводная видеосъемка, флуоресценция, стереоскопическая система, зоопланктон.

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Introduction

Underwater video systems for plankton analysis have made significant progress in their development over the past few decades and continue to improve (Lombard et al., 2019; Moghimi, Mohanna, 2021). This area of research is actively developing thanks to the technical capabilities of modern video cameras (the emergence of high-speed, high-resolution light-sensitive matrices) on the one hand, and to the success of neural network image processing algorithms, on the other. Together, these two factors underpin the strong trend in plankton research based on automated video analysis systems (Orenstein et al., 2022).

There are several underwater systems designed for different tasks of analyzing images of planktonic organisms in the field and laboratory conditions. Systems can be focused on different size groups of planktonic organisms ranging from several micrometers (Orenstein et al., 2020) to tens of centimeters (Cowen, Guigand, 2008) and can be based on different principles of recording optical signals. Line-scan and area-scan cameras (Culverhouse et al., 2015; Pitois et al., 2021; see review Lombard et al., 2019), holographic cameras (Göröcs et al., 2018; Dyomin et al., 2021), and 3D cameras (Simoncelli et al., 2019) are used. The type of light source and its location vary as well. Some systems are unique developments of individual institutes, for example, ZOOVIS-SC (Benfield et al., 2007), SPC (Orenstein et al., 2020), etc.

More than a dozen similar systems, both new and based on older, but significantly upgraded, models, have appeared over the past years. The purpose of this paper is to summarize the systems mentioned in the literature in the last five years, to discuss major challenges and opportunities in underwater video imaging systems used to study zooplankton, and to introduce the new ZooFluoBox system, which expands the capabilities of *in situ* devices for underwater imaging of zooplankton.

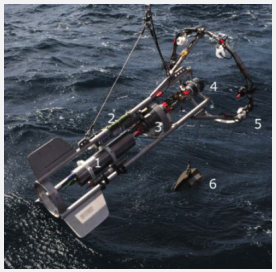
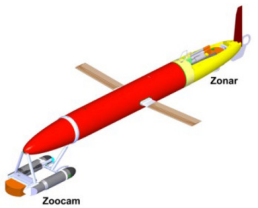
The review of underwater video imaging and zooplankton analysis systems

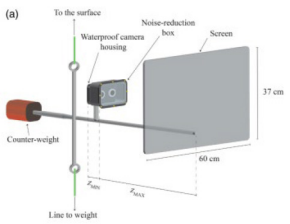
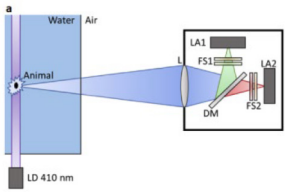
A summary of underwater video imaging and zooplankton analysis systems that have been published since 2019, following the detailed review by Lombard et al. (2019) of different optical and imaging methods to study plankton both in the laboratory and *in situ*, is presented in Table 1. We did not aim to compare in detail all technical characteristics of the systems, because many of them can be easily modified by changing optics and photo sensors and by altering shooting modes.

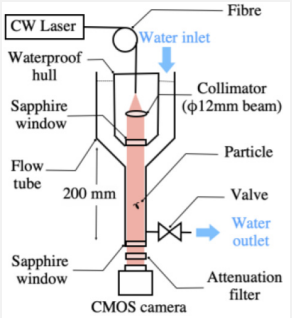
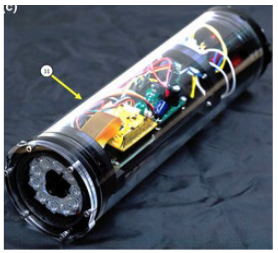
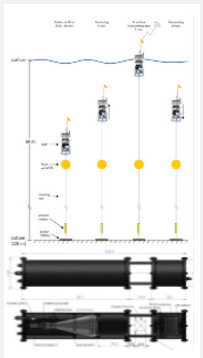
Among the commercial underwater video systems for studying zooplankton, the most famous are the UVP (Underwater Vision Profiler) (Picheral et al., 2010; Picheral et al., 2022) and the *IS*IIS (*In Situ* Ichthyoplankton Imaging System) (Cowen, Guigand, 2008). Both systems are open, i.e. images of zooplankters are taken in the external environment without control of the turbulence and light conditions. The UVP consists of a 4 or 5 MP video camera and two side light sources that produce short flashes in the red range of wavelengths with a duration of 100 μs (http://www.hydroptic.com/index.php/public/Page/product_item/UVP5_DISCONTINUED). Short flashes allow acquiring clear images of objects in motion. The size of the scanned water volume is 15 cm × 20 cm × 3.5 cm (about 1 liter) per frame. The size of one pixel is 145 or 88 μm depending on the system configuration. Recently, a lightweight version of the UVP6-LP was introduced (Picheral et al., 2022), scanning a volume of 15 cm × 180 cm × 2.3 cm (0.6 L) per frame with a resolution of 73 μm.

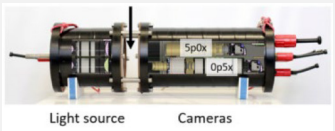
The *IS*IIS, unlike UVP, does not have a lateral light source, but is based on recording of objects in direct transmitted light. The camera

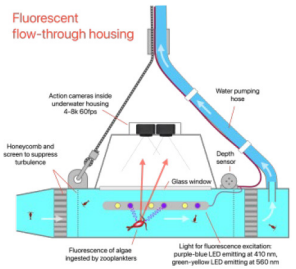
Table 1. Submerged video systems for the *in situ* plankton observations, recording and analysis. Table includes video systems presented since 2019, after the review of Lombard et al. (2019) was published

	System	Image / Outline	Application	Short description (method, size range of objects, sampled volume, resolution, illumination, other important features)	Reference
1	PELAGIOS (The pelagic *in situ* observation system)		The PELAGIOS is suitable for open-ocean observations of gelatinous fauna	Optical imaging with a forward-viewing deep-sea camera. Size range of objects > 1 cm. Recorded average volume is 0.116 m^3/s at a towing speed of 0.5 m/s. High-definition video is used with 50 fps. Illumination – light LED array on the aluminum ring in front of the camera. Additional recording of conductivity, temperature and oxygen. Deploying with two deck persons and a winch operator. Operation depth up to 3000 m	Hoving et al., 2019
2	Zooglider (glider that includes a low-power camera and a dual frequency Zonar)		Optically imaging mesozooplankton and marine snow *in situ*, autonomously profiling the water column in a specified region, over deployment periods as long as 50 d	Shadowgraph imaging. Size range of 0.5–20 mm of equivalent spherical diameter. Sampled volume of 250 mL per frame. Frame size 1296 × 964 pixels, resolution 40 µm. Additional recording of acoustic backscatter, chlorophyll a, temperature, and salinity. Zooglider is a modified Spray glider that includes a low-power camera and a custom dual frequency sonar system. Maximum operating depth of ~ 400 m to the sea surface. In air weight 58.8 kg	Ohman et al., 2019
3	PI (a high-speed color line scan-based imaging instrument)		Collection of mesozooplankton data and biomass estimates over ocean-basin scales, in or near real-time	Optical imaging using a line-scan camera. Size range 100 µm – 20 mm. Maximum field of view of 20 mm, depth of field of 16 mm (Culverhouse et al., 2015). Sampling rate of 22 L/min. Image resolution is 2048 pixels per line and a line-scanning rate of 70 kHz. Resolution of 10 µm. Imaging system flow cell is back-illuminated by strobe LED light to prevent motion blurring. The instrument scans continuously pumped water from a depth of 4 m as the ship is underway	Pitois et al., 2021

	System	Image / Outline	Application	Short description (method, size range of objects, sampled volume, resolution, illumination, other important features)	Reference
4	3D-PTV (a low-cost three-dimensional underwater particle tracking velocimetry system)		Underwater *in situ* videos of moving particles to determine particle settling rate	Optical imaging. Two water-proof cameras that acquire stereoscopic videos of sinking particles at 48 fps over a tunable sampling volume of 45 × 25 × 24 cm	Simoncelli et al., 2019
5	SPC (the Scripps Plankton Camera system)		A framework and platform for *in situ* microscopy, which facilitate observations and insights into the diverse planktonic ecosystem	Optical dark-field imaging. A set of free-space dark field microscopes resolving objects from tens of microns to several centimeters. MICRO-SPC detects objects in the size range of 20 µm – 2 mm. Sample volume of 3 µL/frame, resolution of 2 µm. MINI-SPC detects objects in the size range of 130 µm – 2 cm. Sample volume of 3 mL/frame, resolution of 13.4 µm. MACRO-SPC detects objects in the size range of 500 µm – 10 cm. Sample volume of 0.5 L/frame, resolution of 50 µm. Each system uses a 12 megapixel sensor, frame rate of 8 fps. Illumination – strobed LEDs. The system images particles that enter the sample volume via ambient flow. All SPC instruments can be deployed autonomously – saving data to onboard storage before offloading to the server, or cabled – saving data directly to the remote server via Ethernet. The systems weigh from 11 to 30 kg in air	Orenstein et al., 2020
6	Dual-band fluorosensor		The detection of laser-induced autofluorescence of individual zooplankton	A dual-band fluorescence detection system. Fluorescence is measured on zooplankters of 0.7–1 mm body length. A water volume containing zooplankton is illuminated with a collimated violet 410 nm laser diode with beam size 3 mm × 5 mm. An achromatic doublet lens is used to collect the induced fluorescence. The fluorescence is imaged onto two identical 16-bit linear array detectors, using a long pass dichroic mirror with a cut-on wavelength of 550 nm. The system successfully differentiates the non-eating from algae-eating zooplankton at different ratios in a laboratory setting	Nielsen et al., 2020

7	In-line holographic microscope		Recording of 3-D motion of living/ nonliving particles in seawater over a measurement channel	Holography imaging, using a combination of a continuous wave laser and a fast CMOS sensor with low parasitic gain. The minimal distinguishable size of objects is ~20 µm. The volume observed in each frame is 12 mL. Frame size of 2464 × 2056 pixels, maximum frame rate of 158 fps. The volumetric throughput can achieve up to 1904 mL/s. The instrument records clear holograms of moving particles at a speed of approximately 200 mm/s.	Liu et al., 2021
8	The *In situ* Plankton Assemblage eXplorer (IPAX) Stereoscopic IPAX system with tilted lens		Open-source low-cost imaging platform for zooplankton studies. It can be used to observe phototactic behavior elicited from the different color LEDs. The stereoscopic IPAX system can perform 3D measurements	Optical imaging. The IPAX can resolve 100 µm features with 70 % contrast at the focal plane with 5 cm × 3 cm field of view and 5 mm depth of field. Sampled volume of 7.5 mL, resolution of 20 µm. Camera was tested at 1640 × 1232 pixels resolution and 30 fps. IPAX captured images of open space using backscatter illumination. The LED flash unit is triggered by signal from the Raspberry Pi. Stereo setup with tilted lenses increases the sampling volume by 3.1 times compared to a traditional stereo setup with the same optical parameters. The IPAX unit can be programmed to record videos of different durations on a schedule. Fully charged batteries last for 80 minutes of video recording in total. The versatility of IPAX allows adaptations to many experimental needs for aquatic ecology	Lertvilai, 2020; Lertvilai and Jaffe, 2022
9	The Prince William Sound Plankton Camera (PWSPC)		Autonomous vertical profiling of plankton and particulates	Optical dark-field imaging. The camera system is based on the Scripps Plankton Camera (Orenstein et al., 2020), but with larger optics and a higher resolution camera. The imaged volume of the camera is 450 mL. The camera takes 12-bit color images at a 4 fps with ROI processing. Field of view 93 × 70 mm. Pixel size (object space) – 22.6 µm. Imager resolution 4240 × 2824 pixels. The LEDs are strobed. The weight of total system in the air is 10 kg. The PWSPC is a part of the PWS AMP system based on a WETLabs Thetis profiler. It is capable of conducting ~70 60-m profiles per charge. Profiles were usually done within 15 min	Campbell et al., 2020

	System	Image / Outline	Application	Short description (method, size range of objects, sampled volume, resolution, illumination, other important features)	Reference
10	miniDHC digital holographic camera		Measurement of the following set of background data: plankton concentration, average size and size dispersion of individuals, particle size distribution, water turbidity and suspension statistics	Holography imaging, using in-line holographic scheme in folded configuration. Particle sizes of 0.1–28 mm are fixed. Sampled volume of medium of 0.5 L. Frame rate up to 24 fps. Real-time processing of holograms takes place discretely – every 20 sec. Full processing of holograms is completed after the recording. The device can record particle distribution profiles with the device lowering speed of 0.1–1 m/s up to the depth of 500 m	Dyomin et al., 2021
11	DSPC (Underwater dual-magnification imaging)		Automated *in situ* monitoring of phyto- and zooplankton communities. The DSPC is able to track the dynamics of taxa, mostly at the genus level, covering many components of the planktonic food web (including parasites and potentially toxic cyanobacteria)	Optical dark-field imaging. DSPC is a dual magnification underwater microscope, based on the Scripps Plankton Camera system (Orenstein et al., 2020). MINI- and MICRO-SPC run simultaneously in a single housing. The detection range of objects is between ~10 µm to ~ 1 cm. The image volumes are 0.2–10 µL and 4–200 µL at 5× and 0.5× magnification, respectively. The maximum rate is 10 fps, maximum image size is 3800 × 2600 pixels (recording of videos is also possible). Internal batteries allow autonomous measurements for up to 3 hours without recharging. Automated *in-situ* imaging data are available in real-time. The DSPC weighs 27.2 kg	Merz et al., 2021
12	Probe video system		Vertical distribution of zooplankton *in situ*	Optical imaging. The sampled volume of the marine environment is 60 × 80 × 4 mm per frame with a resolution of 0.03 mm. The objects are illuminated by a set of rod-shaped white LED lamps. Images are transmitted via the fiber-optic core of the cable to the ship. The operator can remotely control the camera parameters and lamp activation. The working depth of the submersible module of the system is 200 m	Olenin, 2021

13	Buoy-Borne Underwater Imaging System		Achieving high-quality images, acquisition of plankton. Integration with a moored surface buoy for long-term *in situ* plankton monitoring of coastal waters.	Optical imaging. Size range of objects from 200 μm to 40 mm depending on the lens installed. Per image volume is 26.7, 1.5, and 0.0625 mL at different magnifications. The imager features a new strobe LED illuminator with 360° inward convergent laminar lighting design. At a rate of 3 fps, the system can detect individual plankton and suspended particles from raw images, and transmit the cropped region of interest (ROI) vignettes instantly to a remote server via a local wireless cellular network. The imager is protected with antifouling measures	Li et al., 2022
14	ZooFluoBox (underwater optic-fluorescent flow system for zooplankton assessment)		Vertical profiling of zooplankton. Recording of fluorescent signals produced by ingested algae in the guts of zooplankters. Measurement of 3D swimming patterns	Optic-fluorescent dark-field 3D imaging in flow housing. The size range of objects is ~200 μm – 5 mm. Sampled volume per frame is 360 mL. Cameras record video at 3840×2160 pixels and 60 fps with pixel resolution of 26 μm. Illumination – LEDs of 410 nm and 560 nm. Fluorescent signal from ingested algae in the guts of zooplankters is distinguishable. The water inside the housing is refreshed by a piston pump. Instrument is operated at a depth of up to 60 m	This project

and light source are located opposite each other, forming a scanned volume between them. Objects in this volume are projected onto the camera sensor as dark silhouettes against a white background. Owing to the collimator lens, which creates a parallel flow of light in the scanned space, the projections of the objects always have the same size, regardless of their position relative to the collimator lens. In various configurations, the camera captures a strip width from 42 to 135 mm, while the scanned profile is effectively continuous. Resolution of the system is 21–46 µm. Both systems (UVP and *IS*IIS) are focused primarily on the study of marine plankton and operate at depths of up to 6000 and 200 m, respectively. The maximum resolution of the system (21 µm, *IS*IIS) is suitable for identifying objects measuring 0.3 mm or larger. Considering the high transparency of water and the fairly large size of marine zooplankton (usually more than 1 mm), the technical characteristics of the devices fully satisfy the tasks of identifying, determining the abundance, and finding profiles of the spatial distribution of marine zooplankton.

Open systems, though, have their drawbacks. They underestimate the number of rare species or individual groups of organisms of the same species that are not constantly present in the field of view of the camera. To mitigate this drawback, it is necessary to increase considerably the volume of scanned water. This can be achieved either by performing repeated manual measurements of zooplankton profiles or by installing an underwater camera at a buoy station for a long time (Li et al., 2022). Zooplankton abundance and depth distribution can also be profiled autonomously, using a robotic winch. However, integrating a camera into a monitoring platform is not always possible and is more costly.

Another limitation of open systems is that they cannot detect weak fluorescence signal of phytoplankton from the gut of animals against a natural light background. Underwater systems with the flow chambers were first designed quite a long time ago, for example, OPC and LOPC (http://www.coml.org/investigating/observing/opcs.html) or LOKI (Schulz et al., 2010). However, they were not developed further. There are several systems using the flow-through principle, in which the recording part is surface-mounted and located onboard. There are several modifications of the LiZA/PIA flow-through system (Culverhouse et al., 2015; Pitois et al., 2018, 2021). Recently, an imaging and classification system for small aquatic organisms based on the flow principle – SAO Imager – was developed (https://www.oceanspacesensors.org/saoimager). It was presented in May 2022 at the Joint Conference of American Societies for Aquatic Ecology, JASM, May 14–20, 2022 (https://jasm2022.aquaticsocieties.org/). The system consists of the imaging chamber with a high-speed line-scan camera located onboard, while water is pumped from the desired depth. The main advantage of the system is its performance in harsh weather conditions. The system is compact and easy to use. However, the imaging chamber of this system is not submersible, which is a major disadvantage. As the water sample is delivered to the imaging chamber via a hosepipe, the fragile organisms can be destroyed because of cavitation, and the organisms can be subjected to stress, leading, for instance, to the shedding of egg sacs by copepods, which will substantially change the characteristics of the zooplankton community. In addition, the bubbles formed when pumping water through a hosepipe create considerable image noise.

Laboratory systems have better image quality compared to submersible underwater systems. The most widely used laboratory systems are FlowCam (Sieracki et al., 1998) and ZOOSCAN (Grosjean et al., 2004). FlowCam is a flow cytometer that captures image of each

particle/object, optical scattering, and fluorescent signal of particles. The interchangeable objectives make it possible to image objects in a wide range of sizes from phytoplankton cells (the minimal size is ca. 0.2 μm) (e.g. Zadereev et al., 2021) to zooplankton (up to 2 mm) (e.g. Wang et al., 2017). The system has its own software that allows the user to identify objects, consider their quantity, and measure physical characteristics.

ZOOSCAN is a scanner for fixed zooplankton samples, built based on a professional photo scanner of the Epson Perfection series. Images have a high resolution of 4 μm and even higher for small scanning areas. The advantage of this system is the low cost of the device with excellent quality of the resulting images (Yolgina et al., 2022).

Thanks to the efforts of developers of systems for the video imaging of zooplankton in the field and in the laboratory, it became possible not only to speed up the acquisition of data on the abundance and biomass of planktonic species, but also to expand the range of tasks related to the functional ecology. The study by Orenstein et al. (Orenstein et al., 2022) addresses the prospects for such research. Based on computer analysis of images, many characteristics can be obtained that are directly related to growth, reproduction, nutrition, survival, physiological state and behavioral responses. Thus, the greater the capabilities of the imaging system (resolution, reproducibility, size of the field of view, etc.), the wider the range of tasks that it can solve. An integral direction in the development of plankton imaging systems is the improvement of software and image processing algorithms.

The current state of computer methods for the imaging of plankton and "sea snow" particles was reviewed by Irisson et al. (2022). At the early stages of image analysis, classical machine learning methods such as support vector machines and random forests were used to identify objects; later, convolutional neural networks (CNNs) were developed widely. Classical machine learning methods require obtaining individual properties/characteristics of objects (size, area, shape indicators, color, texture, etc.), and a classification is built on their basis. Convolutional neural networks are trained on many (tens of thousands) known images, and, subsequently, they do not need to take individual characteristics of objects – only the original image is needed. There are a large number of projects aimed at improving algorithms for recognizing planktonic organisms (e.g. https://kaggle.com/c/datasciencebowl, https://ecotaxa.obs-vlfr.fr/). In particular, the Russian corporation Yandex recently announced the development of a neural network based on Yandex Cloud for the analysis and classification of planktonic organisms in images previously collected from Lake Baikal (https://github.com/baikal-zooplankton). Nevertheless, machine-learning techniques are only as good as the data they are trained on (Irisson et al., 2022). Classifiers can be improved through large, diverse and representative training data sets. A full review of these methods is beyond the scope of our paper.

The main progress in the development of underwater video imaging systems and zooplankton analysis was associated with oceanic and marine research. On the one hand, oceanic systems, compared to inland water bodies, have a considerably larger scale. For large systems, the possibility of reducing the time for sample processing and increasing the coverage of the water area with samples/observations is one of the priorities. Video imaging of zooplankton and other particles, including settling dead organic matter, can significantly reduce the cost of ocean research and increase the accuracy and volume of information collected. In addition, the work on board large marine vessels makes it possible to use bulky equipment, and the first underwater

video imaging systems were often integrated into various underwater probes and gliders.

Considering the uniqueness or low serial production of many such systems, they were quite expensive (for example, the average cost of the *in situ* optical systems for the plankton imaging was ca. US$ 70000 as reviewed by Lombard et al. (2019)). However, in routine ocean research, given the scale of these systems, the use of expensive instruments is justified.

The high price and the bulkiness of the equipment hampered the use of underwater video imaging systems in continental waters (however, see Zadereev et al. (2010) on the use of a relatively cheap system to analyze vertical profiles of amphipods in the stratified lake). However, the reduction in the price of high-speed video cameras and the development of image analysis methods have led to the rapid development of underwater video imaging systems.

With a decrease in costs of consumables and image and video processing algorithms, a trend is emerging towards the development of cheap open systems in which both consumables and software are available to the user. For example, video-based approach Zoobooth was developed to measure the size of individual zooplankton. The device is small, portable, and constructed of affordable and available components. Authors shared all the files to build and use Zoobooth (Broch, Heuschele, 2023). The *In situ* Plankton Assemblage eXplorer (IPAX) is an open-source low-cost imaging platform for zooplankton studies. Material costs of the IPAX are less than US$ 450 (Lertvilai, 2020). We consider that, similar to other areas of biology (see, for example, Jolles, 2021), the development of underwater video surveillance systems will move towards lower cost and openness of hardware and software code. Thus, keeping in mind this trend and comparative analysis of existing systems for *in situ* imaging observation of zooplankton, we propose the design of underwater optic-fluorescent flow system for zooplankton assessment. As we plan to make this system open in terms of both hardware and software, we decided to describe it and make it available to aquatic ecologists and researchers from the very beginning. The progress with the development of the system will be available online at https://zoofluobox.wordpress.com/.

Proposed underwater optic-fluorescent flow system for zooplankton assessment – ZooFluoBox

Idea and background

The development of new instrumental approaches in zooplankton research based on digital image analysis aims to improve the quality and quantity of measured biological parameters. However, the development of new tools required to solve contemporary scientific problems is often limited by the technical capabilities of the recording photo and video equipment (or its high cost). Fortunately, such digital cameras as action cameras have become widespread recently. The combination of high-quality photographic sensors and a relatively low cost of the devices provides the basis for their current practical use in zooplankton bioimage analysis. For example, testing the GoPro 11 camera in the 4k resolution and 60 fps mode with an external macro lens showed that the image of a 1 mm object at a distance of 10 cm is about 38 pixels (or pixel resolution of 26 μm). This resolution is usually sufficient to determine the shape and size of zooplankters and identify them roughly at the level of higher taxonomic groups. The total volume of water that can be simultaneously "scanned" with this camera with an acceptable level of sharpness is about 300 mL. In addition, the sensors of modern action cameras have sufficient light sensitivity to register fluorescent signals emitted by objects. In the case of zooplankton, microalgae in their guts produce the fluorescent signal. Fluorescence

signals can be used to estimate the physiological state of zooplankters and their food sources and to distinguish between live and dead or diapausing individuals in a population.

The underwater fluorescence imaging systems are extensively used to study the abundance and species composition of phytoplankton (e.g. Lunven et al., 2012). However, such systems are designed to study the florescence signal of small phytoplankton cells in a small water volume illuminated with laser beam at specific wavelength (e.g. submersible flow cytometer (Imaging FlowCytobot) records optical properties of individual suspended cells < 100 µm as they pass through a focused laser beam in a 5 mL water volume (Olson and Sosik, 2007)). Such an approach is not suitable for automated underwater imaging of mesozooplankton, as much larger volumes of water need to be visualized. Thus, all systems mentioned above are technically and ideologically different from the proposed ZooFluoBox system. We tested the possibility to record the fluorescence with an action camera using cladoceran *Moina macrocopa* (Straus, 1820) fed with green alga *Chlorella vulgaris* (Fig. 1). The fluorescent signal, which is clearly seen under an epifluorescence microscope (Fig. 1, a, b), is also visible on the frames captured with the action camera (Fig. 1, c, d). The resolution of the action GoPro HERO 11 (GoPro Inc, U.S.A.) camera is also high enough for the identification of zooplankton individuals with the body length ca. 1 mm and longer (Fig. 1 e, f). In addition to fluorescence, video recordings (as opposed to photo images) can be used to assess the swimming activity of zooplankters. The field of view of action cameras is large enough to record the free movement of zooplankters. Swimming pattern is related to physiological state and, sometimes, can be used for taxonomic identification along with other features. The additional parameters listed (swimming activity and fluorescence) are not new to laboratory systems, but in practice, they are not measured for the zooplankton in the field because underwater instruments are most often of the open type and do not control turbulence and light conditions.

The idea behind the new instrument is to transfer some of the capabilities of laboratory systems into the field device. At the same time, researchers should also be able to control and calibrate the measured parameters by laboratory methods if necessary. This opportunity should be implemented in the field system. The desired system can be based on an underwater flow-

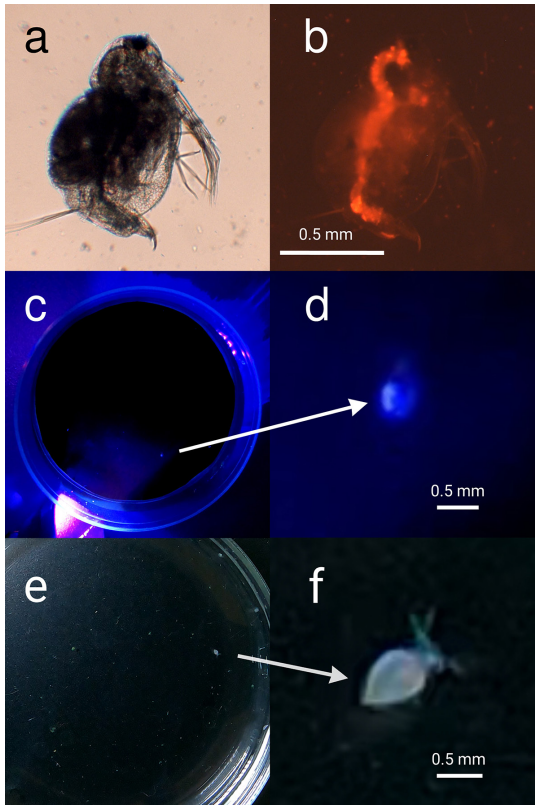


Fig. 1. Images of *Moina macrocopa* (Straus, 1820) fed with green algae *Chlorella vulgaris* acquired with (a, b) epi fluorescent Axioskop 40 (Carl Zeiss, Germany) and (c-f) action camera GoPro HERO 11 (GoPro Inc, U.S.A.). Moina under light-field (a) and fluorescent (b) microscopy. Video acquisition of Moina with GoPro camera illuminated by 410 nm excitation light (c, d) and ambient light (e, f). Fluorescence of algae in guts is distinguishable

through system. Zooplankton should be recorded inside flow housing to control turbulence and light conditions. Two video cameras can be used to obtain 3D images of zooplankters and their swimming patterns. The lighting system should include excitation fluorescent light sources. Water flow through the housing can be performed by means of a hose and a water piston pump. Processing of the video material can be performed by any state-of-the-art methods used in bioimage analysis and machine learning.

Instrument's conception

We propose a new optic-fluorescent flow-through system for zooplankton assessment – ZooFluoBox. The system consists of an underwater node and an on-boat one (Fig. 2). The on-boat node includes a water piston pump and a light switch unit. The underwater node consists of a video camera module and flow-through housing with dark field LED illumination. A depth sensor is added to the underwater node for accurate vertical positioning. A hose connected to an on-boat pump performs the flowing of water with zooplankton through the housing. The length of the hose specifies the depth range of the zooplankton recording. The hose is enhanced with a rope for safe lowering of the underwater part. Along the hose is located the power cable for the light module. The pump drives water

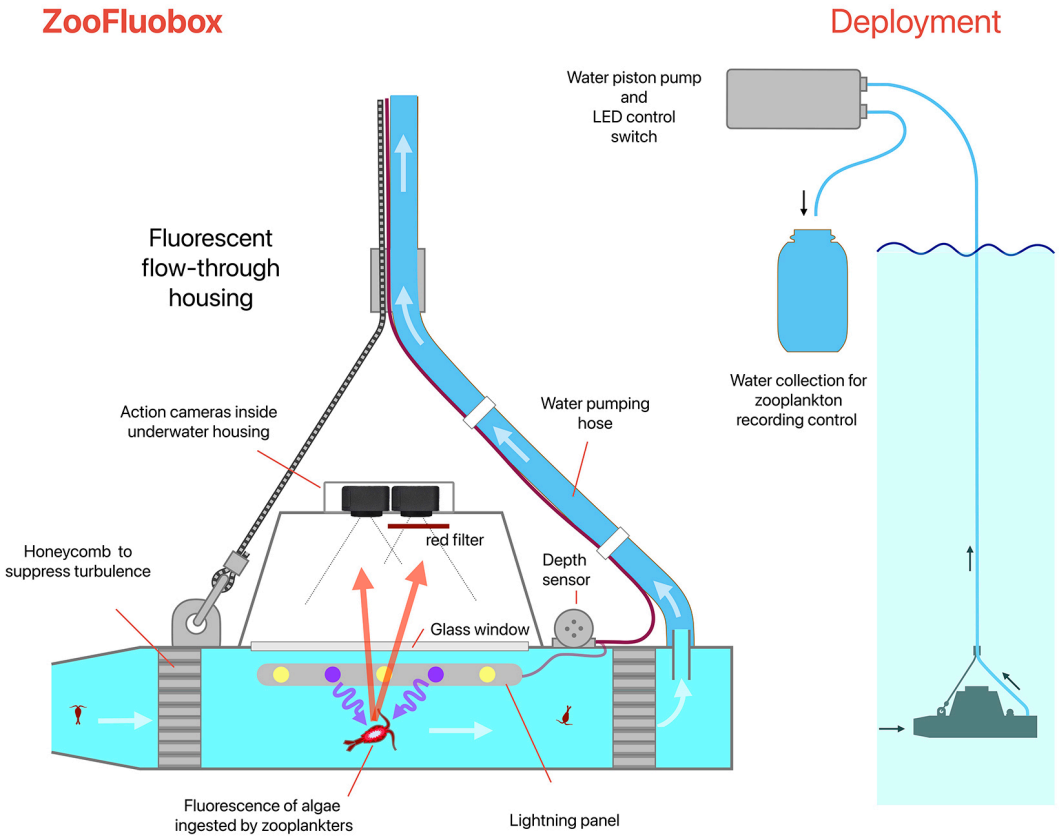


Fig. 2. Structural diagram of ZooFluoBox – optic-fluorescent flow-through system for zooplankton assessment. The system consists of an underwater node and an on-boat node. The on-boat node includes a water piston pump with pulse-width modulation to control flow rate and a light switch unit. The underwater node consists of a video camera module and flow-through housing with dark field LED illumination. A depth sensor is used to determine vertical position

through the housing in cyclic mode. Valves are installed at the inlet and outlet of the pump piston cylinder to prevent water backflow. In the first half of the cycle, the piston is pulled back, creating a vacuum in the cylinder. The vacuum is transferred through a hose to the housing of the submerged part. As a result, the housing is filled with new water from the outside environment. In the second half of the cycle, the pumped water is drained from the pump cylinder. At this time, water movement through the housing is stopped, for the optical-fluorescence characteristics of zooplankters and their natural swimming pattern to be recorded under stable conditions. The water that has passed through the system is collected in an on-boat vessel for subsequent analysis of the captured zooplankton in the laboratory if necessary.

The flow-through housing is a flat parallelepiped about 3 cm high, the top face of which has a glass viewing window for video recording. The front part of the housing is open and narrows horizontally to form a sampling tunnel through which water is drawn from the investigated layer. The opposite side of the housing is connected to the hose that allows the water to be pumped. Damping baffles are located inside the housing to dampen turbulence at the inlet and outlet of the zooplankton video recording area. The baffles include a honeycomb structure wall and mesh screens in a similar manner to work by Zhang et al. (2020). The size of the partition cells certainly limits the size of the recorded objects, but it is large enough (~ 5 mm in diameter) for free passage of freshwater zooplankton. We observed small water swirls in the video recording area for 5–7 seconds after the pump stops. During the water intake, the zooplankters sometimes "freeze", but then they resume their usual movements. Turbulence inside the chamber is minimal due to the damping baffles. In addition, the pump operates with smooth acceleration and deceleration during water intake to reduce turbulence inside the video recording area.

There is a video recording camera module above the viewing window (size 10 cm × 12 cm) of the housing. Two video cameras forming a stereo pair are located at the top of the truncated pyramid body at a distance of 10 cm from the observation window. An air space is kept between the video cameras and the observation window. The use of two video cameras also allows one to determine the exact size of the recorded objects using the triangulation method. The lighting units are located on the side faces of the flow housing. Switching on the LED illumination is related to the water pumping cycle. The housing can be equipped with different types of LEDs to obtain optical and fluorescence characteristics of zooplankters. The following scheme would be optimal for obtaining zooplankton morphometry and swimming pattern and for assessing the qualitative composition of ingested food in a single profiling. One of the two cameras captures objects in the full-light wavelength range and the other in the red region (>650 nm or >590 nm) to record fluorescence from zooplankton gut contents while also retaining outlines of bodies. The blue-violet (400–450 nm) and the green-yellow (550–570 nm) excitation light is proposed to emit the fluorescence. The blue-violet LED is required to record the fluorescence signal from green microalgae, while the green-yellow LED excites the fluorescence from cyanobacteria. Both types of LEDs can be switched either in series or in parallel. The operating depth range of the device is determined by the waterproof properties of the cameras and is usually within 60 meters. In most cases, this is sufficient to study the zooplankton of inland water bodies and to be used in shallow marine ecosystems.

Given the availability of consumables (our system is based on a commercially available

GoPro camera) and the development of image analysis algorithms, we expect this system to be an open source system in terms of software, design, and hardware. With the project development, all drawings, instruction, and program codes will be published on a website (https://zoofluobox.wordpress.com/), which will allow any researcher of inland water bodies to construct and use a similar system at low cost and with available resources.

Conclusion

Until recently, progress in the development of underwater video surveillance systems for planktonic organisms was associated with their use in oceanology. However, as it was noted by John Downing (Downing, 2014), the two fields of aquatic ecology – oceanography and limnology – like two twins, should not be separated by methods, approaches, and ideas. Equipment for inland waters must be substantially more compact and often cheaper than for oceanic and marine ecosystems. However, the development and miniaturization of technology, as well as the emergence of accessible and free image processing algorithms, make this task close to implementation. The following trends can be identified in the development of underwater video imaging systems for planktonic organisms: 1) reduction in the size and price of systems; 2) transition from expensive equipment and licensed software to free hardware and software; 3) expanding the capabilities of systems to solve laboratory and field problems.

The presented underwater optic-fluorescent flow system for zooplankton assessment (ZooFluoBox) is in line with the main trends in underwater imaging development observed in the last five years. Increasing instrumental capabilities in the field of *in situ* studies of zooplankton, combined with the prospects of computerized information processing by machine learning methods, creates the basis for gradually overcoming the problems of costly routine monitoring and assessment of zooplankton by classical hydrobiological methods. It can be expected that automation of zooplankton monitoring will lead, on the one hand, to the collection of more extensive material on the abundance, biomass, and species composition of natural zooplankton, and, on the other hand, to the emergence of qualitatively new tasks in research of zooplankton functioning in aquatic ecosystems based on new quantitative and qualitative material.

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