

1 Underwater dual-magnification imaging for  
2 automated lake plankton monitoring

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19

## 20 Abstract

21 We present an approach for automated *in-situ* monitoring of phytoplankton and zooplankton  
22 communities based on a dual magnification dark-field imaging microscope/camera. We  
23 describe the Dual Scripps Plankton Camera (DSPC) system and associated image  
24 processing, and assess its capabilities in detecting and characterizing plankton species of  
25 different size and taxonomic categories, and in measuring their abundances in both  
26 laboratory and field applications. In the laboratory, body size and abundance estimates by  
27 the DSPC significantly and robustly scale with the same measurements derived by  
28 traditional microscopy. In the field, a DSPC installed permanently at 3 m depth in Lake  
29 Greifensee (Switzerland), delivered images of plankton individuals, colonies, and  
30 heterospecific aggregates without disrupting natural arrangements of interacting organisms,  
31 their microenvironment or their behavior at hourly timescales. The DSPC was able to track  
32 the dynamics of taxa in the size range between  $\sim 10 \mu\text{m}$  to  $\sim 1 \text{ cm}$ , covering virtually all the  
33 components of the planktonic food web (including parasites and potentially toxic  
34 cyanobacteria). Comparing data from the field-deployed DSPC to traditional sampling and  
35 microscopy revealed a general overall agreement in estimates of plankton diversity and  
36 abundances, despite imaging limitations in detecting small phytoplankton species and rare  
37 and large zooplankton taxa (e.g. carnivorous zooplankton). The most significant  
38 disagreements between traditional methods and the DSPC resided in the measurements of  
39 community properties of zooplankton, organisms that are heterogeneously distributed  
40 spatially and temporally, and whose demography appeared to be better captured by  
41 automated imaging. Time series collected by the DSPC depicted ecological succession  
42 patterns, algal bloom dynamics and circadian fluctuations with a temporal frequency and  
43 morphological resolution that would have been impossible with traditional methods. We  
44 conclude that the DSPC approach is suitable for stable long-term deployments, and robust  
45 for both research and water quality monitoring. Access to high frequency, reproducible and  
46 real-time data of a large spectrum of the planktonic ecosystem might represent a  
47 breakthrough in both applied and fundamental plankton ecology.

## 48 Keywords

49 Phytoplankton, zooplankton, size, microscopy, classification, diversity

## 50 Abbreviations

51 DSPC: dual-magnification Scripps plankton camera

52 MIC: inverted microscopy

53 0p5x: 0.5 times magnification

54 5p0x: 5.0 times magnification

55 CNN: convolutional neural network

56

57

## 58 1. Introduction

59 Plankton are a key component of all water bodies and the Earth's biosphere, being crucial  
60 for important ecosystem processes such as carbon and nutrient cycling, and provide  
61 essential services to human society (e.g. clean water and fisheries) (Behrenfeld et al., 2001;  
62 Falkowski, 2012). The combination of relatively short lifespans and strong sensitivity to  
63 environmental conditions makes planktonic organisms effective indicators of environmental  
64 change and ecosystem health, and are used to assess water and aquatic ecosystem quality  
65 worldwide (Xu, 2001; Directive 2000/60/EC). Information about plankton biomass and  
66 community composition, including variation among key functional traits, is essential to  
67 assess the state of ecological systems, their resilience to change, extinctions and invasions,  
68 and to manage the ecosystem services that they provide (Bartley et al., 2019; Cardinale et  
69 al., 2012; Pomati et al., 2011). Assessing plankton community composition is critically  
70 important for the monitoring and forecasting of harmful algal blooms (events of algal  
71 overgrowth that are dominated by toxic species). In freshwaters, cyanobacterial blooms  
72 cause large ecological and economical damage (Huisman et al., 2018), and have been  
73 increasing worldwide due to effects of land and climate change (Chorus and Bartram, 1999;  
74 Ho et al., 2019).

75 Given the importance of plankton for ecosystem processes and services, there is not only  
76 scientific interest, but also a societal need and a policy requirement for monitoring plankton  
77 communities at low cost and ideally in real-time. The assessment of plankton community  
78 composition and taxa relative abundance is however the most difficult, time consuming and  
79 expensive aspect (Pomati et al., 2011). Dedicated laboratories use skilled and trained  
80 taxonomists to identify and count microalgae and zooplankton manually, sometimes taking  
81 several hours to count one sample. Taxonomists frequently misclassify items, count some  
82 objects more than once or overlook others (MacLeod et al., 2010) and mistakes are not  
83 traceable back in time. In many monitoring programs, microscope counts are only conducted  
84 weeks or months after samples are collected, making this approach unable to provide  
85 information in near real-time. Sample collection, transport and storage also demand proper  
86 infrastructure and personnel time. Aquatic ecosystem monitoring would hence tremendously

87 benefit from an automated, reliable, standalone system for plankton identification,  
88 classification and counting that produces transparent and reproducible results (not  
89 dependent on personal judgement), and that does not require sample collection and storage  
90 (additional costs).

91 Several automated quantitative tools for plankton counting have been established:  
92 laboratory-based (e.g. Flow-cam, flow-cytometry, microscopy-imaging, Zooscan) and field-  
93 based (e.g. fluorescence probes, Cytobuoy, Flowcytobot, imaging cameras) (Lombard et al.,  
94 2019). Laboratory-based tools can automate counting and sorting, but still require sample  
95 collection and processing, which changes the nature of the sample and requires time,  
96 resources and infrastructure. Field applications are more promising, but they have their own  
97 limitations. Fluorescence sensors measuring chlorophyll-a, phycocyanin, or other  
98 photosynthetic pigments are common and widely deployed in the field, but they are unable to  
99 resolve taxonomic distinctions beyond coarse levels, and pigment content within and across  
100 species can vary widely, making estimates of abundances and biomass difficult (Johnson  
101 and Martiny, 2015). Automated in situ flow-cytometry has shown encouraging results for high  
102 frequency monitoring of phytoplankton community dynamics and morpho-physiological traits  
103 (Fontana et al., 2018; Hunter-Cevera et al., 2016; Pomati et al., 2011; Sosik et al., 2010).  
104 Flow-cytometry complemented by water physics and chemistry data, and machine learning  
105 for analysis, demonstrated the potential of high-frequency information for modelling  
106 environmental responses and predicting phytoplankton dynamics (Thomas et al., 2018).  
107 Dynamics of single species of phytoplankton and microzooplankton can be tracked and  
108 studied with flow-cytometry if assisted by imaging (Hunter-Cevera et al., 2016; Lombard et  
109 al., 2019). Still, flow-cytometers require constant maintenance and calibration, and may  
110 disrupt natural spatial aggregates while sampling. Additionally, they have limited dynamic  
111 range in terms of size, often being able to follow the dynamics of a single trophic level only  
112 (Lombard et al., 2019). For phytoplankton, particularly, high frequency data of herbivore  
113 zooplankton could be essential to study variation in top-down controls as a response to  
114 environmental change (Murphy et al., 2020).

115 Here we describe a monitoring approach based on dark-field underwater imaging, by means  
116 of a dual-magnification camera derived from the Scripps Plankton Camera (SPC) system  
117 (Orenstein et al., 2020). It was designed to be a flexible, easily configurable imaging system  
118 that can observe objects from 10s of microns to several millimeters, and sample at high  
119 temporal frequency, with minimal influence on the fluid being imaged. Similar cameras have  
120 been successfully deployed in marine systems (Campbell et al., 2020; Kenitz et al., 2020;  
121 Orenstein et al., 2020), but never deployed before in freshwater environments. In this study,  
122 we compared the DSPC to traditional microscopy and high-frequency fluorescence-based

123 monitoring using both field and laboratory comparisons. We then used this tool to observe  
124 plankton dynamics and trait distributions (e.g. size) across the whole planktonic food web *in-*  
125 *situ*, with high temporal resolution. Our results promote underwater imaging as an auspicious  
126 approach to generate empirical high-resolution plankton time series.

127

## 128 2. Methods

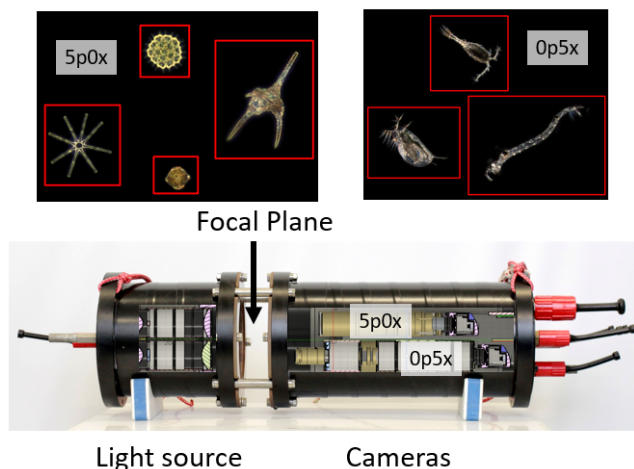
### 129 2.1 Description of the dual magnification camera

#### 130 2.1.1 Instrument's design

131 The Plankton Camera (DSPC) used in this study is a dual-magnification darkfield underwater  
132 microscope, based on the Scripps Plankton Camera (SPC) system (Orenstein et al. 2020).  
133 When compared to the original SPC, the DSPC has several new features designed  
134 specifically to target plankton monitoring in freshwaters. First, it is a dual-magnification  
135 microscope, extending the size range that can be photographed by overlapping spatial  
136 resolution (**Fig. 1**). Based on our trials, the highest magnification (5p0x) has a detection  
137 range between ~ 10  $\mu\text{m}$  - 150  $\mu\text{m}$ , while the lower magnification (0p5x) ranges between ~  
138 100  $\mu\text{m}$  - 1 cm. These estimates depend on the features of the imaged objects (e.g. colour,  
139 transparency) and therefore are not fixed. The imaged volumes per frame for the two  
140 magnifications are (from high resolution to blob detection): 5p0x = 0.2 - 10  $\mu\text{L}$ , 0p5x = 4 -  
141 200  $\mu\text{L}$ , respectively. Second, internal batteries allow autonomous measurements for up to 3  
142 hours without recharging, which is needed for manual depth profiling or sampling of remote  
143 sites (i.e. lakes, ponds). Finally, the distance between light sources and objective has been  
144 reduced to 5 cm to diminish illumination path length, and allow transmission of light in turbid  
145 waters (e.g. during thick algal blooms).

146 The DSPC is relatively affordable and compact enough for one person to carry (57 cm L x 19  
147 W, ~ 27.2 kg weight). The instrument is composed of two housings, one with the LED light  
148 sources and another with the controlling hardware, cameras and lenses (**Fig. 1**). The  
149 instrument has no moving parts; therefore, it has low maintenance requirements (only  
150 cleaning of external surfaces). Housings have facing acrylic viewports that allow transmitting  
151 light from the LED to the microscopes. Water flows freely between the two viewports. To  
152 reduce fouling, our system was complemented with a UV lamp  
153 (<https://amloceanographic.com/biofouling-control/>) installed facing the acrylic viewports (**Fig.**  
154 **1**). The on-board computer uses a Linux based operating system. The camera works  
155 automatically, there is no need for regular calibration and, if installed in a monitoring platform

156 like the DSPC at Lake Greifensee, pictures can be available in real time, if power supply,  
157 computer and internet are available. Acquisition rate for pictures can be easily modified and  
158 the maximum is 10 processed frames per second (recording of videos is also possible). The  
159 actual specifics of the instrument can be found in **Supplemental Table S1**. The  
160 configurations of the machine used for imaging are reported in the Methods section relative  
161 to laboratory and field application.  
162



163  
164 **Fig. 1. Structural drawing of the dual-magnification plankton camera, showing LED light**  
165 **source points, the two cameras with different magnification.** The focal plane is where water flows  
166 through and objects are imaged. The instrument captures full frame pictures, and automatically  
167 performs object detection (red boundaries around objects), cuts out these regions of interest (ROIs)  
168 that are stored, while full frames are usually discarded (Orenstein et al., 2020). Objects depicted as  
169 ROIs are only for display (not to scale).  
170

### 171 2.1.2 Image processing

172 We use a modified version of the original Python SPCCConvert image processing script  
173 (Orenstein et al., 2020), which converts raw regions of interest (ROIs) from the plankton  
174 camera into image mosaics and extracts statistics per processed sample and features of  
175 detected objects. The process consists of color conversion, edge-detection and  
176 segmentation, morphological feature extraction, foreground masking and inverse filtering of  
177 masked foreground. The results are then saved into a new directory and a web app (html  
178 file) is built from the data showing converted pictures. The program creates a spreadsheet  
179 with 64 features (e.g. area, aspect ratio, max. length or orientation, color features, and more)

180 of every detected object. The Python code can be found here:

181 <https://github.com/tooploox/SPCConvert>.

182 Because of their complex shape and moveable appendages, measuring body size in  
183 zooplankton is not straightforward: often, zooplankton can be much wider than they are long  
184 when antennas are included (e.g. copepods), meaning that the major axis cannot reliably be  
185 used. Moreover, the presence of predators and other stressors also influences the overall  
186 shape of zooplankton, and can induce the growth of defensive structures (e.g. spines,  
187 helmets, and elongated tails) further complicating estimation of body size (Tollrian and Drew  
188 Harvell, 1999). To overcome these complexities, we developed The Daphnia Ruler  
189 (<https://github.com/nelstevens/The-Daphnia-ruler>), which effectively removes these  
190 appendages from calculations of zooplankton body size. Using the same edge detection  
191 concepts as SPCconvert, the outline of the zooplankton is refined by repeatedly eroding and  
192 dilating the binary image with round structuring elements of varying sizes, until a threshold  
193 solidity is reached indicating that all protuberances have been sufficiently removed. The  
194 resulting ellipse can then be treated as the core body and measured as for any other  
195 spheroid planktonic object.

196

## 197 2.2. Annotation of images and classification based on deep learning

198 A large number of labelled pictures is needed to create a training database for an automatic  
199 classification based on deep learning. We utilised a free and open source platform, Taxonify,  
200 which expedites the process of species annotation. Through this platform, one can rapidly  
201 label pictures to taxon level, and can add additional attributes to the pictures, i.e colonies,  
202 dividing, and parasites present. More information can be found on <https://www.taxonify.org>,  
203 and software codes are freely available at [https://github.com/tooploox/taxonify\\_gui](https://github.com/tooploox/taxonify_gui). For this  
204 study, we annotated a variable amount of images per class of interest (mainly zooplankton,  
205 in the 0p5x magnification), ranging from a minimum of 10 (*Chaoborus*) to a maximum of  
206 3321 (*Dinobryon*) (**Supplemental Table S2**). We then developed an automated deep  
207 learning classifier for objects detected by the 0p5x magnification of the DSPC. The model we  
208 used was a convolutional neural network (CNN), trained to distinguish 34 different classes of  
209 zooplankton, phytoplankton and other suspended solids (junk) (**Supplemental Table S2**). In  
210 the SI text we describe the used CNN and its hyperparameters, and we show a series of  
211 validation benchmarks. We provide access to the code and a ready-to-use release of the  
212 classifier, at the following address: <https://github.com/mbaitjje/plankifier/releases/tag/v1.1.1>.  
213 The default code settings contain all hyperparameter values used in this study.

214

## 215 2.3 Laboratory calibration of DSPC imaging

216 To use the DSPC in the laboratory, the space between the two viewports (**Fig. 1**) was  
217 covered with a black cloth and flooded with water. Tissue culture flasks containing the  
218 imaged samples were manually held in front of the focal plane of the DSPC between the  
219 viewports to take measurements. For cultures where the sedimentation rate was high, we  
220 mixed the sample every 30 seconds with a Pasteur pipette through the lid of the flask.

### 221 2.3.1 Calibration of plankton body size estimates

222 We compared body size estimates for small (5p0x) and large (0p5x) plankton organisms by  
223 the DSPC, featuring automated object detection and features extraction, to body size  
224 measurements done with images taken by inverted microscopy, using manual object  
225 detection and feature extraction using ImageJ (<https://imagej.nih.gov/ij/>). Having information  
226 on only two dimensions, size was evaluated as the area of objects rather than volume. For  
227 the comparison, only ROIs by DSPC that were comparable to manual microscopy (i.e. not  
228 cropped and imaged from the frontal plane) were selected and used for feature extraction  
229 (i.e. object area). Body size was estimated from DSPC images using SPC convert scripts  
230 (see Section 2.1.2). Taxon specific mean body size was calculated by taking an average  
231 area in pixels over all saved objects, both for DSPC and microscopy.

232 **5p0x magnification.** We used the following phytoplankton cultures: *Aphanizomenon flos*  
233 *aquae*, *Cyclotella meneghiniana*, *Scenedesmus acuminatus*, *Tetraedron minimum*, *Eudorina*  
234 *unicocca*, *Lagerheimia hindakii*, *Staurastrum punctulatum*, *Cosmarium botrytis*, *Oocystis*  
235 *solitaria*, *Planktothrix rubescens*, *Cystodinium sp.*, *Volvox aureus*, *Peridinium sp.* All cultures  
236 were grown in WC medium (Guillard, 1975) under standard conditions (20°C and 10:14  
237 light:dark cycle) for at least 2 weeks. Cultures of two small zooplankton were also imaged at  
238 5p0x magnification, the protist *Euglena sp.* and the rotifer *Cephalodella sp.* These were  
239 grown under standard conditions and with a protozoan pellet medium (provided by  
240 CarolinaTM, Biological Supply Company, Burlington NC, USA). A volume of 2-10 mL  
241 (depending on initial culture densities) was inoculated into a 25 cm<sup>2</sup> tissue culture flask  
242 (vented cap, sterile, VWR 743-2311) and topped with tap water to a final volume of 60 mL.

243 **0p5x magnification.** We used the two large protists *Blepharisma sp.* *Spirostomum sp.*,  
244 which were grown under the same conditions as the protists used for 5p0x calibration. We  
245 also used living samples of *Copepods sp.*, *Daphnia sp.*, *Keratella quadrata*, *Keratella*  
246 *cochlearis* and *Kellicotia sp.* taken from Lake Greifensee (Switzerland).

247 **Traditional microscopy.** An aliquot of the imaged sample was taken and photographed  
248 with a light inverted microscope (Leica DMI8 magnification x160 or x320, depending on the  
249 size of the plankton species). Using ImageJ, the maximum and minimum axes length



250 (perpendicular) were manually measured for 50 to 200 individuals, and then used to  
251 calculate the object area by applying an ellipses formula.

### 252 2.3.2 Calibration of DSPC object detection relative to plankton density

253 We compared the average number of objects detected per frame (i.e. per second) in the  
254 DSPC to density measurements obtained by inverted microscopy for small (5p0x) and  
255 stereomicroscopy for large (0p5x) plankton organisms, in serial dilutions. This DSPC metric  
256 is not sensitive to frame rate and we tested its comparability to volumetric measurements.

257 **5p0x magnification.** The following cultures were used: *Scenedesmus acuminatus*, *Oocystis*  
258 *solitaria*, *Tetraedron minimum*, *Botryococcus braunii*, *Euglena sp.* and *Cephalodella sp.* For  
259 this experiment, we used culturing flasks (as in the previous experiment). An initial sample of  
260 0.5 to 10 mL of the culture was taken and topped to 60 mL with tap water. After imaging  
261 each culture for 5 minutes, 30 mL of the samples were replaced with tap water to prepare  
262 the next dilution step (1:2 dilution), followed by imaging with the DSPC. This was repeated  
263 for five dilution steps for *Scenedesmus acuminatus*, *Oocystis solitaria*, *Tetraedron minimum*,  
264 *Botryococcus braunii* and six dilution steps for *Euglena sp.* and *Cephalodella sp.* Images  
265 were then sorted out manually, whereas cropped or wrong images were not considered. The  
266 initial culture density was estimated from a subsample counted manually under an inverted  
267 microscope. **0p5x magnification.** Cultures of three *Daphnia* species, of different size  
268 classes: *D. magna*, *D. longispina*, *D. cucullata* were used. They were diluted (1:2) in six  
269 decreasing levels from 200 to 7 individuals (counted manually), and imaged in a culturing  
270 flask (total volume of 60 mL) for 5 minutes each. The images were manually sorted: first, all  
271 pictures that were considered as *Daphnia*, including cropped or/and not in focus were used  
272 for density estimation. Second, for the size estimation, only pictures of *Daphnia* in focus and  
273 not cropped were considered.

274

## 275 2.4 Field deployment and comparison with traditional monitoring

### 276 2.4.1 Automated deployment and monitoring station

277 The dual-magnification DSPC was installed at our automated monitoring station located in  
278 Greifensee, a peri-alpine, eutrophic lake in Switzerland (47.35 °N, 8.68 °E). Specific details  
279 about the automated monitoring platform can be found in (Pomati et al., 2011). The station  
280 features a multiparametric probe Ocean7 and an automated profiler  
281 (<https://www.idronaut.it/>), a meteorological station (WS700-UMB from OTT Hydrometrie /

282 LUFFT), a local computer and data network by 4G modem allowing data transmission. All  
283 data are streamlined and published in real-time on our website [www.aquascope.ch](http://www.aquascope.ch).

#### 284 2.4.2 DSPC settings and data

285 The DSPC is permanently installed at a depth of 3 m, monitoring the plankton community  
286 hourly, for 10 minutes at the start of every hour, at an imaging rate of 1 frame / second.  
287 **Phytoplankton:** for comparisons with traditional monitoring, we used only the 5p0x  
288 magnification and selected the images of the DSPC that were taken in the same hour as a  
289 microscopy sample. This was mostly between 10:00-12:00 in the morning. The whole 10  
290 minutes of running time and associated images were used. The images were then classified  
291 taxonomically to the lowest possible classification level. **Zooplankton:** We used only the  
292 0p5x magnification data and aggregated discrete hourly samples over an entire day to allow  
293 for comparisons with traditional twin-net tows. Specifically, we took the mean of a 24 h  
294 running time, 10 minutes every hour. This was to increase DSPC sample size, since net-  
295 tows concentrate hundreds of liters of water. The images of 0p5x magnification were subset  
296 to every 6 second in order to exclude multiple imaging of the same object, since some  
297 individuals were observed to remain in the focal plane of the camera for some seconds,  
298 particularly when the water was calm. The images were then classified at the lowest possible  
299 taxonomic level using the trained CNN described in Section 2.2.

#### 300 2.4.3 Traditional sampling and microscopy

301 Water samples were taken every week for 12 consecutive months starting in April 2019, with  
302 the exception of winter months (November - early March 2020) when we sampled fortnightly.  
303 All samples were taken between 10:00 and 12:00 in the morning. **Phytoplankton:** Water  
304 samples were taken near the DSPC, at 3 m depth, with a 5 L Niskin bottle. An aliquot of 50  
305 mL was fixed with Lugol's iodine solution, and then identified and counted in the laboratory  
306 under an inverted microscope (Utermöhl method). **Zooplankton:** Integrated water column  
307 samples, starting from January 2020 onwards, were taken with a 95 µm twin net, from 17 m  
308 to surface. Samples were concentrated with a 95 µm mesh and fixed in 50 mL of 100%  
309 ethanol. Identification and counting was done under a stereomicroscope.

310

### 311 2.5 Data analysis

312 All data analyses and plotting were performed in the R programming environment  
313 (<https://www.r-project.org/>). All the laboratory and monitoring data from this study will be

314 made available from a public repository before publication (link to the repository will be  
315 reported here).

### 316 2.5.1 Laboratory calibration

317 **Body size:** We calculated the size of objects as 2 dimensional areas of the object mask, as  
318 automatically provided by our image processing scripts for DSPC, or manually per each  
319 taxon in microscopy. We used a least-square linear regression to correlate microscopy  
320 measurements (the benchmark) and measures taken by the DSPC, in  $\text{Log}_{10}$  space. Size-  
321 density curves were done by using `geom_density` from the `ggplot2` package on  
322 untransformed areas (`geom_density`, `ggplot2` 3.2.1). **Density:** Measurements were  $\text{Log}_{10}$   
323 transformed, with zeros set to an arbitrary low value (0.1) before transformation. When  
324 dilution series were run multiple times, we took the mean between technical repeats. Density  
325 estimates by microscopy (the benchmark) and DSPC were then compared using linear  
326 regression for each taxon. Similarly, an overall relationship between microscopy and DSPC  
327 was calculated with linear regression in  $\text{Log}_{10}$  space for each magnification.

### 328 2.5.2 Field application

329 **Seasonal patterns:** We estimated the mean size of the phytoplankton community (as area  
330 of ROIs detected by the 5p0x magnification), and its densities, using the DSPC data from  
331 2018. As a comparison to DSPC density estimates, we used the Chlorophyll-a (Chl-a)  
332 concentration recorded by the CTD-probe fluorimeter (<https://chelsea.co.uk/products/trilux/>),  
333 using averages of Chl-a measurements from 2.9 to 3.1 m from each profile. We classified  
334 objects at 0p5x magnification, including zooplankton taxa and several large colonial  
335 phytoplankton, with the trained CNN using images sampled from the full dataset every 6  
336 seconds, again to avoid multiple images of the same object. Counts were then aggregated  
337 into coarser taxonomic levels (copepods, daphnids, rotifers, predators and nauplia) for  
338 comparison with DSPC data. **Day-night patterns:** To study phytoplankton daily division  
339 (cells grow during the day and divide during the night) and consequent change in community  
340 average size, and zooplankton community day-night migrations, we used phyto- and  
341 zooplankton body size (area) and abundance (ROI / second) data over 24 h across 34 non-  
342 consecutive days extracted with a weekly interval from the data collected in 2020. Again,  
343 0p5x data were down sampled to one frame every 6 seconds, then ROIs were classified with  
344 the CNN model, and finally aggregated into coarse taxonomic groups. **Community**  
345 **diversity:** Community diversity metrics were estimated using ROI counts performed  
346 manually (classification and counting of images) for the 5p0x magnification, and  
347 automatically (CNN) for the 0p5x magnification. For both comparisons, the number of data-

348 points used amounted to the weekly samples taken for traditional plankton monitoring  
349 methods (n = 44 for phytoplankton and 30 for zooplankton). Richness, Shannon diversity  
350 and Pilon's evenness were calculated using functions of the R package vegan (2.5-6) and  
351 linear density data. Relationships between traditional monitoring and DSPC in terms of  
352 plankton abundance and diversity were estimated using least-square linear regression.

353

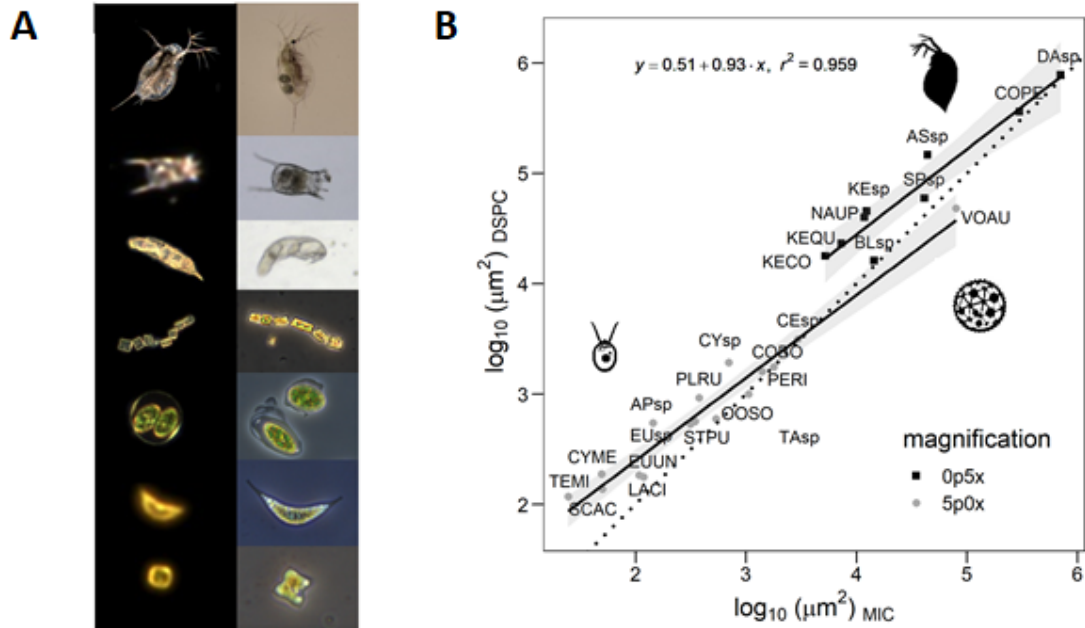
## 354 3. Results

### 355 3.1 Laboratory calibration

356 To assess the DSPC sensitivity in automatically detecting objects, how such detection  
357 scales with plankton densities, and its accuracy in extracting object features such as  
358 plankton body size (i.e. the area of the object in two dimensions), we performed laboratory  
359 experiments to compare DSPC results with traditional (manual) microscopy methods. We  
360 used phytoplankton cultures, protists cultures, daphnia cultures and fresh field samples for  
361 rotifers, copepods and other zooplankton species. We selected species to represent a wide  
362 range of sizes, from *Tetraedron minimum* (0.00018 mm<sup>2</sup>) to *Daphnia magna* (3.2 mm<sup>2</sup>).

363 Overall, we found a strong linear relationship in Log-Log space (Log<sub>10</sub>, R<sup>2</sup> = 0.959) between  
364 inverted microscopy (area calculation based on ImageJ) versus automated imaging and ROI  
365 area extraction using the DSPC (**Fig. 2**). The relationship indicates that size estimates  
366 tended to be larger in the DSPC. For both magnifications, we observed a stronger deviation  
367 from the linear relationship at the smaller size end. **Fig. 2A** shows how the lower resolution  
368 for smaller taxa in DSPC images makes small objects appear overpixelated and blurry, biasing  
369 the automated edge detection and therefore area estimation. The only taxon lying below the  
370 1:1 line was *Volvox aureus*, a very large phytoplankton colony for which we detected many  
371 partial images. Individual size distributions for each taxon confirmed an overestimation of  
372 size (as ROI area) at the lower size end. In both magnifications, density distributions of size  
373 estimates overlapped better for larger taxa, i.e. *Daphnia*, *Cephalodella*, *Oocystis*, compared  
374 to smaller taxa, i.e. *Keratella quadrata*, *Scenedesmus acuminatus*, *Tetraedron minimum*  
375 (**Supplemental Fig. S1**).

376



377

378 **Fig. 2. Relationship between body size estimates by inverted microscopy and the DSPC. [A]**

379 Selected pictures of species by DSPC (left) and inverted microscopy (right), sorted based on size

380 from the biggest taxon (on top) to the smallest (bottom). [B] Mean body size (ROI area) between

381 inverted microscopy (MIC) and the plankton camera (DSPC). Dotted line = 1/1 relationship, solid lines

382 = linear regression estimates ( $p < 0.05$ ) and 95% confidence intervals.

383

384 For all taxa, density estimations by microscopy scaled significantly and accurately with ROI/s

385 detected by the DSPC (**Fig. 3** -  $R^2 = 0.89$  and  $R^2 = 0.73$  for phytoplankton and zooplankton,

386 respectively). We compared the number of objects detected per second (i.e. per frame) in

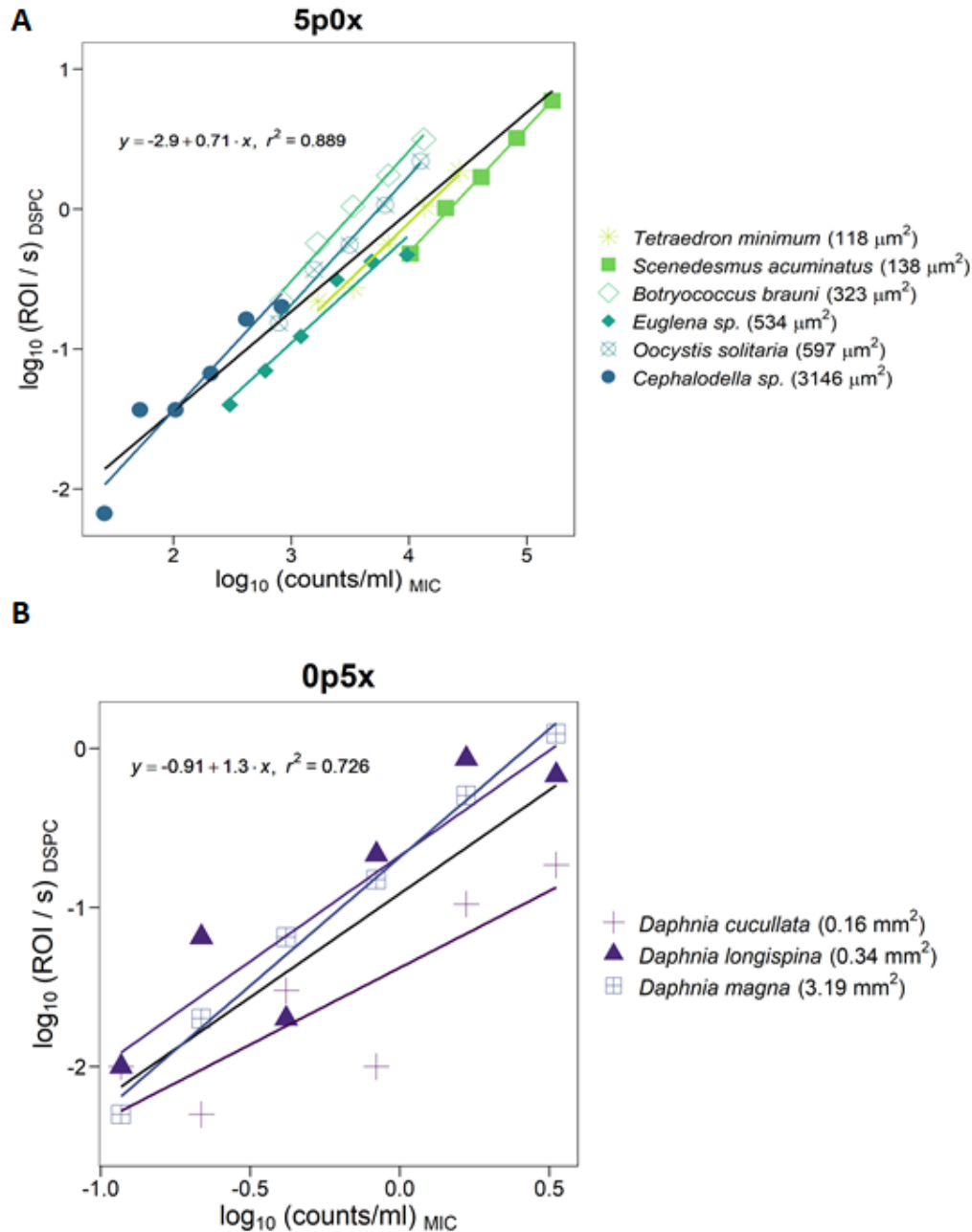
387 the DSPC to density measurements obtained by inverted microscopy for small (5p0x) and

388 stereomicroscopy for large (0p5x) plankton organisms, in serial dilutions. We performed

389 serial dilutions of zoo- and phytoplankton taxa ranging of different sizes ( $118 \mu\text{m}^2$  -  $3.2 \text{mm}^2$ ).

390 The overall scaling was more consistent across taxa for 5p0x magnification than for 0p5x.

391 However, the strength of the relationship did not depend on size.



392

393 **Fig. 3. Calibration of abundance estimates by DSPC relative to microscopic counts.** [A]  
394 Phytoplankton and small protists ordered according to body size (area, measured by DSPC). [B]  
395 Three different species of *Daphnia* ordered according to body size (area, by DSPC). Black lines show  
396 the overall relationship between  $\text{Log}_{10}$  transformed abundance measures by the DSPC (ROI / s) to  
397 density estimates based on inverted microscopy (counts / mL). Colored lines and dots represent the  
398 regression lines for the single taxa. All relationships are significant to  $p < 0.05$ .

399

400

## 401 3.2 Field deployment and biodiversity dynamics

402 To explore the potential of the new *in situ* imaging approach for automated monitoring of  
403 plankton community dynamics, we installed a dual-magnification DSPC permanently, at the  
404 stationary depth of 3 m, at the Eawag platform in the middle of Lake Greifensee  
405 (Switzerland) ([www.aquascope.ch](http://www.aquascope.ch)). The instrument has been operating autonomously, with  
406 rare interruptions of a few days a year for maintenance, since April 2018. During this time,  
407 we have acquired imaging data to describe the dynamics of the planktonic food web at high  
408 temporal frequency, and regularly taken samples with traditional monitoring approaches to  
409 compare biodiversity measurements by the DSPC to standard sampling and analysis  
410 methods. Patterns collected between 2018-2020 highlight seasonal successions and bloom  
411 dynamics, circadian rhythms in abundance and size. Comparisons with traditional methods  
412 reveal advantages and limitations of this new approach.

### 413 3.2.1 DSPC characterization of plankton diversity

414 In over two years of data collection in Lake Greifensee we could identify 28 plankton taxa  
415 with the 0p5x magnification (mostly zooplankton) and 80 taxa with 5p0x magnification  
416 (mostly phytoplankton). Some taxa were present in both magnifications, i.e. ciliates, rotifers  
417 and large phytoplankton colonies. **Fig. 4A** displays a collage of images representing the  
418 diversity of plankton communities as was observed with the dual-magnification DSPC, which  
419 encompassed primary producers, mixotrophs, herbivore grazers and carnivore zooplankton.  
420 Qualitatively, the high resolution and dark field background of images made taxon  
421 identification possible and facilitated image processing and training of a deep-learning  
422 classifier for zooplankton (see further Sections), with both morphology and color playing an  
423 important role.

424 The DSPC imaging approach represented a non-invasive and unbiased method to study  
425 plankton in the field, since taxa and their microenvironment were monitored without  
426 disturbing natural behavior, heterospecific aggregates and colonies structures (**Fig. 4B-D**).  
427 Some potentially toxic algae, such as the cyanobacteria *Mycrocystis* or *Planktothrix*, were  
428 successfully imaged and clearly recognized (**Fig. 4B**). Apart from colonies and natural  
429 aggregates (such as lake snow - **Fig. 4C**), we observed direct interactions between, for  
430 example, hosts and parasites (**Fig. 4D**) or predators and prey (**Fig. 4F**). Some of these  
431 images might be the first ever to capture direct individual trophic interactions in freshwater  
432 microbes from the field, such as ciliates and rotifers preying on phytoplankton (**Fig. 4F**).  
433 Colored images enabled us to distinguish between living and dead cells, and to observe  
434 plankton division and reproduction, i.e. by imaging dividing cells or individuals carrying eggs  
435 (**Fig. 4E**). For some large zooplankton like *Daphnia*, it is possible to count eggs from images.

436 The images also allowed us to identify and count different life stages of zooplankton, as is  
437 the case for juvenile copepods and nauplii.

438



439



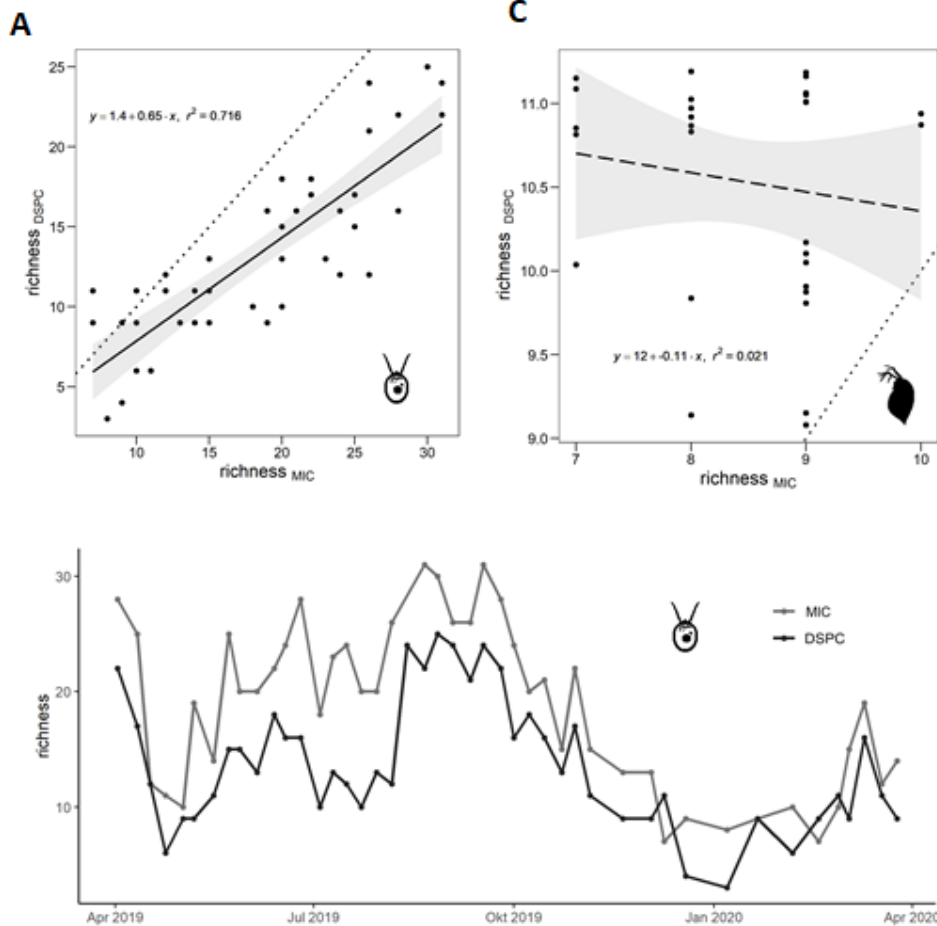
440 **Fig. 4. DSPC images of plankton taxa and natural taxa aggregates from Lake Greifensee**  
441 **(pictures are not to scale).** [A] Collage of different images describing the large range of taxa and  
442 forms that can be imaged with the dual-magnification DSPC. [B] Potentially toxic cyanobacteria, left to  
443 right, top to bottom: two colonies of *Microcystis sp.*, *Dolichospermum sp.*, *Aphanizomenon flos-aquae*  
444 and *Planktothrix rubescens*. [C] Colonial algae - left to right, top to bottom: : *Uroglena sp.*, *Pediastrum*  
445 *sp.*, two colonies of *Coelosphaerium*, (bottom row) *Dinobryon sp.*, natural aggregates of centric  
446 diatoms and aggregates of *Asterionella sp.* [D] Hosts and parasites (or epibionts) - left to right, top to  
447 bottom: parasite on a fish fin, *Epistylis sp.* on copepods, chytrid fungi on *Asterionella formosa* and  
448 *Fragilaria crotonensis* colonies, and unknown epibionts on *Staurastrum sp.* [E] Reproduction and life  
449 stages from left to right, top to bottom: *Daphnia sp.*, *Cyclops sp.* and *Keratella cochlearis* (with eggs),  
450 *Coelosphaerium sp.*, a cyst of *Ceratium hirundinella*, two dividing stages of *Cosmarium sp.*, two  
451 *Staurastrum sp.* - a dividing one and a dying individual. [F] Individual trophic interactions - from left to  
452 right, top to bottom: dinoflagellates eating green algae, two *Peridinium sp.* after eating centric  
453 diatoms, *Cyclops sp.* Eating a rotifer colony, a rotifer eating algal aggregate, *Leptodora kindtii* hunting  
454 for a *Daphnia*, *Ceratium hirundinella* interacting with a ciliate and a copepod nauplius interacting with  
455 an unknown organism.

456

### 457 3.2.2 Comparison with traditional sampling and microscopy

458 We compared phytoplankton and zooplankton biodiversity metrics estimated by the DSPC at  
459 3 m to those obtained by traditional monitoring methods. The taxonomic identification in  
460 DSPC images was variable depending on the taxon and on whether the characters that are  
461 used for classification were distinguishable from the images (**Supplemental Tab. 5**). For  
462 both magnifications, taxonomic classification was harder at the lower end of the detection  
463 spectrum of the cameras, but was on average possible at the genus level. Phytoplankton  
464 richness estimates by the two approaches, however, agreed well overall, considering the  
465 differences in both sampling and analysis methods, with DSPC data systematically  
466 underestimating richness compared to traditional methods (**Fig. 5A**). Temporal trends in  
467 phytoplankton richness highlighted this offset, which however varied over time likely  
468 depending on phytoplankton community composition: data showed a better agreement  
469 between the two approaches in winter, when fewer and easily detectable taxa, such as  
470 *Cryptomonas sp.*, were present (**Fig. 5B**). Zooplankton communities are in general less rich  
471 than phytoplankton, and both approaches detected low richness levels in our samples (max.  
472 11 species). We did not find a significant correlation between zooplankton richness  
473 estimates by DSPC and traditional methods, and the relationship appeared even negative  
474 (**Fig. 5C**). In the case of zooplankton richness estimation, the DSPC, which averaged data  
475 over a 24 h period, detected higher richness than the traditional method based on a daytime  
476 net-tow and microscopy.

477



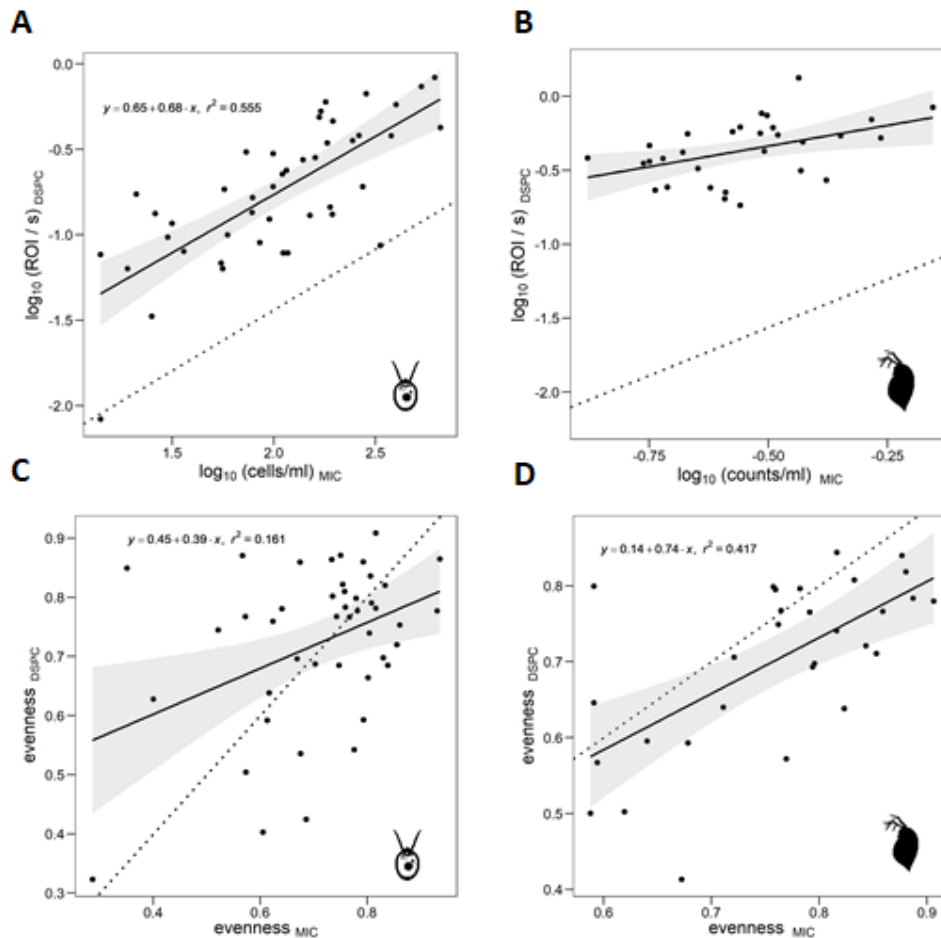
478

479 **Fig. 5. Comparison of species richness from DSPC relative to traditional methods.** [A]  
480 Phytoplankton richness (DSPC from 5p0x magnification - manual counting of images). [B]  
481 Zooplankton richness (DSPC from 0p5x magnification - manual counting of images). [C]  
482 Phytoplankton richness by DSPC compared to weekly traditional plankton monitoring data during  
483 2019-2020. Dotted line = 1/1 relationship; solid lines = linear regression estimates ( $p < 0.05$ ) and 95%  
484 confidence intervals; dashed lines = not significant ( $p > 0.05$ ).

485

486 Total phytoplankton and zooplankton community densities estimated by the DSPC agreed  
487 well with data from traditional sampling methods (**Fig. 6A-B**). The phytoplankton relationship  
488 was statistically significant and robust: ROI/s generated by the DSPC correlated strongly  
489 also with Chl-a concentrations measured *in situ* next to the instrument (**Fig. 5** and  
490 **Supplemental Fig. S2**). For zooplankton, the relationship between density estimates by  
491 daytime net-tows and DSPC 24 h averages was statistically significant, but poor (**Fig. 6B**).  
492 The relationship between phytoplankton taxa relative abundances (i.e. evenness) by DSPC

493 and traditional methods was statistically significant but poor, with data however scattered  
494 around the 1/1 line (**Fig. 6C**). Zooplankton taxa evenness, instead, showed a significant and  
495 relatively strong relationship between the two approaches (**Fig. 6D**).  
496



497

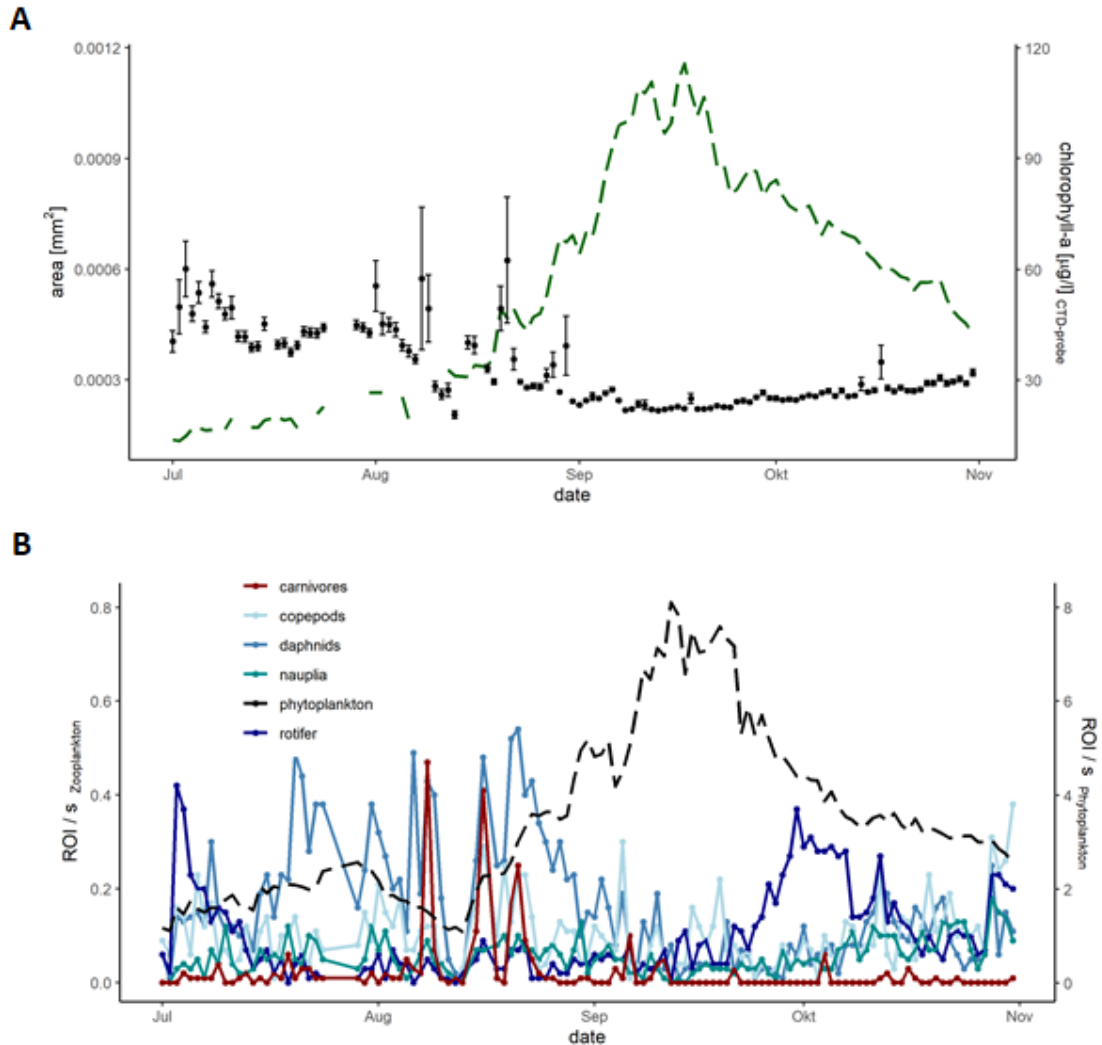
498 **Fig. 6. Comparison of total and relative abundance estimates by DSPC to traditional methods.**

499 [A] Total phytoplankton community density. [B] Total zooplankton community density. [C]  
500 Phytoplankton relative taxa abundances (Pielou's evenness). [D] Zooplankton relative taxa  
501 abundances (Pielou's evenness). Density data were Log<sub>10</sub> transformed for A and B. Pielou's  
502 evenness in C and D was calculated using linear density data. DSPC data: phytoplankton from the  
503 5p0x magnification and zooplankton from 0p5x magnification (considering only crustaceans) - images  
504 were manually counted. Dotted lines = 1/1 relationships in C and D, and calibration relationships from  
505 laboratory serial dilutions in A and B (Fig. 3), solid lines = linear regression estimates ( $p < 0.05$ ) and  
506 95% confidence intervals.

### 507 3.2.3 Seasonal patterns

508 The DSPC data in 2018 depicted the largest algal bloom in Lake Greifensee since the early  
509 1990s in terms of phytoplankton densities (see Chl-a levels in **Fig. 7A**). ROI/s collected by  
510 the DSPC for the 5p0x magnification correlated highly with the Chl-a measured by the CTD  
511 fluorimeter at 3 m depth ( $R^2 = 0.94$ ,  $p < 0.001$ ; **Supplemental Fig. S2**). Both Chl-a  
512 concentration and ROI/s by the DSPC (**Fig. 7**) showed a relatively small increase in  
513 phytoplankton at the end of July, and then a large accumulation in the middle of September,  
514 which was characterized by a relatively smaller mean phytoplankton community size (ROI  
515 area) compared to non-bloom periods (**Fig. 7A**). The late summer bloom was dominated by  
516 the small single-cell taxon *Oocystis* sp., as identified by DSPC images, while pre-bloom  
517 communities were more heterogeneous (**Supplemental Fig. S3**). Patterns of co-occurring  
518 zooplankton taxa and their abundance (ROI/s) showed that relatively high density of  
519 herbivore grazers (particularly daphnids) in the pre-bloom phase (middle of August) was  
520 followed by a decrease in their abundance after the DSPC detected the presence of  
521 carnivore species (particularly *Leptodora*) at the onset of the phytoplankton bloom (**Fig. 7B**).  
522 This release from top-down grazing pressure might have been involved in the emergence of  
523 the bloom, which was dominated by a small edible taxon. At the end of September, the algal  
524 bloom declined as we observed an increase in small fast-growing herbivores (i.e. rotifers),  
525 which might have been involved in bloom termination (**Fig. 7B**).

526



527

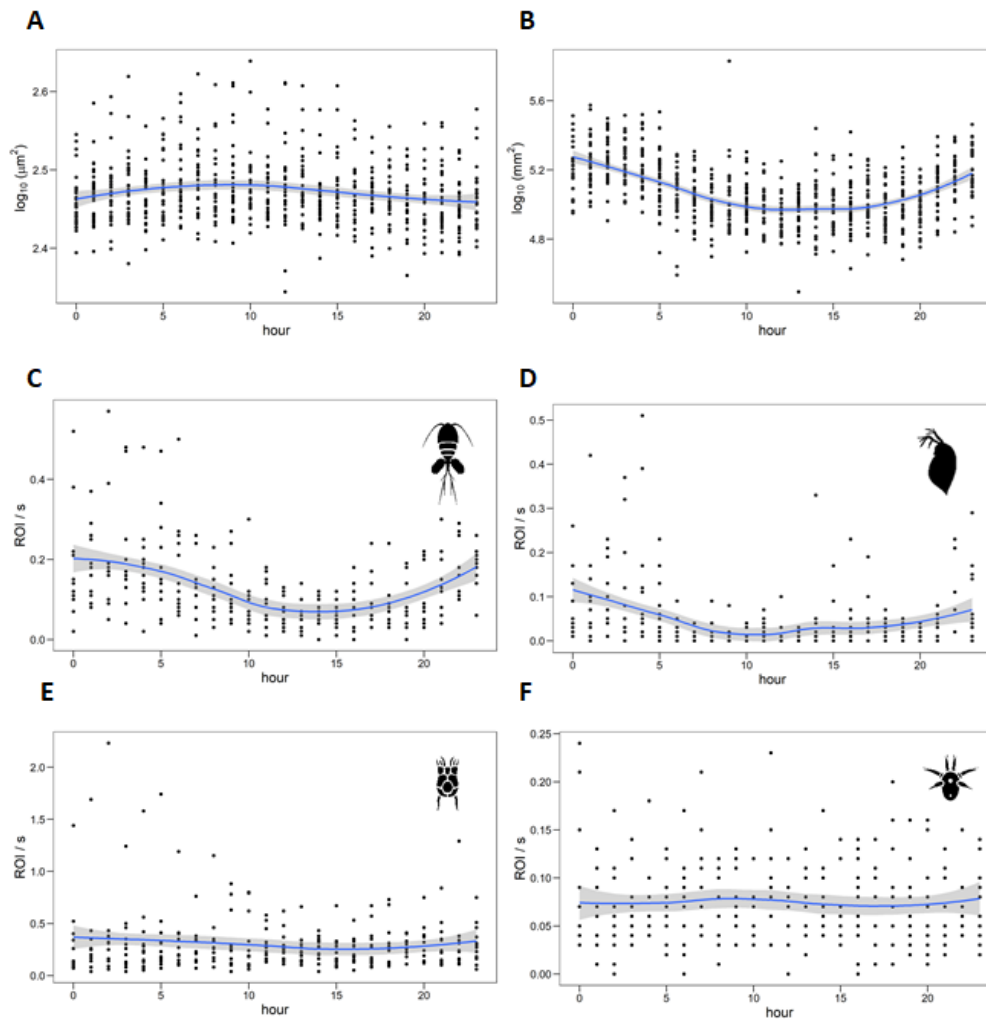
528 **Fig. 7. Patterns from DSPC monitoring data from Lake Greifensee (2018).** [A] Chl-a data from the  
529 CTD and fluorimeter (dashed green line), and phytoplankton community size by DSPC (points  
530 represent the mean ROI size in the 5p0x magnification +/- standard error of the mean). [B] Patterns in  
531 plankton density (ROI/s) dynamics from the DSPC: colored lines represent different zooplankton taxa  
532 (based on CNN automated classification, from 0p5x magnification, see **Supplemental Table S3**),  
533 dashed black line represents phytoplankton (from the 5p0x magnification). Data represent one DSPC  
534 measurement per day (10 min running time, subset to 6 seconds for zooplankton) taken during the  
535 night (01:00).

536

### 537 3.2.4 Circadian patterns

538 Time series generated by the DSPC, which was set for 10 min of measurements every hour,  
539 24/7, allowed study of circadian size dynamics and of zooplankton day/night migratory  
540 behavior (**Fig. 8**). Phytoplankton mean community size showed to be slightly larger during

541 the day compared to the night (when both grazing and cell division mostly occur) (**Fig. 8A**).  
542 Zooplankton mean community size was larger, instead, during the night than during the day  
543 (**Fig. 8B**). This was likely due to different migration behaviors of large relative to small  
544 herbivore grazers (**Fig. 8C-F**). Copepods and daphnids were more abundant (higher ROI/s)  
545 during the night, and peaked between midnight and 3 am (**Fig. 8C-D**). During the day,  
546 daphnids were almost undetectable. Conversely, nauplia and rotifers (the most abundant  
547 group at 3 m) did not show any significant circadian patterns (**Fig. 8E-F**).  
548



549

550 **Fig. 8. Circadian patterns in plankton size and abundance.** Change in phytoplankton community  
551 mean size over 24 hours (from 34 days with a weekly interval during 2019 and 2020) [A]. Zooplankton  
552 community mean size change over 24 hours [B], and density (ROI/s) of copepods [C], daphnids [D],  
553 rotifers [E], and nauplia [F] (from 34 random days in 2020). Zooplankton counts based on CNN  
554 automated classification, from 0p5x magnification, see **Supplemental Table S3**.

## 555 4. Discussion

556 The dual magnification DSPC allowed imaging plankton individuals, colonies, and  
557 aggregates of taxa and other suspended solids (e.g. lake snow) without disrupting natural  
558 arrangements of interacting organisms, their micro-environment or their behavior. The  
559 instrument reliably delivered high-resolution images in real-time, and measurements of traits  
560 like size, shape and color. This included detection capability across a wide-reaching size  
561 range of the planktonic food web. Detecting, with high resolution, particles from  
562 nanoplankton to mesoplankton, is a dynamic range never achieved before in other *in-situ*  
563 imaging systems (Lombard et al., 2019). Taxonomic identification was variable depending on  
564 detected taxon and the resolution of the image. In general, however, the colored images with  
565 dark background afforded high object definition and contrast, qualities that facilitate the  
566 identification of taxa and the annotation of ROIs for training of deep-learning models.  
567 Automated image classification was achieved for plankton with the 0p5x magnification in this  
568 study (**Supplemental methods**) and elsewhere (Campbell et al., 2020; Orenstein et al.,  
569 2020). The training of a phytoplankton classifier will be manageable with relatively low effort  
570 (Kenitz et al., 2020), however more tedious due to the larger number of taxa to annotate. An  
571 approach based on imaging has the potential to yield high frequency and standardized  
572 plankton monitoring data (Lombard et al., 2019) and, boosted by continuous (and open-  
573 source) advances in image processing and artificial intelligence, offers the prospect to  
574 automate and standardize also taxonomic classification of plankton (MacLeod et al., 2010).

575 Time series collected by the stationary DSPC in Lake Greifensee depicted seasonal  
576 succession patterns, bloom dynamics and circadian fluctuations with a temporal and spatial  
577 (i.e. microenvironment and morphology) resolution that would have been virtually impossible  
578 to collect based on traditional methods. In particular, the DSPC data included dynamics of all  
579 the planktonic groups that are important to understand and predict community dynamics  
580 (including algal blooms), but are often overlooked in routine monitoring (e.g. ciliates, chytrid  
581 fungi) (**Fig. 2**). In addition, for all the monitored plankton taxa, automated underwater  
582 imaging allows following the dynamics of the distribution of important traits such as size,  
583 shape, and pigmentation, which are extremely hard to measure manually on large numbers  
584 of individuals. Morphological characteristics of phytoplankton such as length, surface,  
585 coloniality, and pigmentation correlate with species functional properties, such as resource  
586 acquisition, growth rate, sinking rate, grazing resistance, and therefore with population  
587 abundances and responses to environmental gradients (Fontana et al., 2019; Kruk et al.,  
588 2010; Pomati et al., 2013).

589 In this study, DSPC imaging and feature extraction allowed calculation of bi-dimensional  
590 areas for ROIs, which related well to areas of plankton taxa based on traditional microscopy  
591 and manual measurements. Although measured in two dimensions, the body size estimates  
592 by one or two linear dimensions scale with biovolume estimates by traditional methods  
593 (Mittler et al., 2019). Body size was overestimated by the DSPC at the lower size end, where  
594 both regression lines for the 0p5x and 5p0x magnification deviated for smaller species from  
595 a perfect 1/1 relationship. This was due to a lack of resolution in pixels for smaller  
596 individuals, particularly when slightly out-of-focus, which can be corrected using the  
597 relationship derived by laboratory calibration (**Fig. 2**). The overestimation of size would be  
598 even greater when using estimates of 3-D biovolume (rather than 2-D area), so care should  
599 be given when calculating biovolume estimates.

600 Similarly, our measure of plankton densities (ROI / s, or alternatively ROI / frame), can be  
601 translated into a number of individuals per unit of volume (e.g. L) based on the laboratory  
602 calibration (**Fig. 3**). Note, however, that the initial densities in our laboratory experiments  
603 were much higher than those usually found in nature: for the goal of an *absolute quantitation*  
604 of plankton densities, we suggest a laboratory calibration targeted towards specific  
605 instrument settings and realistic densities. Measuring densities based on detected ROI / s  
606 would be suitable to estimate *relative abundances*. Other issues emerging from the  
607 comparison of DSPC data with traditional plankton sampling include: i) the difference  
608 between methods in terms of sampled volume, which is used to estimate diversity metrics; ii)  
609 no detection of phytoplankton < 10 µm, with loss of some small but common species; iii) the  
610 spatial heterogeneity and circadian behavior of zooplankton.

611 The DSPC imaged volume (**Supplemental Table S1**) is small compared to traditional  
612 monitoring samples, and variable depending on water optical conditions (Orenstein et al.,  
613 2020). The DSPC cannot easily change sampled volume to respond to changes in densities,  
614 unlike traditional counting methods. While we can apply a space for time substitution to  
615 increase imaging sampling effort, it is difficult to assess the actual differences in sample size  
616 between DSPC and traditional methods. This limitation, which applies to almost all  
617 automated *in-situ* methods, has been already acknowledged (Lombard et al., 2019). While  
618 automated imaging data allow the scaling of measures of diversity based on sample size  
619 (e.g. by temporally aggregating data and applying rarefaction and probability estimate of  
620 metrics) (Chase and Knight, 2013), this is generally impossible with traditional sampling  
621 methods. Those differences in scaling hamper our understanding of why imaging and  
622 traditional methods sometimes fail to converge to the same diversity estimates (**Fig. 5-6**).

623 The DSPC showed difficulties in detecting small species for the 5p0x magnification, and in  
624 quantifying densities of large rare species, including carnivorous zooplankton, for the 0p5x



625 magnification. The lack of taxonomic resolution for small phytoplankton taxa (in 5p0x) is a  
626 limitation of the optical system itself, solvable by an extra (higher) magnification. The  
627 correlations between DSPC and microscopy estimates for phytoplankton richness and total  
628 abundance were however robust (**Figs. 5A and 6A**), with the limitations in taxonomic  
629 resolution becoming more evident in the comparison of relative abundance estimates (**Fig.**  
630 **6C**). The lack of reliable quantitation of large zooplankton taxa in the 0p5x magnification,  
631 instead, resides in the relationship between spatial density and body size of organisms  
632 (White et al., 2007). Imaging with the DSPC for a longer time, particularly at night when  
633 these organisms are more active, might ameliorate this problem by increasing the sampling  
634 effort. Due to a relatively small focal volume, however, large individuals tend to be imaged  
635 and cropped multiple times. We suggest that large carnivorous zooplankton can only be  
636 studied with presence/absence data or during periods when their densities reach very high  
637 levels.

638 Zooplankton species have patchy distributions and exhibit diel vertical and horizontal  
639 migrations, which could lead to systematic biases in measuring community and population  
640 properties, if these are based on non-replicated local samples, or only daytime samples  
641 (Doubek et al., 2020). We propose that the poor correlation between DSPC and traditional  
642 sampling for zooplankton richness and abundances was caused by the heterogeneous  
643 distribution of taxa over space and time, evident also by high levels of variation among  
644 replicated samples, particularly for traditional methods (**Fig. S7**). In this perspective, DSPC  
645 data (which have been integrated over 24 h and include both day and night samples) may be  
646 more trustworthy than the traditional daytime net-tows, which have been previously  
647 suggested not to be an appropriate approach for monitoring (Doubek et al., 2020).

648 Availability of high resolution real-time plankton data represent a potential breakthrough in  
649 both applied and fundamental plankton ecology and may be more important for water quality  
650 monitoring than a fine taxonomic resolution. High-frequency data have shown to provide  
651 exciting prospects for modelling, understanding and predicting plankton dynamics and  
652 ecosystems processes (Fontana et al., 2018; Thomas et al., 2018). The seasonal patterns  
653 depicted in **Fig. 7** highlight the rich description of the ecological succession of taxa that  
654 DSPC data can offer for studying plankton communities. Natural processes fostering high  
655 plankton biodiversity on one hand, and leading to the emergence of harmful algal blooms on  
656 the other hand, represent unresolved questions in aquatic ecology (Burford et al., 2020; Fox  
657 et al., 2010; Huisman et al., 2018; Li and Chesson, 2016). The DSPC was able to detect  
658 (and count) all the most common and potentially toxic cyanobacterial taxa forming blooms in  
659 lakes, including their large colonies (**Fig. 2**). If supported by an automated deep-learning  
660 classifier and paired with meteorological and CTD data (and/or a calibrated lake process

661 models), the DSPC could be instrumental to develop forecasting models for harmful  
662 cyanobacterial blooms, and the stability and biodiversity of natural plankton communities  
663 (Pomati et al., 2011; Thomas et al., 2018).

664 Time series generated by instruments like the DSPC can be combined with novel data  
665 analysis approaches, including equation free modelling and machine-learning, to reverse  
666 engineer community ecology based on observational information and to develop effective  
667 forecasting tools (Deyle et al., 2016; Martin et al., 2018; Sugihara et al., 2012; Thomas et al.,  
668 2018). The combination of high-frequency data, broad taxonomic spectrum and artificial  
669 intelligence (for data assimilation and modelling), presents unparalleled potential for  
670 capturing the processes acting in natural ecosystems. This approach offers great  
671 opportunities to advance our understanding of the mechanisms controlling the dynamics of  
672 the complex networks of interacting plankton species by suggesting novel and realistic  
673 hypotheses to be tested experimentally and through theory development.

674

## 675 5. Conclusions

676 The approach presented in this article, based on dual-magnification underwater microscopy,  
677 allowed us to obtain automated plankton data at the food web level with high temporal  
678 frequency and adequate image resolution. The plankton camera employed here was  
679 demonstrated to be very robust and reliable, showed low requirements in terms of  
680 maintenance and running costs and offered complete automation. We conclude from our  
681 comparison with traditional monitoring methods that the approach is mature as a research  
682 tool, and suitable for stable continuous deployment, with great potential for application in  
683 water quality monitoring.

684 Automated *in-situ* imaging data are available in real-time, and they are shareable, traceable,  
685 transparent, and independently verifiable. Biases in the data processing and analysis can be  
686 corrected by simply re-processing and re-analyzing archived raw data. On the other hand,  
687 new-generation monitoring tools like the DSPC present the challenge of managing and  
688 analyzing large datasets. This might prove particularly relevant if underwater imaging  
689 cameras become more affordable and, to increase sampling effort or account for spatial  
690 variability in water bodies (both vertical and horizontal), multiple instruments are employed  
691 locally and used in full-time operation (e.g. to gain power for density estimates of rare taxa).  
692 Recent publications suggest that this transition from relatively expensive tools to more  
693 affordable, and therefore scalable, instruments is already underway (Lertvilai, 2020).  
694 Streamlined and open source data management protocols, image processing software and  
695 automated image classification algorithms are becoming more and more popular, and the

696 approach that we propose can be employed for both fundamental and applied ecology,  
697 allowing calibration of lake ecological models, and supporting water quality monitoring with  
698 real-time plankton data.

699

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707

## 708 7. Contributions

709 E.M., T.K., M.R., C.E., P.I., S.D. and F.P. planned the study. T.K. and M.R. performed field  
710 sampling and taxonomic classifications. C.E. installed the instrument in Lake Greifensee and  
711 ensured constant functioning. P.R. and J.S.J. designed and built the instrument. M.B.-J., T.L.  
712 and T.H. developed and trained the CNN. N.S. and S.D. created a script for zooplankton  
713 appendix erosion. E.M., T.K., M.R., P.I., M.B.-J., S.D. and F.P. drafted the manuscript. All  
714 authors provided critical feedback and approved the final version of the manuscript.

715

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820

## 821 Appendix. Supplementary Materials and Methods.

822 **Table S1.** Specifications of the plankton camera. The instrument is derived from SPCP2 and  
 823 SPC cameras, for the high magnification <http://spc.ucsd.edu/cameras-2/spcp2-camera/>, and  
 824 for the low magnification <http://spc.ucsd.edu/cameras-2/spc-camera/>, respectively (Orenstein  
 825 et al., 2020).

Camera	<b>Matrix Vision mvBlueFox3 (3.45um pixel size)</b>
Sensor	<b>Sony IMX253</b>  [Point Grey GS3 12 MP, (GS3-U3-120S6C-C) – this is the standard for SPC & SPCP. Eawag Pcam was custom made and has other hardware]
Objective Lens	5p0x: <b>Olympus 5x Long Working Distance M Plan Semi-Apochromat (LMPLFLN5xBD)</b>  0p5x: <b>Opto Engineering High resolution telecentric lens for 1” detectors, magnification 0.508x, C-mount (TC2MHR024-C)</b>

Pixel Size (Object Space)	5p0x: 0.62 um 0p5x: 6.2 um
Tube Lens	Thor Labs TTL200
Computer	Nvidia Jetson TX1
Operating System	Ubuntu 16.04, Linux For Tegra R24.2.1
Power Requirements	9-36VDC (AC Adapter provided)
Battery Pack and Controller	Inspired Energy 98 Whr (NH2054) with EB335 controller About 3 hour runtime at full load
Depth Rating	Viewports: 50 m. Housings: 200 m Original design tested at 200 m.
Power Input	10-24 VDC; < 3A inrush @ 24V, Testing done at 24V.
Power Consumption	<b>0.2 W</b> : CTRL on, system off <b>13 W</b> : CTRL on, system on, idle. <b>25 W</b> : Full system load Jetson consumes ~ 9 W, each camera consumes ~ 4 W, and UV LEDs consume about 7W.
Weight in Air	Approx 60 lbs (27.2kg)
Weight in Seawater	Approx 5 lbs buoyant (2.3kg)
Viewport Material	Acrylic due to limited resolution through sapphire for 5x magnification.
Housing Material	Acetal, Tubes (Natural) and Endcaps (Black)
End Plate Material	6061-T6 Hard Black Anodize
Port Retaining Ring	110 Copper
Bulkhead connectors	Subconn, brass
Anode material	Magnesium

External Hardware	Titanium Grade 2. Delrin spring lock washers
Strobes	Custom Darkfield Illuminator, CBT-140 Luminus LED for each magnification, roughly 4000k color temp.
Manufacturer Costs	~ 80'000 \$ (in 2018)
Video Performance	10 FPS, raw 16 bit to disk, Approx 160 MB/s, per camera Max 3800 x 2600 pixels in 16 bit mode
ROI performance	Up to 600 (small) ROIs per second to disk Small ROIs are those will size roughly 100 x 100 pixels or less.
Detection range	5p0x: ~10 $\mu$ m - 150 $\mu$ m 0p5x: ~ 100 $\mu$ m - 7 mm
Imaged volume	5p0x: 0.2 - 10 $\mu$ L / frame 0p5x: 4 - 200 $\mu$ L / frame

826

827 CNN classifier for 0p5x images

828 The model we used for classifying zooplankton objects is a neural network with two  
829 convolutional layers, with kernel size (number of filters) of respectively 24 and 12 (64 and  
830 32) and relu activations, followed by batch normalization and max pooling modules, and a  
831 dense layer with softmax activation<sup>1</sup>. The loss function was a categorical cross-entropy. We  
832 provide our code and trained model at:

833 <https://github.com/mbaitye/plankifier/releases/tag/v1.1.1>

834 The default code settings contain all the hyperparameter choices we made. The dataset we  
835 used consisted of 17909 images (**Table S2**), of which 80% were used for training, and 20%  
836 for testing. The training data and further benchmarks on this model, along with more  
837 sophisticated ones which reach higher performance, will be published separately<sup>2</sup>. The  
838 performances that we show in this section are based on further independent data.

839 **Table S2.** Classes and number of ROIs used for CNN training per class.

Class	Number of images	Class	Number of images
dinobryon	3321	conochilus	264

<sup>1</sup> Despite its simplicity, this model obtains satisfactory performances, and has the additional advantage of being very lightweight. This allows us to share it, already trained, in our github repository [github.com/mbaitye/plankifier](https://github.com/mbaitye/plankifier). The consequence is that practitioners can use it directly through the simple commands provided in the release documentation.

<sup>2</sup> Kyathanahally, S., Merz, E., Hardemann, T., Reyes, M., Kozakiewicz, T., Isles, P., Pomati, F., Baity-Jesi, M., In preparation



nauplius	1507	trichocerca	255
maybe_cyano	1364	unknown	245
diaphanosoma	1089	aphanizomenon	225
asterionella	1055	fish	222
uroglena	953	keratella_cochlearis	215
cyclops	866	leptodora	203
ceratium	814	synchaeta	142
rotifers	744	dirt	131
daphnia	721	bosmina	80
asplanchna	607	polyarthra	80
eudiaptomus	537	unknown_plankton	71
kellikottia	519	daphnia_skins	46
paradileptus	424	copepod_skins	33
keratella_quadrata	420	hydra	18
filament	405	diatom_chain	17
fragilaria	306	chaoborus	10

840

841 We rely on abstention to be able to tune the precision-recall tradeoff in a simple manner.  
 842 This consists of labeling as unclassified all the images for which the classifier has a  
 843 confidence lower than a threshold  $\theta$ . In **Fig. S7** we show the precision and recall of our  
 844 model with  $\theta=0$ , on the classes that are relevant for our study. The average precision is 0.84,  
 845 and the average recall is 0.8. If we set  $\theta=0.8$ , the average precision is 0.91, and the average  
 846 recall is 0.67, which means that around 30% of the data is not being classified, but the data  
 847 that gets classified has a very low error rate. The data shown in this paper uses  $\theta=0.8$ .

848 Since in this study we were interested in a higher-level taxonomic description of the  
 849 observed organisms, we created some macro-classes, each comprising more than one of  
 850 the classes described in **Table S3**. Obviously, the classifier performances with the macro-  
 851 classes are at least as good as those of **Fig. S7**.

852 **Table S3.** Composition of the zooplankton functional groups used in the main text.

Section in main text	Macro-class	Classes
<b>Fig. 5</b>	copepods	cyclops + eudiaptomus
	daphnids	diaphanosoma + daphnia + bosmina
	rotifer	kellikottia + keratella_quadrata + keratella_cochlearis + trichocerca + conochilus + asplanchna + rotifers
	predators	chaoborus + leptodora
<b>Fig. 6</b>	copepods	cyclops + eudiaptomus
	daphnids	daphnia + bosmina + diaphanosoma
	rotifers	kellikottia + keratella_quadrata + keratella_cochlearis + conochilus + asplanchna + rotifers + synchaeta + polyarthra + trichocerca
	nauplius	nauplius

853

854 **Table S4.** Linear regression summary.

figure	y-variable	x-variable	group	intercept	slope	r-sqr	p-value	significant
1B	body size log10(um2)DSPC	body size log10(um2)MIC	0p5x	1.3529	0.7734	0.9038	0.00005202	yes
1B	body size log10(um2)DSPC	body size log10(um2)MIC	5p0x	0.8965	0.75061	0.9481	1.336E-10	yes
1B	body size log10(um2)DSPC	body size log10(um2)MIC	overall	0.52642	0.92377	0.9572	< 2.2e-16	yes
2A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	TEMI	-3.34127	0.8112	0.9732	0.001215	yes
2A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	SCAC	-3.87528	0.89204	0.9965	0.00005738	yes
2A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	BOBR	-3.30509	0.93043	0.9793	0.0008224	yes
2A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	Eusp	-3.2663	0.7702	0.9406	0.0008598	yes
2A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	OOSO	-3.4492	0.92219	0.9865	0.000435	yes
2A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	Cesp	-3.2581	0.9104	0.8886	0.003071	yes

2A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	overall	-2.86184	0.7107	0.889	7.40E-16	yes
2B	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	DACu	-1.3782	0.9654	0.6431	0.03406	yes
2B	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	DALO	-0.6746	1.3255	0.7806	0.0123	yes
2B	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	DAMA	-0.68311	1.61262	0.9894	0.0000269	yes
2B	density log10(ROI/s)DSPC	density log10(counts/ml)MIC	overall	-0.912	1.3012	0.726	7.153E-06	yes
5A	richnessDSPC	richnessMIC		1.4	0.65	0.716	4.81E-13	yes
5C	richnessDSPC	richnessMIC		11.5062	-0.1149	0.021	0.444	no
6A	density log10(ROI/s)DSPC	density log10(counts/ml)MIC		0.65	0.68	0.555	1.254E-08	yes
6B	density log10(ROI/s)DSPC	density log10(counts/l)MIC		-1.7346	0.5594	0.206	0.01181	yes
6C	evennessDSPC	evennessMIC		0.45	0.39	0.161	0.006888	yes
6D	evennessDSPC	evennessMIC		0.1403	0.7395	0.417	0.000115	yes
S2	density ROI/s DSPC	chlorophyll-a (ug/l)CTD- probe		-0.23361	0.062248	0.9387	< 2.2e-16	yes
S4B	countsML	countsMA	rotifer	0.42767	0.49976	0.7481	< 2.2e-16	yes
S4B	countsML	countsMA	daphnids	1.32638	0.69017	0.8646	< 2.2e-16	yes
S4B	countsML	countsMA	copepods	0.43969	0.55061	0.8221	< 2.2e-16	yes
S4B	countsML	countsMA	carnivores	0.7658	0.32412	0.7389	< 2.2e-16	yes
S4B	countsML	countsMA	nauplia	0.78086	0.65868	0.6551	< 2.2e-16	yes

855

856

857 **Table S5. Phyto- and zooplankton taxa in Lake Greifensee.** 1 taxa was to some degree (genus or  
858 species) identified on images taken with the 5p0x magnification.

859

Taxa MIC	Taxa DSPC	Seen in DSPC?	Comments
<b>Cyanobacteria</b>			
cyanobacteria small		0	too thin/small for DSPC
blue filament		0	too thin/small for DSPC
cyanobacteria colony	cyanobacteria colony	1	
aphanizomenon flos-aquae	aphanizomenon flos-aquae	1	we see it because it's mostly forming mats
aphanocapsa sp.	cyanobacteria colony	1	
aphanothece sp.	cyanobacteria colony	1	

chroococcus sp.	chroococcus sp.	1	
coelosphaerium sp.	coelosphaerium sp.	1	
chroococcales diverse	cyanophyceae	1	
dolichospermum sp.	dolichospermum sp.	1	
leptothrix echinata		0	too thin/small for DSPC
leptothrix ochracea		0	too thin/small for DSPC
merismopedia sp.	merismopedia sp.	1	
microcystis wesenbergii	microcystis sp.	1	
microcystis aeruginosa	microcystis sp.	1	
microcystis sp.	microcystis sp.	1	
planktothrix sp.	planktothrix sp.	1	
pseudoanabena sp.		0	too thin/small for DSPC
phormidium	phormidium sp	1	
snowella lacustris	snowella lacustris	1	
spirulina sp.		0	too thin/small for DSPC
synechococcus		0	
woronichinia naegeliana	woronichinia naegeliana	1	old name: Gomphosphaeria, able to see when changing light settings
<b>Gold algae</b>			
chrysophyceae div	chrysophyceae div	1	

bicosoeca sp.		0	too thin/small for DSPC
bitrichia sp.		0	too thin/small for DSPC
chrysochromulina		0	too thin/small for DSPC
chromulina sp.		0	too thin/small for DSPC
dinobryon bavaricum	dinobryon sp.	1	to be sure we identify to genus level
dynobryon sp.	dinobryon sp.	1	to be sure we identify to genus level
chrysoflagellaten div		0	too thin/small for DSPC
erkenia sp.		0	too thin/small for DSPC
kephyrion sp.	kephyrion sp.	1	
mallomonas big	mallomonas big	1	
mallomonas acaroides	mallomonas big	1	
mallomonas akrokomos	mallomonas akrokomos	1	
ochromonas sp.	ochromonas sp.	1	
pseudopedinella	pseudopedinella sp.	1	too thin/small for DSPC
salpingoeca sp.		0	
uroglena	uroglena	1	almost too big for 5p0x magnification
<b>Diatoms</b>			
stephanodiscus	centrales	1	impossible to see differences between cyclotella and stephanodiscus with DSPC
stephanodiscus big	centrales	1	

asterionella formosa	asterionella formosa	1	
aulacoseira	aulacoseira	1	
cyclotella comta	centrales	1	
centrales medium	centrales	1	
centrales small	centrales	1	
diatoma	pennales	1	
fragilaria crotonensis	fragilaria sp.	1	
gomphonema	gomphonema	1	
gyrosigma	gyrosigma	1	
navicula	pennales	1	
nitzschia	pennales	1	
pennate	pennales	1	
synedra acus	synedra sp.	1	
synedra acus angustissima	synedra acus angustissima	1	
synedra cyclopum	synedra cyclopum	1	
synedra sp.	synedra sp.	1	
<b>Dinoflagellates</b>			
dinoflagellates div	dinoflagellates	1	
ceratium hirundinella	ceratium hirundinella	1	

gymnodinium helveticum	dinoflagellate	1	
gymnodinium lantzschi	gymnodinium lantzschi	1	
peridinium aciculiferum	dinoflagellate	1	
peridinium diverses	peridinium sp.	1	
peridinium willei	peridinium willei	1	
dinocysten div	dinocysten div	1	
<b>Chrytophyceae</b>			
cryptophytes div	chrytophyceae	1	
chroomonas sp.		0	too thin/small for DSPC
cryptomonas 1	cryptomonas 1	1	
cryptomonas 2	cryptomonas 2	1	
cyathomonas		0	too thin/small for DSPC
katablephris sp.		0	too thin/small for DSPC
rhodomonas big	rhodomonas sp.	1	
rhodomonas lacustris	rhodomonas lacustris	1	
<b>Green algae</b>			
ankyra ancora	ankyra sp.	1	too thin to see details for sp identification
ankyra lanceolata	ankyra sp.	1	too thin to see details for sp identification
ankyra sp.	ankyra sp.	1	

carteria sp.	chlorophyte	1	
chlamydocapsa planctonica	chlorophyte	1	
chlamydomonas sp.	chlorophyte	1	
chloro diverse small	chlorophyte	1	
chloro diverse	chlorophyte	1	
chloro colonial	chloro colonial	1	
chloro diverse flagellated	chlorophyte	1	
chloro star shaped sheath	chlorophyte	1	
coelastrum microporum	coelastrum microporum	1	
coelastrum reticulatum	coelastrum reticulatum	1	
crucigenia	crucigenia	1	
crucigeniella	crucigeniella	1	
elakatothrix gelatinosa	elakatothrix gelatinosa	1	
eudorina	eudorina sp.	1	
gonium sp.	gonium sp.	1	
hormidium	hormidium	1	
lagerheimia	oocystaceae	1	
monoraphidium		0	too thin/small for DSPC
nephrocytium	nephrocytium	1	



eutetramorus	chlorophyte	1	
green colony	chlorophyte	1	
oocystis sp.	oocystaceae	1	
oocystis marsoni	oocystaceae	1	
pandorina sp.	pandorina sp.	1	
phacotus lenticularis	phacotus lenticularis	1	
pediastrum boryanum	pediastrum sp.	1	
pediastrum duplex	pediastrum sp.	1	
pediastrum simplex	pediastrum sp.	1	
planktosphaeria galatinosa	planktosphaeria galatinosa	1	
raysiella sp.	raysiella sp.	1	
scenedesmus armatus	scenedesmus sp.	1	
scenedesmus ellipticus	scenedesmus ellipticus	1	
sphaerocystis	chlorophyte	1	
tetrachlorella alterans	chlorophyte	1	
tetraedron minimum	tetraedron sp.	1	
tetraedron sp.	tetraedron sp.	1	
ulothrix	chlorophyte filament	1	
willea irregluaris	willea sp.	1	

<b>Zygnematales</b>			
zygnemophyceae	chlorophyte filament	1	
closterium acutum	closterium acutum	1	too difficult to see details for sp identification
closterium acutum var. Variabile	closterium acutum	1	too difficult to see details for sp identification
cosmarium sp.	cosmarium sp.	1	
stauratsrum sp.	stauratsrum sp.	1	
<b>Ciliates</b>			
ciliates div	ciliphora	1	
askenasia sp.	askenasia sp.	1	
ciliates big	ciliphora	1	
ciliates small	ciliphora	1	
coleps sp.	coleps sp.	1	
didinium sp.	didinium sp.	1	
epistylis sp.	epistylis sp.	1	
halteria sp.	ciliphora	1	
lagynophrya	ciliphora	1	
lionotus	ciliphora	1	
strobilidium	ciliphora	1	
strombidium viride	strombidium sp.	1	

tintinnidium fluviatile	tintinnidium sp.	1	
tintinnopsis lacustris	tintinnopsis lacustris	1	
urothricha sp.	ciliphora	1	
vorticella	vorticella	1	
zooflagellaten div	zooflagellaten div	1	
<b>Rotifer</b>			
rotatorien div	rotifer	1	
asplachna sp.	asplachna sp.	1	
brachionus sp.	brachionus sp.	1	
conochilus sp.	conochilus sp.	1	
gastropus sp.	gastropus sp.	1	
filinia terminalis	filinia sp.	1	
kellikotia sp.	kellikotia sp.	1	
keratella cochlearis	keratella cochlearis	1	
keratella quadrata	keratella quadrata	1	
polyarthra sp.	polyarthra sp.	1	
paradileptus sp.	paradileptus sp.	1	Never seen in traditional sampling and microscopy but very common in DSPC 0p5x magnification
trichocerca sp.	trichocerca sp.	1	
synchaeta sp.	synchaeta sp.	1	

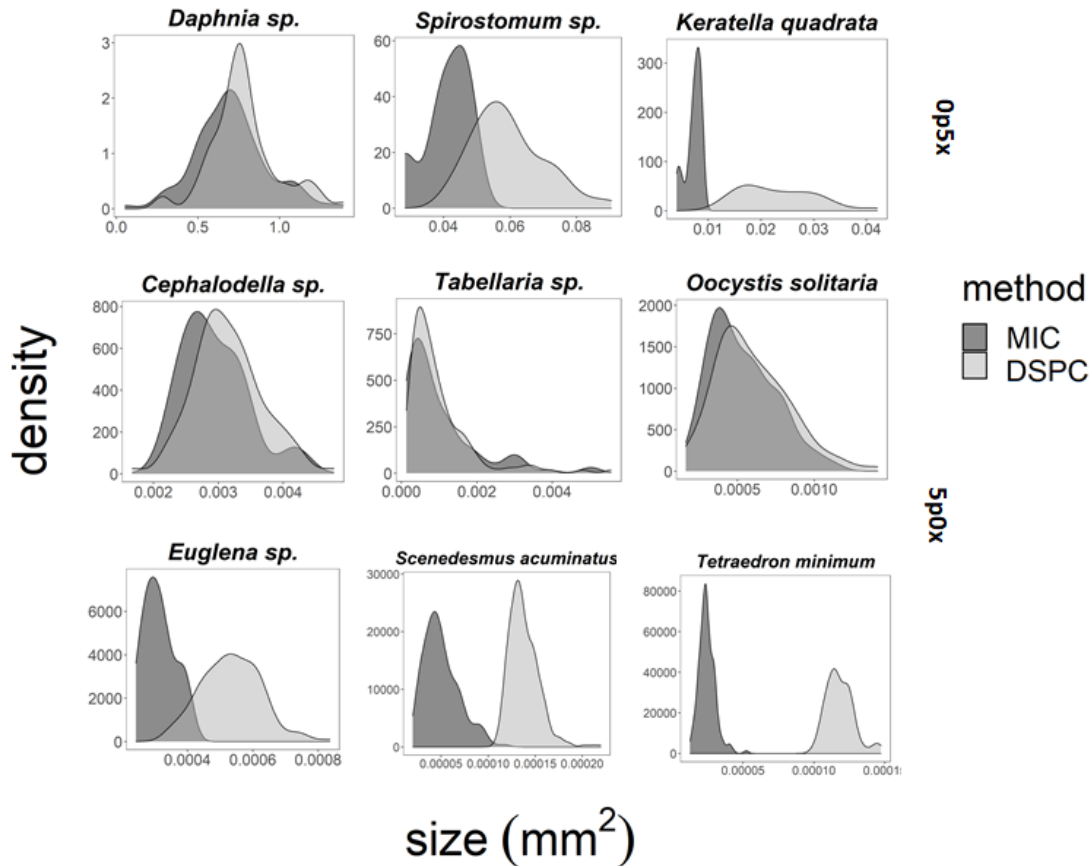
<b>Larger zooplankton</b>			
bosmina	bosmina	1	
diaphanosoma	diaphanosoma	1	
daphnia sp.	daphnia sp.	1	
cyclops	cyclops	1	
eudiaptomus	eudiaptomus	1	
nauplia	nauplia	1	
leptodora kindtii	leptodora kindtii	1	
chaoborus	chaoborus	1	
<b>Other invertebrates</b>			
hydra sp.	hydra sp.	1	

860

861

862 Fig. S1

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864

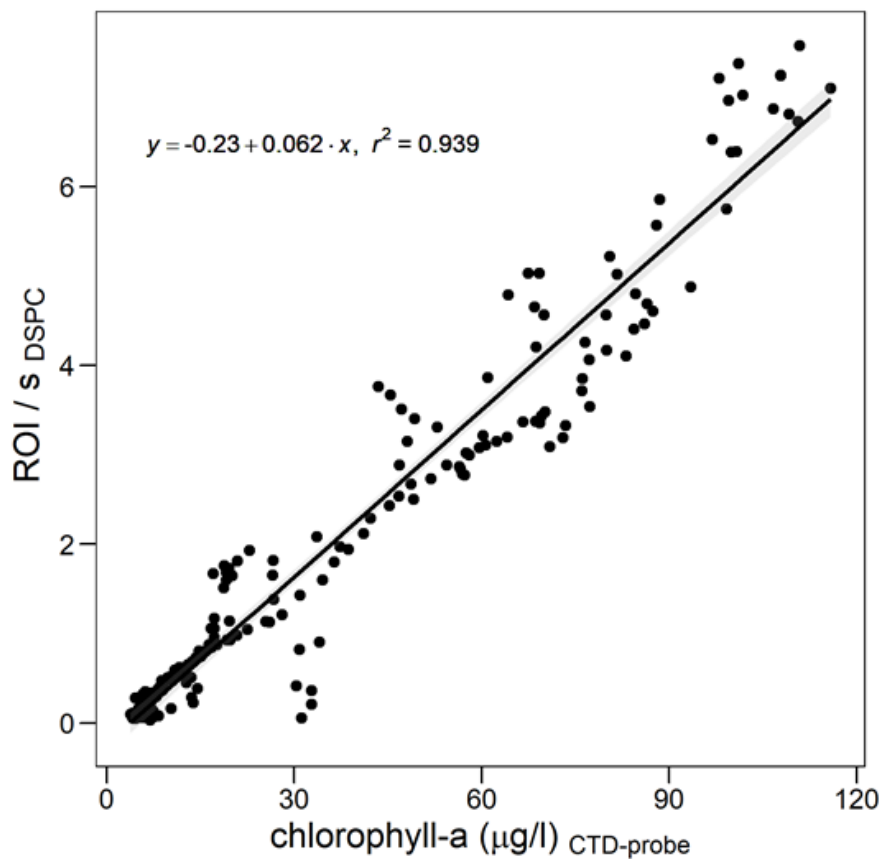
865

866 **Fig S1. Plankton body size distribution estimated by the DSPC and microscopy.** We  
867 calculated size density curves for plankton taxa covering a large size spectrum. We used  
868 both magnifications, 0p5x and 5p0x and ordered them according to size, with the largest  
869 organisms in the left top corner and smallest in the bottom right corner. Curves are colored  
870 according to the method used, microscopy or the plankton camera. Size distributions overlap  
871 better for larger than smaller taxa in both magnifications.

872

873 Fig. S2

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879 **Fig. S2. Chlorophyll-a measured by CTD against images per seconds taken by the**  
880 **DSPC.** Each point represents a sample taken in the year 2018. Amount of Chl-a scales well  
881 with ROI/s taken by the higher magnification (5p0x) of the plankton camera ( $R^2 = 0.94$ ).

882

883 Fig. S3

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885

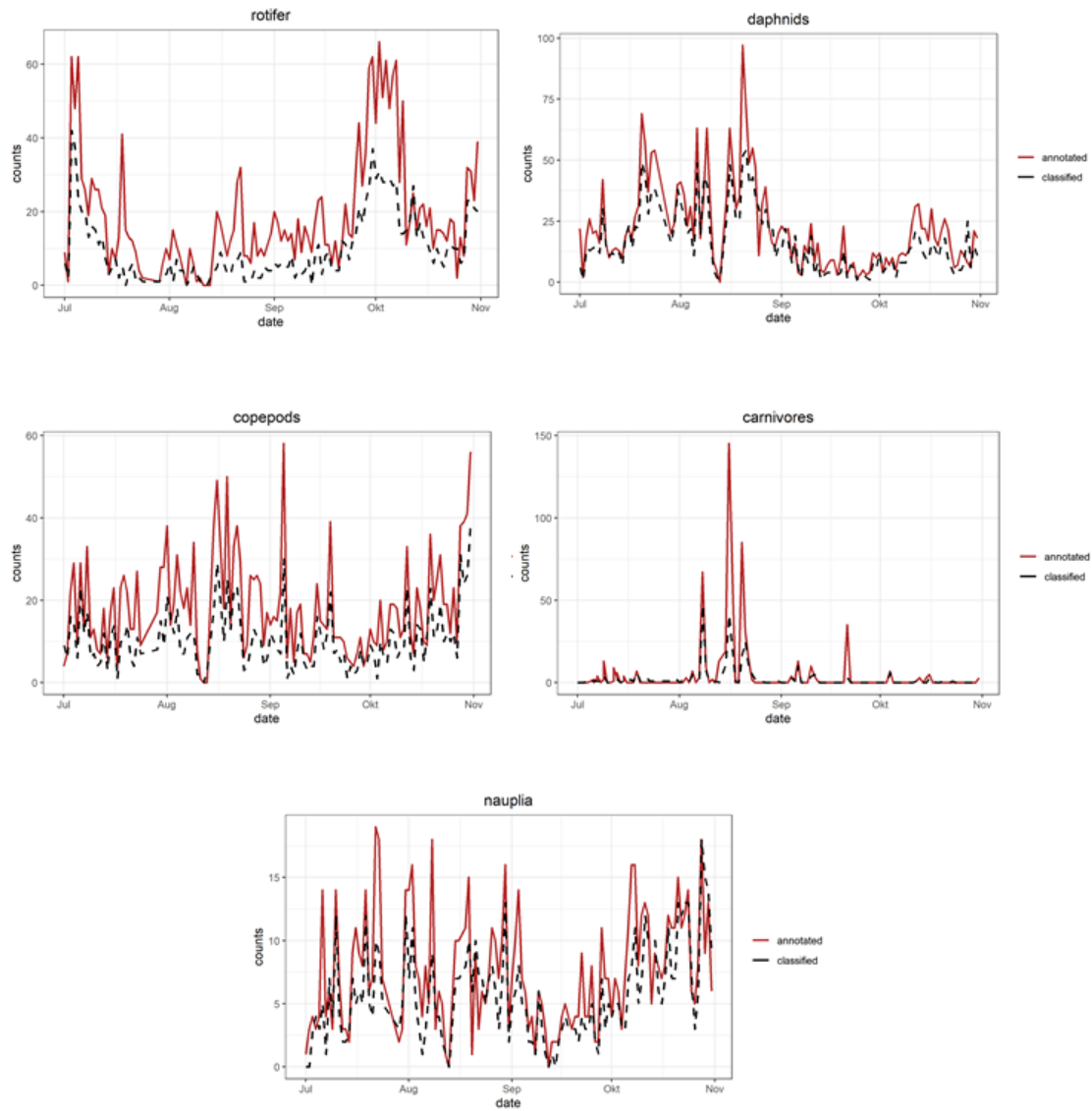
886 **Fig. S3. Phytoplankton community before and during the bloom of 2018 seen through**  
887 **the plankton camera. A Community before the bloom, mostly dominated by *Hormidium***  
888 ***sp.* B Community during the bloom, mostly dominated by *Oocystis sp.***

889

890

891 Fig. S4

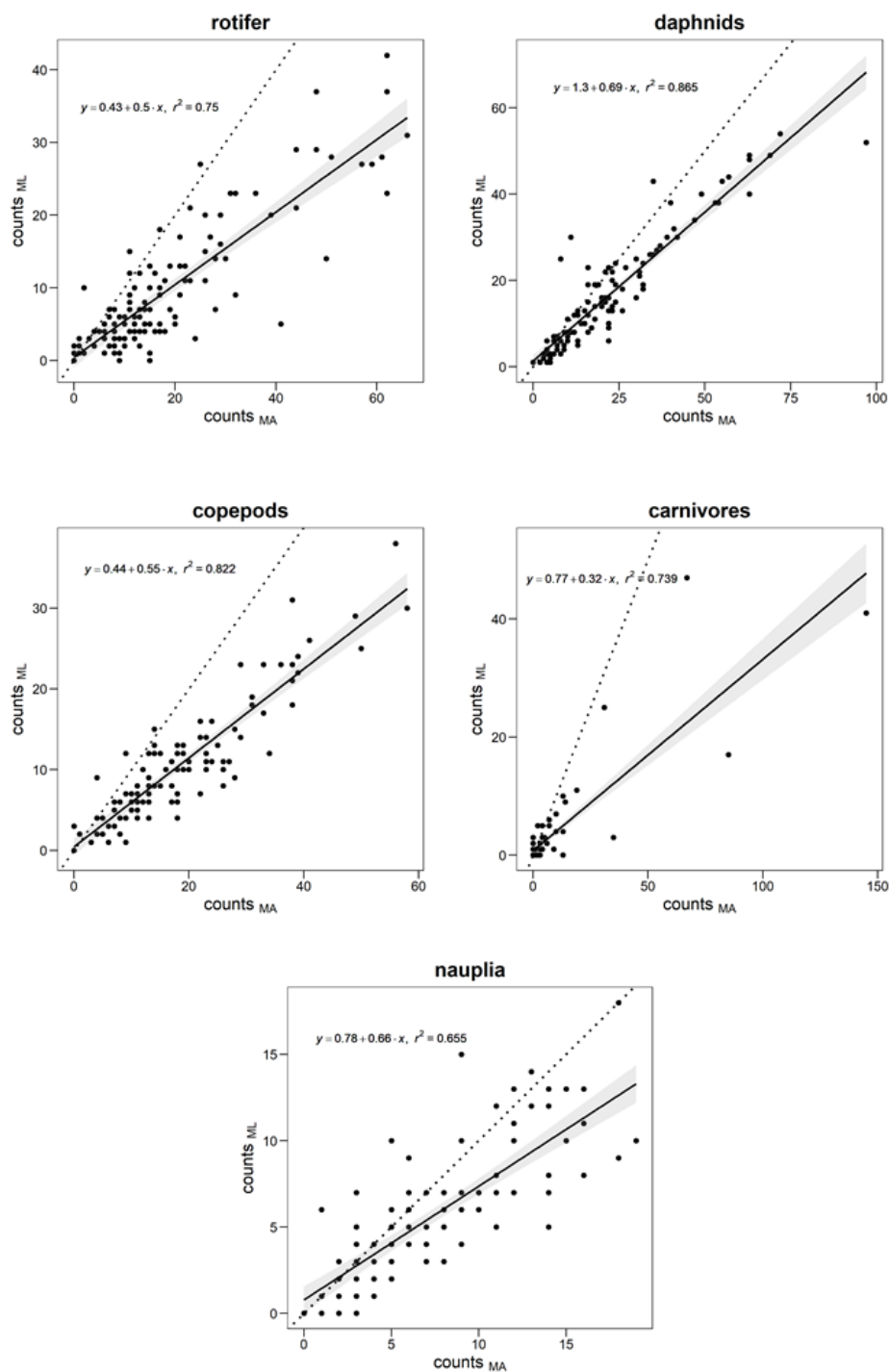
A



892



**B**



893

894 **Fig. S4: Comparison of manual annotation (MA) and classification by CCN (ML) for**  
895 **zooplankton taxa.** Images of 4 months of consecutive plankton camera data (one sample  
896 per day, taken during the night at 00:00 or 01:00) were manually annotated by 10 different  
897 people guided by taxonomists. The same images were run through a CNN. Zooplankton  
898 taxa were aggregated into higher taxonomic groups. **A:** Time series estimated by manual  
899 annotation (red) and CNN (black) for higher zooplankton taxa in July-November 2018, **B:**

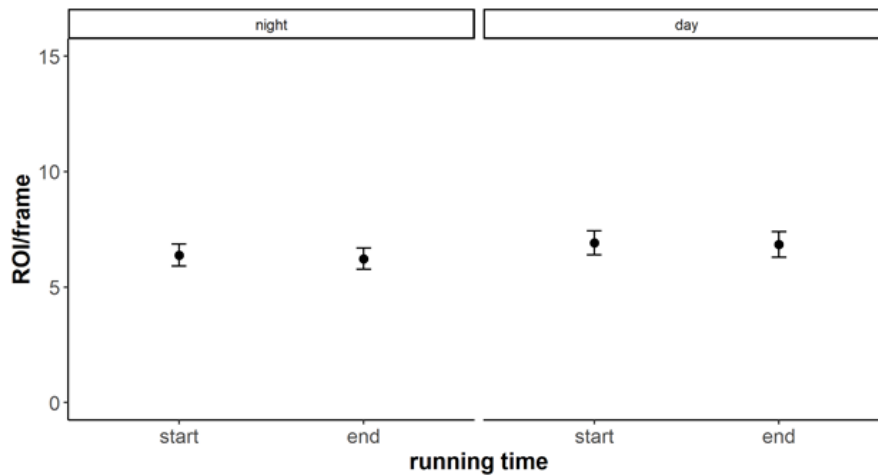
900 Linear regression plots comparing manual annotation to CNN for aggregated zooplankton  
901 taxa. Each dot represents one sample between July-November 2018. Data is the same as in  
902 Fig. 5 B, seasonal patterns and changes in planktonic food web during an algal bloom.

903

904 Fig. S5

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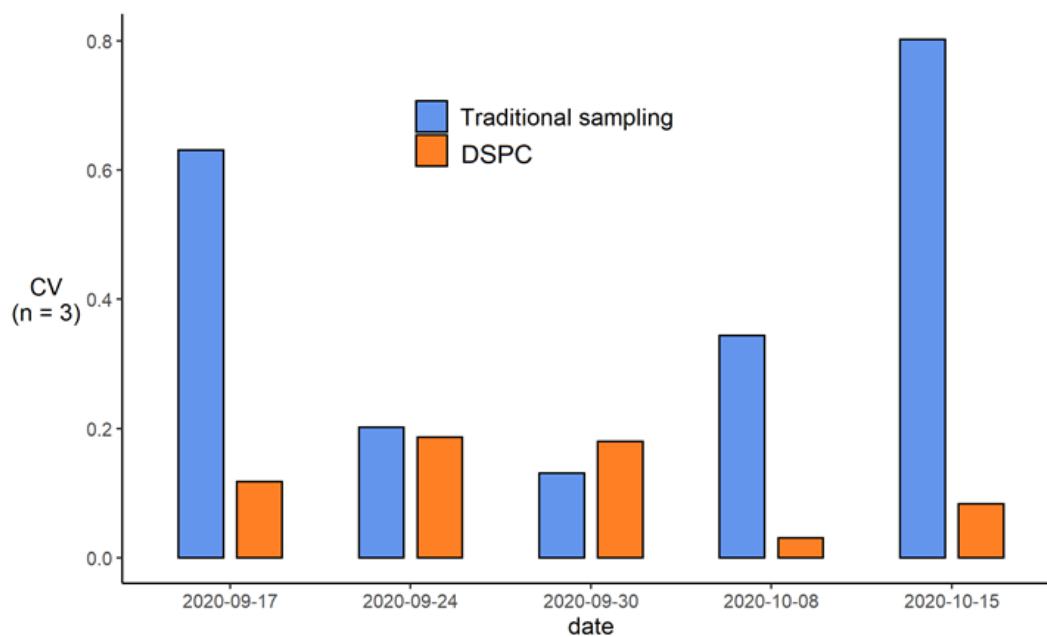
907

908

909 **Fig S5. Zooplankton phototaxis.** Some zooplankton species, such as *Daphnia sp.*, may be  
910 attracted by the flash of the DSPC and bias results. We calculated ROI/s from the stationary  
911 DSPC in Lake Greifensee at the start and end of running time (1st minute and 9th minute).  
912 We used the whole plankton data of 2019 (n=563), March 21 - December 2019, taking a  
913 sample at 04:00 (night) and 16:00 (day) per day. Images were subset to every 6' in order to  
914 reduce duplicated ROI. There was no significant difference in ROI/s between day and night  
915 samples nor at the start and end of DSPC running time.

916

917 Fig. S6



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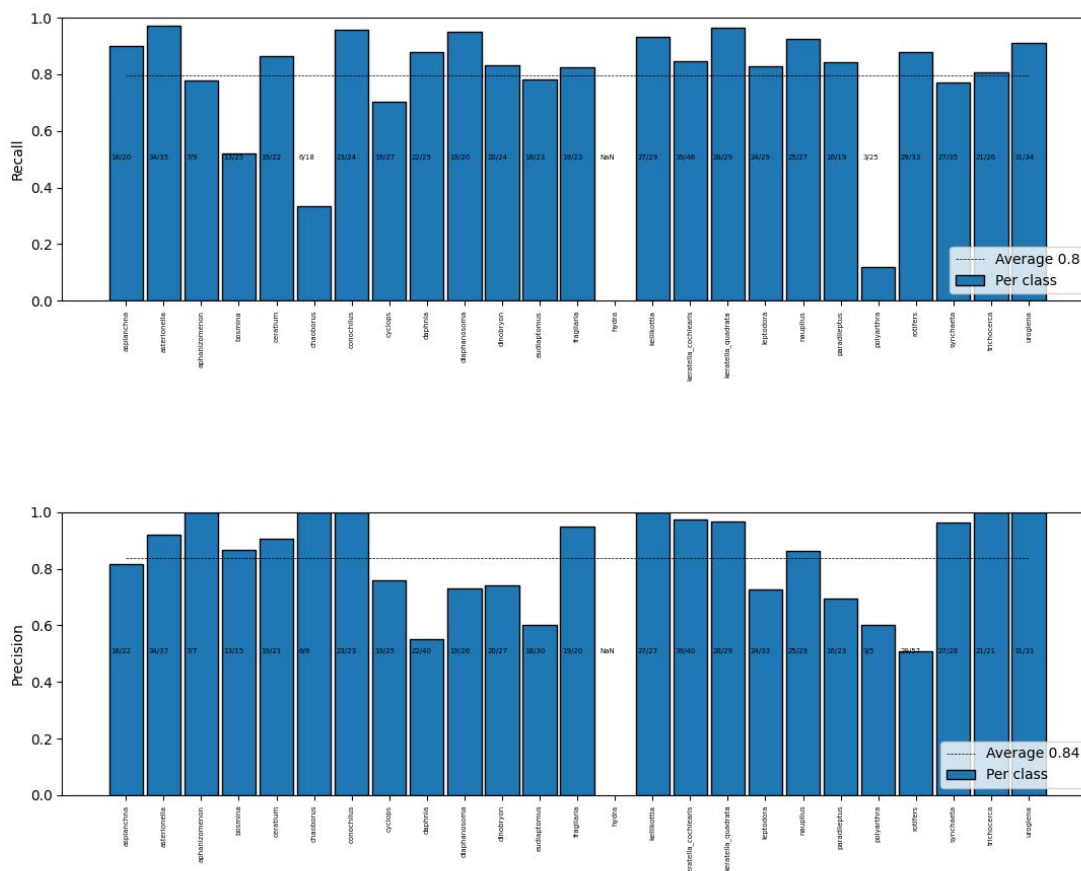
920

921 **Fig. S6. Reproducibility of abundance estimates (sampling by niskin bottle and**  
922 **microscopy relative to DSPC).** CV represents the coefficient of variation in zooplankton.  
923 The coefficient of variation between traditional samples was obtained from stereomicroscopy  
924 counts of three independent niskin bottles taken consequently, during routine monitoring  
925 work (middle of the morning). DSPC data come from measurements at 09:00, 10:00 and  
926 11:00 am on the same day.

927

928 Fig. S7

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931 **Fig. S7. Base performance of the deep-learning classifier.** The top plot shows the per-  
 932 class recall of our classifier. The bottom one shows the per class precision. The horizontal  
 933 lines are the macro-average, over all the classes. The numbers X/Y on each bar indicate  
 934 how many examples were correctly classified (X), out of the total (Y). This validation was  
 935 performed on a completely new batch of field data, which did not contain hydra.

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