Test of in situ (underwater) automated imaging, as provided by the Scripps Plankton Camera, for monitoring and analysis of lake phytoplankton

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1. Introduction

We tested a Scripps Plankton Camera (SPC) (http://spc.ucsd.edu) from the laboratory of Underwater Imaging of Dr Jules Jaffe (http://jaffeweb.ucsd.edu/), at the Scripps Institution of Oceanography (University of California, Sand Diego). This report concerns with the testing of the underwater microscope in two different systems and operational conditions. For this work, we used a machine that we obtained on loan from Dr Jaffe’s group, with the objective of assessing advantages and limitations of the instrument, and define specifications for a new SPC to purchase.

We studied the relationship between imaging data and microscopy concerning the assessment of phytoplankton abundance and number of species detected.

Test 1 - In collaboration with the Wasserversorgung (WVZ) - Stadt Zürich (Dr Oliver Köster and his team), we installed the SPC online at the WVZ plant in Zürich Giessen and analysed water from the surface of lake Zurich for 7 days.

Test 2 - We deployed the SPC (underwater) into five experimental ponds at Eawag, which represent different ecosystems with varying levels of productivity. For each pond, one point measurement was done and compared to microscopy.

Below we summarise the outcomes of these trials, organised in Methods, Results and Conclusions.

2. Methods

2.1. Description of SPC

The SPC is an automated underwater microscope consisting in two modules: a camera with a magnifier and a light source (Fig. 1). From the group of Jules Jaffe at Scripps, we obtained a second-generation SPC system, the SPCP2 (see http://spc.ucsd.edu/cameras-2/spcp2-camera/ for details),
with a plankton size detection range from circa 0.05 mm to 0.1 mm. This configuration allowed to image and study medium to large sized phytoplankton.

The SPC employs dark-field illumination to enhance contrast of transparent objects, has real-time image processing, and object detection, operated by an internal computer. When measurements are done and images acquired, data from the SPC are transferred to a laptop computer, and a code written in Phyton allows extracting and batch processing all the images. Result are summarised in an html file (Fig. 2), where the user can browse the images and explore samples, or extract raw data. Details about the specific configurations of the SPC are not reported here, however the protocol with detailed instructions can be made available.

Fig. 1. The SPC at the Eawag phytoplankton ecology lab.

Fig. 2. Screenshot of the SPC data output.
2.2. Test-1 Lake Zürich

The WVZ plant Giessen is located about 2 km downstream of Lake Zürich, on the river Limmat. The Limmat represents the outflow of Lake Zürich surface water, which can be used by the WVZ for drinking water provision in case of necessity. Therefore, Limmat water flows through the WVZ plant and we were allowed to install the SPC online with other monitoring instruments. We built a simple flow through system that allowed the camera to take pictures in real time of the water flowing through the WVZ plant. A plastic cover was arranged around the camera and Limmat water was flowing into it and overflowing from above and below to assure the water renewal (Fig. 3). The monitoring campaign lasted 7 days, from the 4th to the 10th of October, for a total of 7 samples.

Fig. 3. Set up of the SPC as online monitoring system at WVZ.

2.2.1. Microscopy

Water samples (50 mL) for phytoplankton identification under the microscope were collected during the time the camera was taking pictures except for Saturday and Sunday, when samples were taken few hours later. A total volume of 25 mL were poured into an Utermöhl sedimentation chamber (HYDRO-BIOS) and left for sedimentation overnight before proceeding to identification. We used an inverted Zeiss Microscope (Axiovert 135) to count algae, rotifers and ciliates. A first screening with 160X magnification was carried out in order to count the biggest taxa and check that the algal distribution was homogeneous. For smaller cells, a magnification of 320X or 640X was used. At each of these two magnifications a total of 40 field views (out of 1686 or 6900 respectively) were counted and from that extrapolated the total abundance of each species in the 25 mL.

2.2.2. SPC

The SPC was programmed to automatically run for 2 hours each day, starting at 11 am and acquiring images at a rate of 1 frame/second. Using the provided software, data were converted to an html
files. With a visual screening, we estimated the proportion of non-phytoplankton particles as well as a list of the species richness for each sampling date.

2.3. Test-2 Eawag experimental ponds

The experimental ponds at Eawag have a capacity of 15 m$^3$, and are currently being used for an experiment in which aquatic ecosystems with different food-web structures are tested for their resilience to eutrophication. This set of experimental ponds offered the possibility to deploy the SPC underwater in environments with different productivity. The SPC was submerged into the shallow area of five ponds (circa 50 cm below water surface – Fig. 4). The monitoring occurred between the 16$^{th}$ and the 20$^{th}$ of October, and a total of 7 ponds were monitored, of which 2 did not give useful results due to the high cell densities (see Section 4). Therefore a total of 5 samples were collected for comparison between microscopy and SPC.

![Fig. 4. The SPC deployed in Eawag experimental ponds.](image)

2.3.1. Microscopy

Water samples (50 mL) for phytoplankton identification with the microscope were taken at the end of each measurement with the SPC. A total volume of 3 mL was poured into an Uttermöhl chamber and left for sedimentation at least 2 hours before proceeding to identification. Algal density in the ponds is much higher than lake water and that is why the used volume was much lower. We used the same procedure described in 2.2.1.
2.3.2. SPC
The camera was set to acquire images for 1 hour (instead of 2 h as previously, because of higher cell densities compared to Lake Zurich) in each of the ponds at a rate of 1 frame/second.

3. Results

3.1. Test-1 Lake Zürich
We encountered no problems with either hardware or software, therefore the SPC worked as expected. Imaging was executed by the SPC computer every day at the right time as programmed. The images produced by the camera were of good quality and we could clearly recognise, in the size range between 20 to 150 µm, the same species that were also observed by microscopy. Particularly, it is important to note how well we could detect and recognise the toxic and bloom forming cyanobacterium *Planktothrix rubescens* (Fig. 5).

![Fig. 5. P. rubescens in Lake Zürich, from microscopy (A) and SPC (B) imaging.](image)

3.2. Test-2 Eawag ponds
The SPC worked well also during this test, no problems with either hardware or software. The images produced by the camera were of good quality and we could recognise, also in this case, a number of species that were also observed by microscopy, in the size range between 20 to 150 µm. We however found out that we could not image phytoplankton in the most productive ponds. Here the cell density was so high (1200 particles per µL, note that 1 µL is also the imaged volume per frame, so ~ 1000 particles per frame) that the computer could not identify particles from the background due to high light scattering and, possibly, high particle packaging in the imaged field.

3.3. Density and richness comparison between microscopy and SPC
Considering only the range of taxa that could be detected by the SPC (20-150 µm), we made a comparison between phytoplankton density and richness obtained by the camera relative to traditional microscopic counts. We considered microscopy and SPC results from both trials.
combined, in order to cover the widest possible range of density and richness of species in our studied systems, and the largest range of operative conditions. We also counted phytoplankton particles for density estimation, not cells: this means that colonial forms we considered as one individual entry in our counts. This was done to simplify the comparison between the two different methods. Results are reported in Fig. 6.

**Fig. 6.** Relationship between microscopic counts and SPC imaging for phytoplankton density (logged) and richness (number of species), in the size range 20-150 µm.

The relationship between **density derived by microscopy and SPC** was positive and significant, but not clearly a 1:1 ratio (Fig. 6A):

\[ Y = 0.726 X + 0.341 \quad (p-value = 0.00069, \text{Adjusted R-squared: } 0.6701) \]

It has to be noted that the SPC estimates the number of particles detected per unit of time, not a concentration estimate (per unit of volume) like the one we obtain for microscopy.

In addition, the relationship between **number of species derived by microscopy and SPC** was positive and extremely significant, and approached very closely a 1:1 ratio (Fig. 6B):

\[ Y = 0.989 X - 1.4217 \quad (p-value = 1.001e-05, \text{Adjusted R-squared: } 0.856) \]

The relationship in phytoplankton richness between microscopy and SPC had a negative intercept, which suggests a systematic tendency of SPC to underestimate phytoplankton number of species.

4. **Conclusions**

Our trial tests suggest that the SPC can be used to monitor, count and identify plankton with good prospects to become a reliable and standardisable method. We envision applying it for research on drivers of phytoplankton biodiversity, to study the effects that phytoplankton dynamics have on lake pelagic food-web processes, and individual level microbial interactions. We have good confidence that this new technology will prove to be innovative and efficient for research, although it requires more development particularly on the image analysis side. For example, the user should be able to extract more features from the imaged particles and directly use them for analysis and classification.
of plankton taxa. An automated classification tool for the images would also be essential in the future, to identify and count taxa. It is a promising tool that can complement existing monitoring efforts, but substantially more research would be needed before it can be considered as an alternative for phytoplankton monitoring (i.e. a replacement of existing approaches).

The approach has also some technical limitations that will be the topic for future research and development. For example, the perfect match (1:1 ratio) between density and richness estimated by the two methods depends on the correlation between number of particles imaged over time and those present per unit of volume (as counted by microscopy). More calibration is needed to clearly establish the relationship between abundances over time and volume. The issue with the camera not detecting particles at high-densities could theoretically be solved by reducing the path length between the light source and camera to reduce the light scattering due to the cells present in suspension.

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