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SHORT COMMUNICATION

Application of autonomous underwater vehicle and image analysis for detecting the three-dimensional distribution of freshwater red tide *Uroglena americana* (Chrysophyceae)

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The spatial structure of Uroglena americana was detected using an autonomous underwater vehicle equipped with a submersible microscope, video recording system and water quality monitoring sensors. Uroglena americana colonies were enumerated via image analysis and pattern recognition methods. This quantitative technique is useful for understanding plankton assemblages, distributions and their potential to become nuisance algal blooms.

INTRODUCTION

Monitoring of nuisance algal blooms is important for understanding aquatic ecosystems as well as decreasing their potential for causing environmental and economic damage. Generally, cell or colony counting through an optical microscopy in a laboratory has been prevalent in the determination of plankton biomass, but offers only discrete point-source information. Optimal monitoring methods, which would detect plankton patchiness, have not been proposed. Recently, automated plankton monitoring systems with an autonomous underwater vehicle (AUV) have been developed for applied marine and freshwater ecological research (Kumagai *et al.*, 2001; Fornari, 2003; Rudnick and Perry, 2003). These systems simultaneously obtain biogeochemical, physical, environmental and biological data and can immediately analyse large data sets from a three-dimensional (3D) water body. Also, advanced biological sensors, for instance bio-optical sensors, hologrammetry technologies and image analysis are under development for detecting target microorganisms and their distributions in water without chemical treatments (Gorsky *et al.*, 2000; Hobson *et al.*, 2000; Beutler *et al.*, 2002; Embleton *et al.*, 2003). These novel technologies for water research are seen to be of value for understanding natural plankton ecology.

Lake Biwa, Japan's largest lake, has suffered from freshwater red tide since 1977 (Yoshida *et al.*, 1983a). Chrysophycean *Uroglena americana* Calkins [*Uroglenopsis americana* (Calkins) Lemmerman] are the main cause of spring freshwater red tides. The *U. americana* usually found in deep lakes and reservoirs tended to occur in waters with high TN : TP, DIN : DIP and TP : DIP ratios, and with low water temperature, DIP and TN : DIN ratios (Yoshida *et al.*, 1995). Also, iron and the presence of bacteria for their phagotrophic ingestion are important for *U. americana* bloom development (Kimura and Ishida, 1986; Bird and Kalff, 1987; Nishio and Ishida, 1990; Urabe *et al.*, 1999). The blooms induce problems that affect fish gills causing mortality, undesirable fish smell, disturbing of water filtration, as well as being aesthetically displeasing

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(Nakahara *et al.*, 1988; Yano *et al.*, 1988). They have presented a serious social problem because the lake water is supplied to ~14 million people for drinking.

In spite of enthusiastic studies on the development of freshwater red tide by direct sampling and remote sensing, it has not been easy to predict when and where red tide would appear in this large lake, because the blooms are strongly affected by metrological conditions (Yoshida *et al.*, 1983b; Yoshida *et al.*, 1983c; Yoshida, 1997). To enhance our understanding of these events, we observed *U. americana* distributions using an AUV system in order to quantify the 3D bloom size and structure *in situ*. In this communication, we describe the 3D dynamics of this nuisance microalgae using a video image analysis system mounted on an AUV and evaluate the colony distribution structure.

Our AUV was designed by the Institute of Industrial Science of the University of Tokyo and the Lake Biwa Research Institute and was constructed by Mitsui Ship Builder Co. Ltd. in 1998–2000 (Kumagai *et al.*, 2001). The AUV is equipped with a submersible underwater microscope, CTD, GPS in the air and 3D positioning system supported by Doppler Velocity Log (DVL) when submerged and is controlled via microwave wireless communication when on the surface and ultrasonic communication when submerged. It can also measure water currents using its Acoustic Doppler Current Profiler (ADCP; 1200 kHz, RD Instruments) when the DVL data is available. The submersible microscope (SM-201) for capturing plankton images was constructed by KISTEM Co. Ltd. This microscope consists of a greyscale CCD camera (Toshiba IK-M42), a C-mount macro lens adjusted to a field of view of 4.3 mm (width) × 3.2 mm (height) × 1 mm (depth) and a stroboscope with 6000 μd red LED lightning. The strobe illumination duration was set to 10 μs for stop-motion recording to a Hi-8 video recording system (Sony, GV-D300) at 29.97 frames per second. The CTD obtained simultaneous data from a temperature sensor (AQTE-PT100 Aquamatic) and fluorescence chlorophyll (Chl)sensor (AQFL-100MG Aquamatic).

Plankton images were encoded to 60-minute duration AVI video files. Each flame of the video files was de-interlaced and converted to separate BMP files. All objects in the BMP images were segmented, and *U. americana* objects were detected and analysed by extracting 130 statistical and morphometrical features using an image analysis and pattern recognition system (Walker and Kumagai, 2000). The numbers of *U. americana* objects per-unit-time were counted and combined with the recorded AUV route trajectory data. A 100 × 100–m area of water was surveyed at depths of 0.5, 4, 8, 12 and 16 m. *Uroglena americana* counts throughout this volume were interpolated into 1 m (length) × 1 m (width) × 0.16 m (depth) bins using Inverse Distance Weighting and Spline Interpolation methods.

RESULTS AND DISCUSSION

Field research was carried out in April 27, 2001 in an offshore area of the north basin of Lake Biwa (Fig. 1a). The AUV moved horizontally at 1 ms^{-1} throughout

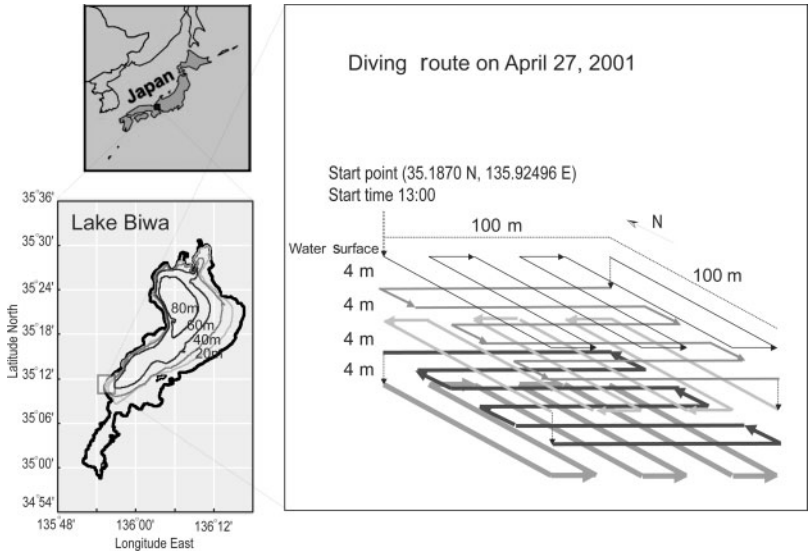

Fig. 1. Map of Lake Biwa, Japan and the diving route of the autonomous underwater vehicle from 13:00 to 14:00 on April 27, 2001.

the surveyed water volume (Fig. 1b). The total survey duration was 1 hour.

An example image captured from the on-board submersible microscope's video camera is shown in Fig. 2(a). The circular objects are the target species *U. americana*, whereas the other numerous small thin objects are diatoms and green algae. From the digital videotape, we frame-captured 29.97 images per second. That is 107 892 greyscale images of dimensions 720 × 280 pixels for the 1-hour video. A training set consisting of 190 examples of well-imaged *U. americana* (Fig. 2b), 139 examples of poorly imaged *U. americana* (Fig. 2c) and 218 examples of non-*U. americana* (Fig. 2d) objects was used to select a subset of highly discriminant features for classification purposes. In this study, two of the 130 features (Walker and Kumagai, 2000)—a Fourier feature of the boundary of the object (Feature 6) and the ratio of boundary length divided by object area (Feature 4)—were used to discriminate *U. americana* objects from other objects by using pattern recognition (Fig. 3). Also, to confirm the accuracy of our automated classification, *U. americana* counts via image analysis were compared with manually counted *U. americana* colonies from 4680 flames of video averaged over 10 s using the naked eye (Fig. 4). As can be seen, there was a strong linear relationship between the image analysis results and the manual results ($r^2 = 0.9481$, $n = 13$, $P < 0.001$). However, the image analysis system over-estimates the counts. The factor of 0.8802 from Fig. 4 was used to calibrate the automated count.

After interpolating data in the 100 × 100 × 16–m water mass taken at 0.5, 4, 8, 12 and 16 m depths, *U. americana* densities >6.5×10^4 colonies L^{-1} (Fig. 5a), we plotted Chl *a* concentrations >2.0 mg m^{-3} (Fig. 5b) and water temperatures of >12.4°C (Fig. 5c) were plotted in colour density maps. The densities of *U. americana* were in the range of 5.0–8.0×10^4 colonies L^{-1} (Fig. 5a); these are much less than the typical densities recorded during red tide outbreaks (Ichise *et al.*, 2002) of ≥3.0×10^5 colonies L^{-1}. According to Yoshida *et al.* (Yoshida *et al.*, 1983b), they observed 7.0×10^6 colonies L^{-1} on June 9, 1978 near the research area. The Chl a concentrations, at <5.0 mg m^{-3}, were also less than those recorded for red tide outbreaks of ~10–20 mg m^{-3} in the lake (Yoshida *et al.*, 1983a). The temperature of the water body was between 10.4 and 13.3°C (Fig. 5-c), which seems to be suitable for *U. americana's* growth (Yoshida *et al.*, 1983b).

The distribution of *U. americana* colonies was heterogeneous and of high density around the 4-m-depth layer (Fig. 5a). However, Chl *a* concentrations detected by a fluorescence sensor did not show a maximum in the 4-m-depth layer, but around the 12-m-depth layer (Fig. 5b). The water surface temperature was highest in

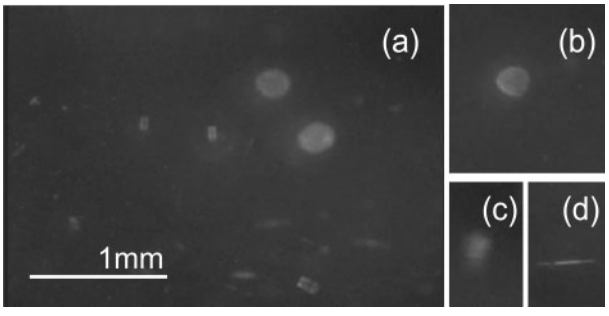

Fig. 2. (**a**) A frame image from submersible microscope, (**b**) well-imaged *U. americana*, (**c**) poorly imaged *U. americana*, (**d**) non-*U. americana*.

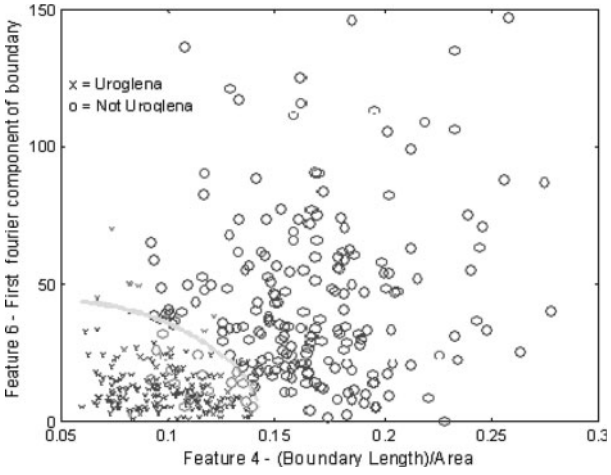

Fig. 3. Quadratic discriminate of *U. americana* objects against the other objects

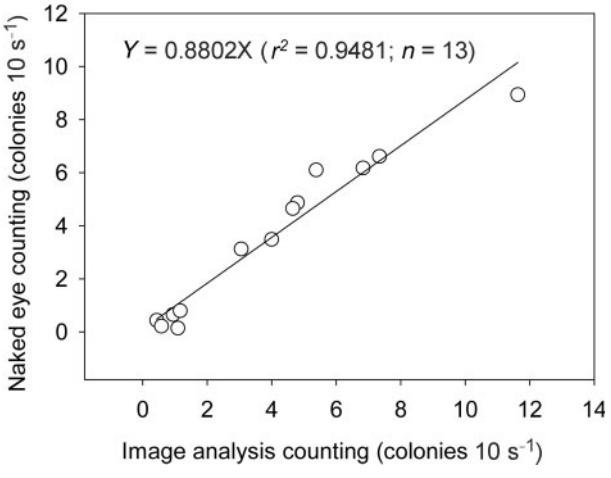

Fig. 4. Relationship between the image analysis counting and the naked eye counting of the colonies of *U. americana*.

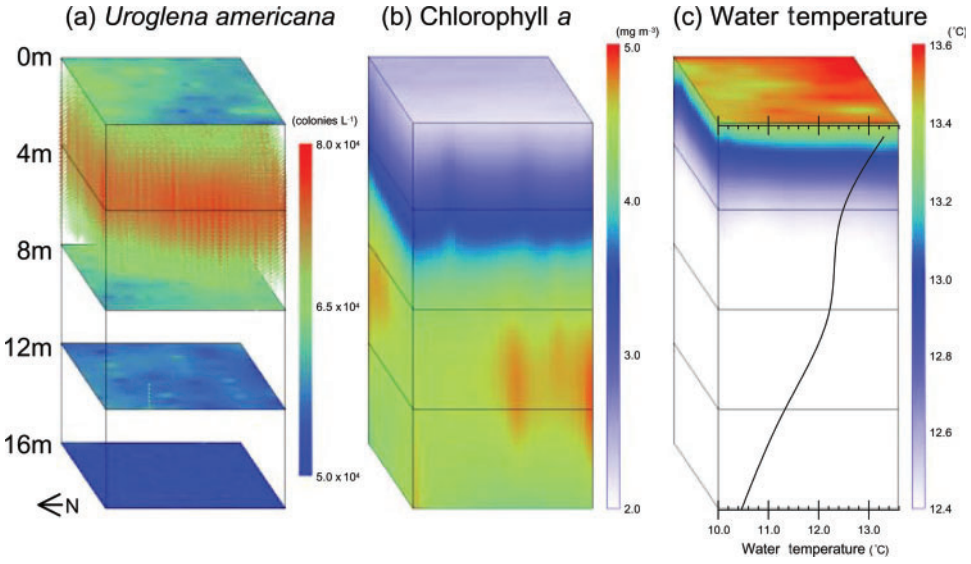

Fig. 5. (**a**) Spatial distribution of *U. americana* colony densities, (**b**) chlorophyll *a* concentrations, (**c**) water temperature in 100 × 100 × 16–m depth area.

the water column and decreased with increasing depth. The change in water temperature over the 5–8-m layer was much lower than that of other depth layers (Fig. 5c).

We believe that there is a relationship between *U. americana* density distribution and water temperature distribution that will lead to a better understanding of accumulation mechanisms of *U. americana*. Colony density was highest in waters of 12.4°C (Fig. 5c). The vertical gradation of water temperature shows that the water column was likely to be stable, but the southern part of the water surface was slightly higher in temperature (Fig. 5c) and lower in colony density (Fig. 5a). This might suggest that the warmer surface layer shifted to the south because of the prevailing mild wind and, in the northern areas, was replaced by water from just below the surface. Accumulation patterns of *U. americana* were well studied when they first appeared in this lake. Yoshida (Yoshida *et al.*, 1983b) suggested that there were three types of accumulation:

(i) Type 1: During windy and poor weather, *U. americana* accumulate downwind and have an uniform vertical distribution.
(ii) Type 2: At the end of the bloom season, they become less active and float to the water surface, where they are subsequently transported downwind. Therefore, their vertical distribution is predominantly at the water surface.
(iii) Type 3: During calm and fine weather, they stay in the 2–3-m depth band because of their sensitivity to light. Colonies in the surface layer sink in response to strong sunlight, and colonies at deeper levels float toward the light (Yoshida and Uda, 1987). When the wind blows, warmer surface waters move downwind, and as a result the blooms are forced up to the surface to replace the loss.

In our field research on April 27, 2001, the distribution pattern seemed to be the Type 3 distribution that is likely to be seen under calm and growth-promoting conditions in the early stages of *Uroglena* blooms. The mild wind of $\sim$1.6 ms^{-1} was blowing from the northeast during the research period, 13:00–14:00 on April 27, 2001. However, it is important to note that the 3D snapshot of plankton distributions shown in Fig. 5(a) may have been influenced by lake water currents, because sampling by the AUV throughout the 3D area of water was obviously not instantaneous. The horizontal water velocities at each depth measured by the ADCP on the AUV are shown in Table I. It appears that the water body was transported to the north and east directions during the observation period. However, these data also support our hypothesis regarding the formation of 3D distribution patterns of *U. americana* in Fig. 5(a).

Ichise *et al.* (Ichise *et al.*, 2002) reported that *U. americana* blooms were observed at the water surface in the lake with increased density of $\sim$1.0–3.0 × 10^5 colonies L^{-1} from May to early June in 2001, which is one month after our research. This shows that we monitored the early stages of seasonal blooms of *U. americana*.

Chl *a* concentration was highest around the 12-m depth (Fig. 5b), and was not concordant with *U. americana*

Table I: Mean water velocity measured by Acoustic Doppler Current Profiler on autonomous underwater vehicle (AUV)

AUV depth (m)	Measuring depth (m)	Mean water velocity (m s^{-1})	
		North–South	East–West
0.5	0.8–2.0	$+6.2 \times 10^{-3}$	$+8.9 \times 10^{-3}$
4	4.3–5.5	$+20.3 \times 10^{-3}$	$+1.2 \times 10^{-3}$
8	8.3–9.5	$+34.1 \times 10^{-3}$	$+25.3 \times 10^{-3}$
12	12.3–13.5	$+20.9 \times 10^{-3}$	$+10.1 \times 10^{-3}$
16	16.3–17.5	No data	No data

distribution patterns. We assume that a large biomass of diatoms *Fragilaria crotonensis* and green algae *Closterium aciculare* in the water body contributed to these high Chl *a* readings (Fig. 2c). Also, it is well known that *in vivo* fluorescence measured by *in situ* Chl fluorescence instruments is likely to be affected by bright light during the daytime and will form a maximum Chl depth band in a subsurface layer under stratified water conditions (Falkowski and Raven, 1997). This is called non-photochemical quenching (Schreiber *et al.*, 1986), which might explain the observed Chl *a* distribution pattern (Fig. 5b). However, we unfortunately did not have enough data to confirm or deny these possibilities.

In summary, we were successful in separating and visualizing the 3D distribution of *U. americana* from other phytoplankton using an AUV and image analysis. We were also able to observe the precursors to a potential algal bloom that was developing in the 4-m-depth layer. Our research has shown that the monitoring of phytoplankton using an AUV is useful to predict noxious algal blooms in a large lake, and that automated image analysis makes possible the quantification of phytoplankton numbers in large volumes of water that would be difficult to quantify via traditional manual methods. To facilitate future progress in this research, it is necessary to improve image quality, expand our phytoplankton database and improve algorithms for more precise detection of targeted species.

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