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SHORT COMMUNICATION

FlowCAM as a tool for studying small (80–1000 μm) metazooplankton communities

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FlowCAM was assessed as a tool for studying small (80–1000 μm) metazooplankton communities by comparing the abundances estimated using FlowCAM with those estimated using a stereomicroscope for eight groups of organisms. With the exception of poecilostomatoid copepods, estimates of the number of organisms in samples using FlowCAM were similar to those using the stereomicroscope. These results suggest that FlowCAM is an effective tool for enumerating small metazooplankton.

KEYWORDS: imaging system; FlowCAM; microscopy; zooplankton; abundance

INTRODUCTION

Metazooplankton (multicellular zooplankton *sensu* Sieburth *et al.*, 1978) communities have widely been studied in various marine areas and ecosystems throughout the world. Microscopy is generally used for the identification and enumeration of zooplankton organisms. However, this method is time-consuming and could be biased by human factors (wrongly identified species owing to fatigue, boredom or limited knowledge of taxa). Various plankton imaging systems and automatic recognition programs have been developed to reduce sample processing time (see Culverhouse *et al.*, 2006 and Benfield *et al.*, 2007 for reviews), ZOOSCAN (Grosjean *et al.*, 2004; Gorsky *et al.*, 2010) being one of the most popular systems (Garciá-Comas *et al.*, 2011; Nowaczyk *et al.*, 2011; Forest *et al.*, 2012). However, ZOOSCAN cannot detect particles with an equivalent spherical diameter (ESD) of <300 μm. The Flow Cytometer And Microscope (FlowCAM) system (Sieracki *et al.*, 1998) is able to detect smaller particles as well as large particles (ESD from 2 to 2000 μm), but is mainly used for

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studying unicellular microplankton (20−200 μm, marine photosynthetic organisms and ciliates) (See *et al.*, 2005; Buskey and Hyatt, 2006; Zarauz *et al.*, 2009) by combining flow cytometry and microscopy. So far as we are aware, no study using this system to investigate metazooplankton communities has been published, even though the FlowCam developers consider that this would be technically feasible (Fluid Imaging Technologies, 2011). Nauplii were observed using FlowCAM by Álvarez *et al.* (Álvarez *et al.*, 2012), but these were in a negligible proportion of all the organisms observed. This study evaluated the efficiency of FlowCAM for identifying and enumerating small metazooplankton communities.

Zooplankton was vertically and horizontally sampled in the Ouano lagoon (ca. 165°56′E, 21°59′S, New Caledonia, South West Pacific Ocean) using a WP2 zooplankton net with a 80 μm mesh from 2 to 10 October 2013. There were five sampling stations: external ocean, St Vincent pass, Tẽnia channel, lagoon and barrier reef (17 samples). Samples were preserved in a 4% formaldehyde solution and sieved to remove organisms over 1000 μm, which were not taken into account in this study. Each sample was first analysed using FlowCAM and then using microscopy. Both analyses were carried out by the same person. Two zooplankton size classes (80−200 μm, 200−1000 μm) were separated and rinsed using sieves and preserved in a given volume of distilled water (V_i) prior to being processed using FlowCAM. An aliquot of each size class from each sample was analysed using FlowCAM (VS-IV; Fluid Imaging Technologies, Inc.) using the auto-image mode (4 and 2 frames/s respectively), with ×4 and ×2 objectives and 0.3 × 3 and 2 × 4 mm flow cells for the two size classes, respectively. Analysis was stopped when more than 2000 particles had been counted or when the complete sample had been used. The aliquot analysed was then returned to the sample. Particles (metazooplankton, unicellular organisms, eggs, debris and undetermined items) were identified (if possible to genus level for copepods) using VisualSpreadsheet®. Only metazooplankton organisms were taken into account in this study. The organisms observed were classified manually, which gave precise taxonomic identification but did not test the efficiency of automatic classification of organisms as in previous studies. The proportion of the volume analysed was given by the ratio of volume digitized by FlowCAM (V_a) to V_i, while the abundances of organisms in the samples were determined by dividing the number of organisms counted by this ratio. The abundances of organisms in both size classes were pooled and then divided into eight groups (Fig. 1: calanoid copepods, *Oithona* copepods, poecilostomatoid copepods, harpacticoid copepods, gelatinous zooplankton, other organisms, nauplii and meroplankton). The total abundance, i.e. the sum of the eight groups, was also calculated.

The samples for both size classes were then grouped into a new volume (V_i') and two successive aliquots (volume V_{a1}' and V_{a2}' as defined by the person carrying out the experiment: $V_{a1}' < V_{a2}'$, e.g. 5 and 20 cm^3) were analysed using a stereomicroscope (Leica M205C). The most abundant organisms in the first aliquot ($n \geq 30$) were not counted in the second aliquot. The aliquots analysed were then returned to the sample. The abundances of the organisms identified using the stereomicroscope were estimated by dividing the number identified by the ratios $V_{a1}' : V_i'$ for abundant organisms ($n \geq 30$ in the first aliquot) and $(V_{a1}' + V_{a2}'):V_i'$ for rarer organisms that were counted in both aliquots. These abundances were then added to the groups previously defined and the total abundance was also calculated.

The number of zooplankton taxa identified for each sample using both methods was compared using paired Student *t*-tests. The estimated abundances of each group of organisms and of the total sample recorded using both methods were compared using paired Wilcoxon tests. This was used because the differences between the values obtained using FlowCAM and those obtained using the microscope were not normally distributed according to Shapiro tests. Levels of significance were corrected with sequential Bonferroni corrections (Rice, 1989) for the tests on the eight groups of organisms.

A greater number of zooplankton taxa per sample was identified by microscopy (mean ± SEM = 21.71 ± 1.49) than with FlowCAM (14.71 ± 1.17) (Student *t*-test, $P < 0.001$). The two methods provided a similar estimate of the total abundance in the samples (Wilcoxon test, $P = 0.378$) and, for each group of organisms, no significant differences in estimated abundances were observed between the two methods, except for poecilostomatoid copepods which, for each sample, were more abundant when counted using FlowCAM rather than the microscope (Fig. 2. FlowCAM: 597.33 ± 156.84 individuals, stereomicroscope: 252.24 ± 93.89 individuals, Wilcoxon test, $P < 0.001$).

The lower number of zooplankton taxa identified using FlowCAM is probably due to smaller subsampling, and thus, lack of rare taxa detection. The proportion of the volume analysed using FlowCAM ranged from 0.57 to 12.60% for the 80−200 μm size class and from 1.88 to 72.70% for the 200−1000 μm size class. However, the proportion of the volume analysed using stereomicroscope ranged from 10 to 100% of each sample. For the poecilostomatoid copepods, differences in subsampling strategies may also explain the differences in the abundances estimated using the two methods but it is also possible that species may not have been correctly identified,

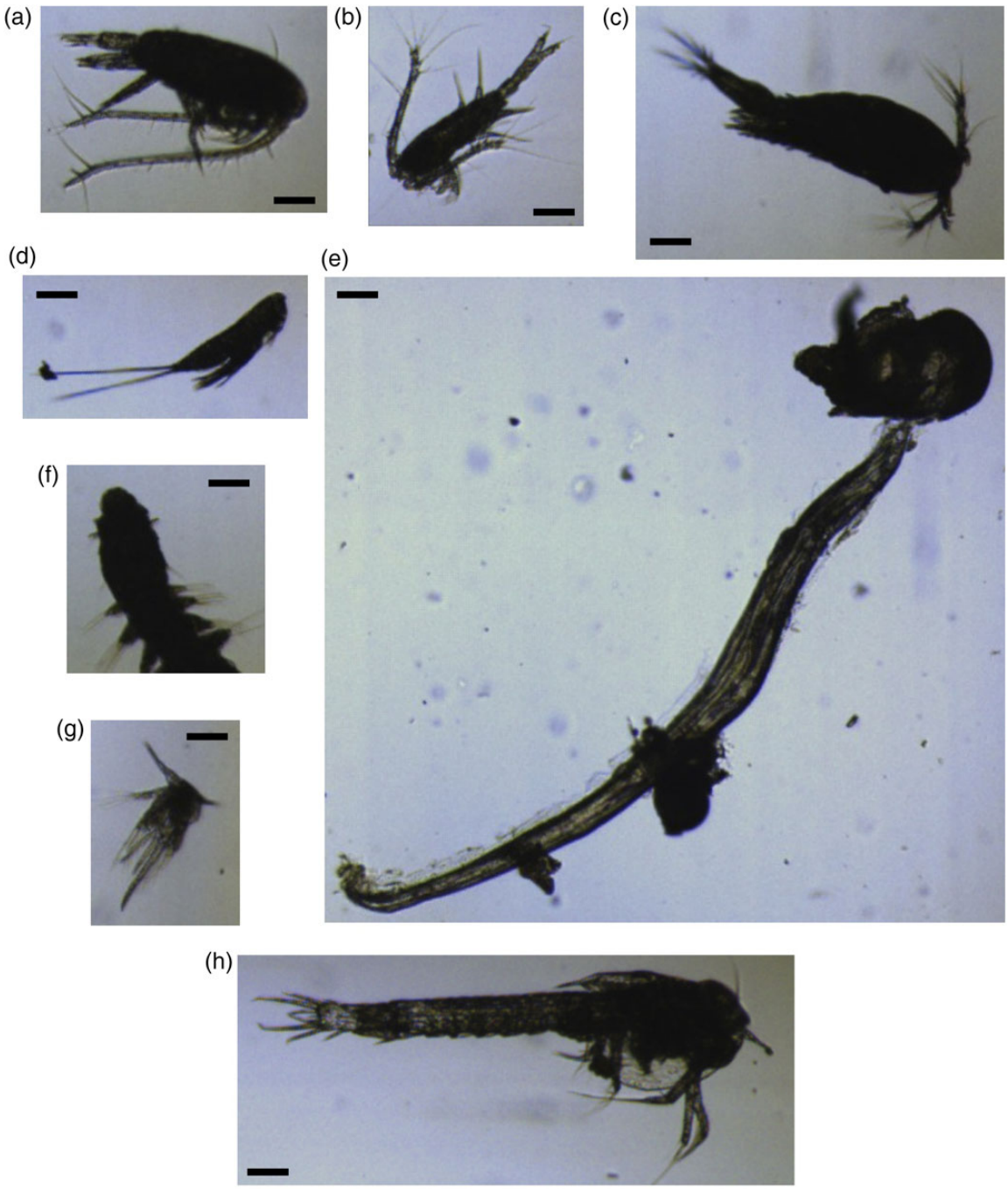

Fig. 1. Examples of images produced by FlowCAM of organisms in the various groups: (**a**) calanoid copepods (*Clauso/Paracalanus*); (**b**) *Oithona*; (**c**) poecilostomatoid copepods (*Oncaea*); (**d**) harpacticoid copepods (*Microsetella*); (**e**) gelatinous zooplankton (appendicularian); (**f**) other organisms (annelid); (**g**) nauplii; (**h**) meroplankton (protozoea). Scale = 100 μm.

even though, in this study, both analyses were performed by the same person. For example, species of the poecilostomatoid copepod genus *Oncaea* may be confused with species of the cyclopoid genus *Oithona* and *vice versa*. However, with the exception of poecilostomatoid copepods, these differences were not significant. On the other hand, Gislason and Silva (Gislason and Silva, 2009) found that an imaging system similar to ZOOSCAN underestimated or overestimated abundances in comparison with counts using a microscope. The better results in our study are probably explained by the fact that Gislason and Silva (Gislason and Silva, 2009) used

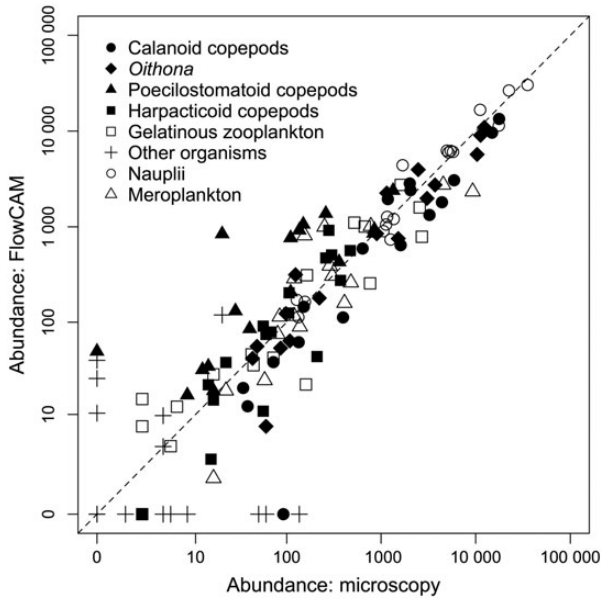

Fig. 2. Comparison of estimated abundances (number of individuals in samples) using a stereomicroscope (*x*-axis) and FlowCAM (*y*-axis) for each group. The dashed line shows the 1:1 line.

automated classifications whereas we used manual classification.

Previous studies on unicellular organisms have reported relatively good correlation between abundances estimated using FlowCAM and microscopy (Sieracki *et al.*, 1998; Buskey and Hyatt, 2006; Ide *et al.*, 2008, Álvarez *et al.*, 2014). However, See *et al.* (See *et al.*, 2005) found that values estimated by FlowCAM showed better agreement with values estimated by epifluorescence microscopy than with those estimated by light microscopy. Our results indicate that the FlowCAM system is suitable for studying zooplankton communities as it produced similar results to those obtained by microscopy for nearly all the groups of organisms considered. This system provides a method for classifying organisms using recorded images and for checking the accuracy of results at any time. Furthermore, images of organisms can be archived and shared within the scientific community. Another potential advantage of FlowCAM over microscopy is the ability to take account of and enumerate particles such as debris that are hard to count or generally ignored using microscopy. However, as FlowCAM has not, so far as we are aware, been previously used for studying metazooplankton, further investigations with larger sampling are required to test the reliability and variability of this method for enumerating small metazooplankton. Furthermore, the efficiency of automated classification should be assessed as soon as a library of zooplankton images is available, because manual classification of images taken by the FlowCAM is time-consuming, particularly if images are blurred, duplicated or contain more than one organism.

Previous studies using FlowCAM or ZOOSCAN assessed the efficiency of these systems for estimating abundances by comparison with using a microscope, mainly by using correlation coefficients and linear regression models (Sieracki *et al.*, 1998; Buskey and Hyatt, 2006 for FlowCAM; Gislason and Silva, 2009; Garciá-Comas *et al.*, 2011; Nowaczyk *et al.*, 2011 for ZOOSCAN). These comparisons of abundances should continue in future studies but paired tests should be used systematically to compare relative differences in abundance, as in the study by See *et al.* (See *et al.*, 2005) and in this study.

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REFERENCES

Álvarez, E., López-Urrutia, Á. and Nogueira, E. (2012) Improvement of plankton biovolume estimates derived from image-based automatic sampling devices: application to FlowCAM. *J. Plankton Res.*, **34**, 454–469.

Álvarez, E., Moyano, M., López-Urrutia, Á., Nogueira, E. and Scharek, R. (2014) Routine determination of plankton community composition and size structure: a comparison between FlowCAM and light microscopy. *J. Plankton Res.*, **36**, 170–184.

Benfield, M. C., Grosjean, P., Culverhouse, P. F., Irigoien, X., Sieracki, M. E., Lopez-Urrutia, A., Dam, H. G., Hu, Q. *et al.* (2007) RAPID: research on automated plankton identification. *Oceanography*, **20**, 172–187.

Buskey, E. J. and Hyatt, C. J. (2006) Use of the FlowCAM for semi-automated recognition and enumeration of red tide cells (*Karenia brevis*) in natural plankton samples. *Harmful Algae*, **5**, 685–692.

Culverhouse, P. F., Williams, R., Benfield, M., Flood, P. R., Sell, A. F., Mazzochi, M. G., Buttino, I. and Sieracki, M. (2006) Automatic image analysis of plankton: future perspectives. *Mar. Ecol. Prog. Ser.*, **312**, 297–309.

Fluid Imaging Technologies. (2011) Zooplankton analysis using FlowCAM®. FlowCAM® Application note 108.

Forest, A., Stemmann, L., Picheral, M., Burdorf, L., Robert, D., Fortier, L. and Babin, M. (2012) Size distribution of particles and zooplankton across the shelf-basin system in southeast Beaufort Sea: combined

results from an Underwater Vision Profiler and vertical net tows. *Biogeosciences*, **9**, 1301–1320.

Garciá-Comas, C., Stemmann, L., Ibanez, F., Berline, L., Mazzochi, M. G., Gasparini, S., Picheral, M. and Gorsky, G. (2011) Zooplankton long-term changes in the NW Mediterranean Sea: decadal periodicity forced by winter hydrographic conditions related to large-scale atmospheric changes? *J. Mar. Syst.*, **87**, 216–226.

Gislason, A. and Silva, T. (2009) Comparison between automated analysis of zooplankton using ZooImage and traditional methodology. *J. Plankton Res.*, **31**, 1505–1516.

Gorsky, G., Ohman, M. D., Picheral, M., Gasparini, S., Stemmann, L., Romagnan, J. B., Cawood, A., Pesant, S. *et al.* (2010) Digital zooplankton image analysis using the ZooScan integrated system. *J. Plankton Res.*, **32**, 285–303.

Grosjean, P., Picheral, M., Warembourg, C. and Gorsky, G. (2004) Enumeration, measurement, and identification of net zooplankton samples using the ZOOSCAN digital imaging system. *ICES J. Mar. Sci.*, **61**, 518–525.

Ide, K., Takahashi, K., Kuwata, A., Nakamachi, M. and Saito, H. (2008) A rapid analysis of copepod feeding using FlowCAM. *J. Plankton Res.*, **30**, 275–281.

Nowaczyk, A., Carlotti, F., Thibault-Botha, D. and Pagano, M. (2011) Distribution of epipelagic metazooplankton across the Mediterranean Sea during the BOUM cruise. *Biogeosciences*, **8**, 2159–2177.

Rice, W. R. (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

See, J. H., Campbell, L., Richardson, T. L., Pinckney, J. L., Shen, R. and Guinasso, N. L. (2005) Combining new technologies for determination of phytoplankton community structure in the northern Gulf of Mexico. *J. Phycol.*, **41**, 305–310.

Sieburth, J. M., Smetacek, V. and Lenz, J. (1978) Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanogr.*, **23**, 1256–1263.

Sieracki, C. K., Sieracki, M. E. and Yentsch, C. S. (1998) An imaging-in-flow system for automated analysis of marine microplankton. *Mar. Ecol. Prog. Ser.*, **168**, 285–296.

Zarauz, L., Irigoien, X. and Fernandes, J. A. (2009) Changes in plankton size structure and composition, during the generation of a phytoplankton bloom, in the central Cantabrian sea. *J. Plankton Res.*, **31**, 193–207.