

ANALYSIS AND SORTING OF ZOOPLANKTON

AUTOMATED SORTING AND QUANTIFICATION OF DIFFERENT ZOOPLANKTON SPECIES COMPARED TO MICROSCOPIC ANALYSIS

OBJECTIVE

The purpose of this experiment was to test the feasibility of using the COPAS™ PLUS instrument to analyze and distinguish between different species of zooplankton from water samples. In order to verify the experimental results, zooplankton were sorted for microscopic inspection. Results were compared to concentration numbers and species found using manual collection methods and microscopic analysis.

INTRODUCTION

The COPAS PLUS instrument is able to analyze and sort large objects (200-750 microns) on the basis of size, density, and fluorescence signals. In this experiment we analyzed and sorted various species of zooplankton from aquatic samples. These organisms were dispensed into Petri dishes and visually inspected for accuracy and viability. The COPAS' gentle pneumatic sorting mechanism provides a method for analyzing and handling these sensitive organisms with a high level of recovery and viability.

Zooplankton live suspended in the water column in all aquatic ecosystems. The organisms, which range in size upwards of 200 microns, provide an important food link between the phytoplankton and the higher trophic levels of the ecosystem.

Presently, monitoring water quality is done by several methods such as fish counting, and phytoplankton and chemical analysis. In Europe, these results are used to classify safety and purity of waters. In lake "Veerse Meer" for example, where these experiments were performed, it has been found that during the summer months, some types of blooming of algae can have an effect of food production or collection (mussels) or can make the lake unhealthy to swim in. The Royal Institute for Coastal and Sea research (RIKZ) measures the phytoplankton levels and has proposed that European guidelines for measuring water quality should include counting species and concentrations of zooplankton.

MATERIALS

Two aquatic samples, VP1 and SP1, were collected for this analysis.

Sample VP1a: fixed (formaline 4%), untreated

Sample VP1b: fixed, stained with WGA-Alexa 488

Sample SP1a: fixed, untreated

Sample SP1b: fixed, stained with WGA-Alexa 488

WGA-Alexa 488; Molecular Probes

METHOD

Zooplankton sample populations were collected from two different locations on lake "Veerse Meer" (Zeeland, Netherlands): Sample VP1 from location Vrouwenpolder (VP); and Sample SP2 from location Soelekerkepolder (SP). Both zooplankton samples were collected using a 50 micron mesh filter and contained various contaminating parts including eggs and diatoms.

Samples were fixed in 4% formaline. The portions of both of the samples that were stained were incubated with 50 µg WGA-Alexa 488 per ml for 60 minutes and washed with 1x PBS. The WGA-Alexa 488 dye is known to stain (parts of) all species, and has a notable affinity for polychaete worm larvae (Figure 5). Prior to running the samples on the COPAS instrument, L. Peperzak took a small portion of each sample and classified the different species and their sample concentrations by microscopic analysis. This was used as a control

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Peperzak, L.¹, Rutten, Th.¹, Bongaarts, R.²

¹ Royal Institute for Coastal and Sea research (RIKZ); Vlissingen, The Netherlands

² Union Biometrica; Geel, Belgium

group.

RESULTS

Microscopic analysis:

VP:	Copepodite (Figure 1)	400/ml
	Copepod (Figure 2)	1000/ml
	Worm larvae (Figure 3)	100/ml
SP:	Copepodite	2200/ml
	Copepod	3100/ml
	Worm larvae	50/ml
	Ostracode (Figure 4)	1000/ml



Figure 1 Copepodite



Figure 2 Copepod



Figure 3 Worm-larvae



Figure 4 Ostracode

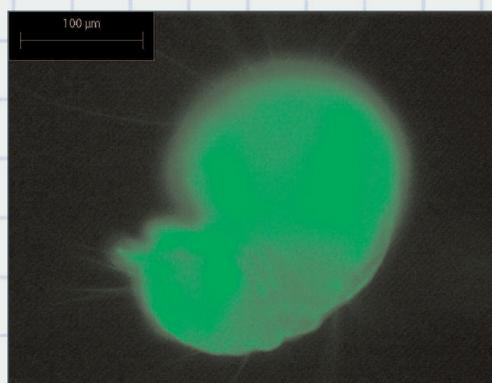
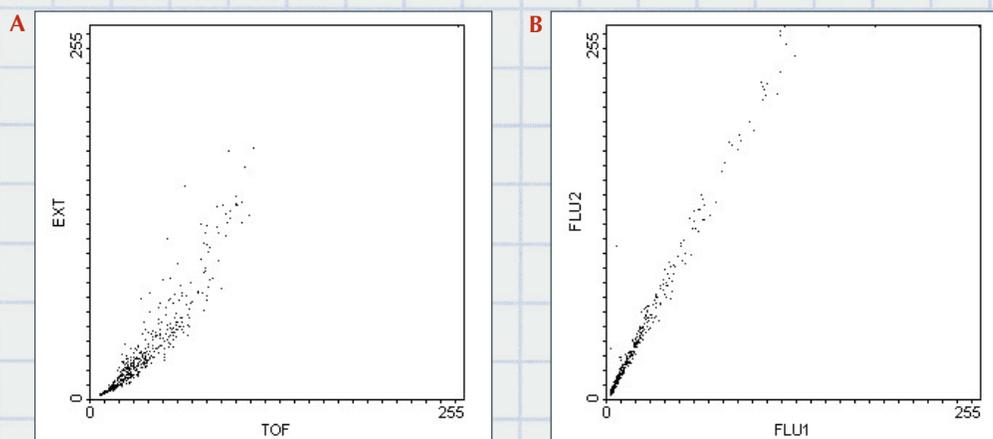


Figure 5 Fluorescent Worm-larvae

COPAS analysis and sorting:

Small Copepodites (Plots 1a & b) were analyzed and sorted using a combination of the parameters: Time of Flight (TOF) which is a measurement of object length, Extinction (EXT) which is a measurement of optical density, and two fluorescence parameters, FLU1 and FLU2, where FLU1 represents the green emission (515 nm) and FLU2 represents the red emission (585 nm). The purities we achieved were around 60-70%. Contaminating diatoms and eggs could not be completely eliminated. The sample was run at a rate of 20 events per second with a fraction of 11% selected. We sorted 250 events (out of 3429) in 150 seconds. The volume of sample was 7.5 ml. The concentration was calculated on these parameters.

Sample VP1a, which was used for COPAS instrument setup and initial sorting, was based on object size, density, and autofluorescence (Plots 1a & b). The same sample was mixed with 169 micron high fluorescence beads for a general size identification (Plots 2a & b). Sample VP1b was also run on the COPAS instrument, which excited the sample with a 488 nm laser in order to sort fluorescence positive worm-larvae for purity. Visual quantification was done on small copepodites and worm-larvae.



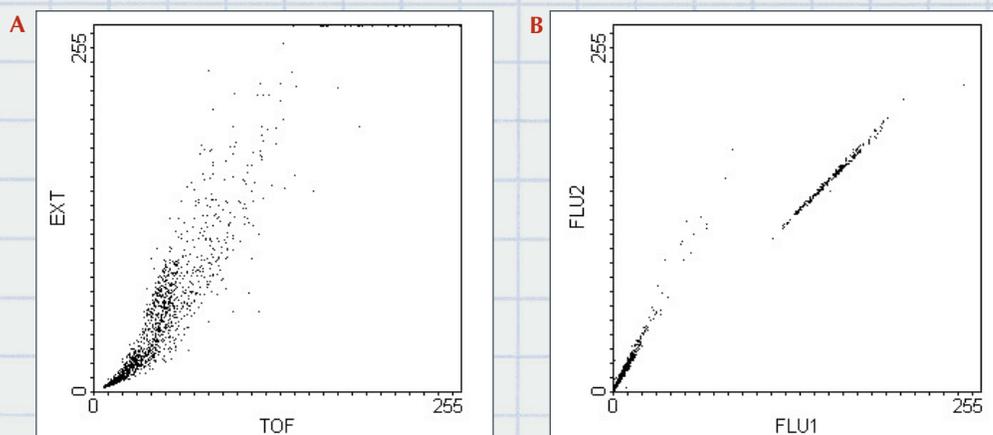
Plot 1: VP sample of small copepodites (unstained). a) TOF/EXT; b) FLU1/FLU2.

VP1a: Copepodite 457 per ml (400 per ml by microscope)

The sample VP1b (Plots 2a & b) was used to sort FLU1 positive worm-larvae. Purity achieved was 80%, contamination occurred by large copepods. The percentage selected was 6%. 100 worms were sorted in 2 minutes at a processing speed of 25 events per second. The sample was diluted 4x and the calculated concentration was 25 per ml.

VP1b: Worm-larvae 100 per ml (100 per ml by microscope)

Sample SP1a was used to sort the ostracodes (4% of the total population) based on the objects' size, density, and autofluorescence. The purity achieved was 75%. Contamination occurred by small copepodites. The large and dense objects sorted in this sample were all large copepods. The purity was 98%.



Plot 2: VP sample of small copepodites (unstained) mixed with 169 micron high fluorescence beads. a) TOF/EXT; b) FLU1/FLU2.

Sample SP1a was run on the COPAS instrument to determine if the Ostracodes could be distinguished and sorted. SP1b was run on the COPAS instrument to determine if the fluorescence positive worms could be distinguished and sorted.

Sample SP1b was used to sort the FLU1 positive worm-larvae (Fig. 5). The sample was diluted 10x and a purity of 95% could be achieved. The selected percentage was 1%.

CONCLUSIONS:

Experience with flow cytometry (Optical Plankton Analyzer, RIKZ, Netherlands) has demonstrated that it is very difficult to separate and sort phytoplankton since there are not enough distinguishing characteristics among the different species and stages. The sort experiment on the COPAS PLUS demonstrates that the same difficulty applies for zooplankton although sorting and counting can be performed to a certain level of reliability and purity. Sorting and verification by microscope however, remains necessary. If, as demonstrated in this experiment, a group of zooplankton such as the worm larvae, can be labeled with a specific fluorescence probe, determination, sorting, and counting are possible.

REFERENCES:

Peperzak, L., Vrieling, E.G., Sandee, B., Rutten, T., 2000, Immuno flow cytometry in marine phytoplankton research. *Sci. Mar.* 64: 165-181.

Union Biometrica, Inc., 35 Medford Street, Ste. 101, Somerville, MA 02143 (USA)

Tel: +617-591-1211 • Fax: +617-591-8388 • sales@unionbio.com • http://www.unionbio.com