## **III. Biological Data**

## 9. Protozoa

## (by Noriko Takamura)

Water samples were taken from the surface to 2.0 m depth with an acrylic column sampler at Sts. 3 and 9. The water sample (100ml) for counting ciliates was fixed with a few drops of Lugol's iodine solution. The sample was put in a 10ml of sedimentation chamber (Utermöhl 1958). The upper water was removed after the sample was kept for 24 hours. 40 cells of ciliates were counted for each sample with an inverted microscope. The number per one ml was converted as follows:

Cells\*ml<sup>-1</sup> = Counted number (cells)\*area of sedimentation chamber (mm<sup>2</sup>)/ total counted areas (mm<sup>2</sup>)/volume of sedimentation (ml)

Counting procedures were completed within three months.

The water sample (100 ml) for counting nanoflagellates was fixed with glutarardehide (final concentration, 1%) and then it was kept cool (4-6°C) until it was counted (up to 1 week). The water sample (approx. 5 ml) was filtered with nuclepore filters (pore size; 1.0µm), previously dyed with Sudan Black B. The filter was put in FITC solution (2 g of FITC powder dissolved in 50ml phosphate buffer) for one minute, and then rinsed with phosphate buffer. The filter was placed onto a clean slide, a small drop of the immersion oil added, and a coverslip was mounted on the top of the filter. 100 cells of flagellates were counted for each sample with an epifluorescence (BV-filter) microscope. The number per one ml was converted as follows:

Cell number (cells\*ml<sup>-1</sup>) = Counted number (cells)\*area of filtration (mm<sup>2</sup>)/ total counted areas (mm<sup>2</sup>)/volume of filtration (ml).

Counting procedures were completed within two weeks.

## References

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