SUPERFLUX CHLOROPHYLL *a* ANALYSIS:

AN ASSESSMENT OF VARIABILITY IN RESULTS

INTRODUCED PRIOR TO FLUOROMETRIC ANALYSIS

S. J. Cibik and C. K. Rutledge
Department of Biological Sciences
Old Dominion University
Norfolk, Virginia

C. N. Robertson
NOAA, NMFS, Northeast Fisheries Center
Sandy Hook Laboratory
Highlands, New Jersey

SUMMARY

During the Superflux II cruise (June 17-27, 1980), several experiments were undertaken to identify variability in results that came from procedural differences in the processing of chlorophyll samples prior to fluorometric analysis. Specifically, the questions to be addressed were: a) did failure to initially pass the seawater sample through a 300-μm mesh nylon screen to remove large zooplankton cause significant differences in chlorophyll *a* and phaeopigment *a* concentrations over a specified period of time; b) did samples which were immediately filtered through the Whatman glass fiber filters and held for a specified time period yield significantly different results from unfiltered seawater samples held for the same period; c) is there a significant difference in results of samples processed immediately and those held for a 24-hour extraction period?

T-tests on group means indicated that significant differences ($\alpha = 0.05$) in phaeopigment *a* concentrations did result in samples not initially screened, but not in the chlorophyll *a* concentrations. Highly significant differences ($\alpha = 0.001$) in group means were found in samples which were held in acetone after filtering as compared to unfiltered seawater samples held for the same period. No difference in results was found between the 24-hour extraction and samples which were processed immediately.

INTRODUCTION

The intent of the Superflux program was to monitor the fate of the effluent from the Chesapeake Bay. In an attempt to achieve a synoptic view of the plume, smaller support craft were utilized for simultaneous sampling. The samples for fluorometric evaluation were then transferred to the R/V Kelez for

subsequent analysis. One result of this program was the introduction of a time variable between sampling and chlorophyll a determination. In addition, samples exhibited various degrees of preparation, i.e., some arrived as just samples of seawater without any processing, some were initially screened with a 300-μm mesh screen to remove larger zooplankton, and, if facilities were available, some were further processed by filtration, the filters placed in acetone, and held in the dark.

An attempt was made to design experiments aboard ship to simulate these factors and possibly indicate whether they contributed to variability in pigment analysis results. The tests were not an extensive study of the situation; however, they provided some insight into the conditions under which the analyses were conducted and may provide the groundwork for further investigation.

MATERIALS AND METHODS

In order to investigate the effects of larger zooplankton being included in a seawater sample during transport, ten replicate samples without initial screening were held for a period of three hours along with ten replicates in which the seawater had been passed through a 300-μm mesh screen. The samples were held in the one-liter opaque containers which were being used for sample transport.

At the end of the time period the unscreened seawater was passed through the 300-μm mesh screen prior to analysis. Chlorophyll a and phaeopigment a were measured using the standard fluorometric techniques as described by Strickland and Parsons (ref. 1). Extraction was facilitated by the use of a tissue grinder. In each test 400 ml of seawater were filtered for analysis.

To contrast samples which arrived aboard ship already filtered with screened seawater samples, five replicate samples were held in 0.5-l light-proof bottles for six hours. At the same time five 400-ml replicate samples from the same source were filtered through the glass fiber filters, the filters were then folded with the plankton inside, placed in 15-ml centrifuge tubes, and held in the dark in 10 ml of 90% acetone for a similar period. Subsequently, the seawater samples were filtered and processed as described above.

The 24-hour extraction technique was compared with immediate processing by filtering five 400-ml replicates and holding the filters in acetone for 24 hours in a freezer. Five 400-ml samples were processed immediately for comparison.

Experimental results were subjected to a standard t-test to identify significant differences in group means.

RESULTS

The mean chlorophyll *a* concentration for the ten replicate samples which had been screened prior to analysis was 6.52 ± 0.435 mg/m^3*; the mean for the unscreened replicates was 6.88 ± 0.435 mg/m^3. These group means are not significantly different ($\alpha = 0.05$). The mean concentrations for phaeopigments *a* for screened vs. non-screened samples were 2.42 ± 0.244 mg/m^3 and 2.99 ± 0.328 mg/m^3, respectively, which are significantly different at $\alpha = 0.05$.

A highly significant difference ($\alpha = 0.001$) was found between group means for both chlorophyll *a* and phaeopigment *a* concentrations for the samples in which the filters were held for six hours vs. the seawater samples held for the same period. The group means for chlorophyll *a* were 0.76 ± 0.025 mg/m^3 for the filtered samples and 0.45 ± 0.106 mg/m^3 for the non-filtered samples. Group means for phaeopigments *a* were 0.20 ± 0.024 mg/m^3 and 0.10 ± 0.022 mg/m^3, respectively.

No significant difference ($\alpha = 0.05$) was found between the 24-hour technique and those samples processed immediately. Group means for chlorophyll *a* were 0.68 ± 0.068 mg/m^3 and 0.75 ± 0.107 mg/m^3, and those for phaeopigment *a* concentrations were 0.15 ± 0.018 mg/m^3 and 0.17 ± 0.045 mg/m^3.

In summary, the results of these experiments indicate that significant variability in results can be introduced in chlorophyll *a* analysis by differences in the processing of samples prior to fluorometric analysis. It is therefore recommended that uniformity in handling be emphasized when transferring samples from the support craft. Specifically, samples should be filtered aboard the support craft and transported under refrigeration in the absence of light.

REFERENCE

1. Strickland, J. D. H., T. R. Parsons. A Practical Handbook of Seawater Analysis, 2nd ed. Ottawa: Fisheries Research Board of Canada (Bull. 167), 1972.

*Group means are given with their associated standard deviations.