International Commission for the Northwest Atlantic Fisheries

Serial No. 3893
(D.c.1)

ICNAF Res.Doc. 76/VI/81[1]

ANNUAL MEETING - JUNE 1976

Variation in subsampling of zooplankton from the ICNAF Area[2]

by

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Introduction

In 1974 Poland and the United States established a joint Plankton Sorting and Identification Center in Szczecin. During the past two years the staff of the Center has sorted and identified some 160,000 fish larvae from samples collected during the ICNAF Larval Herring Surveys. The Center is now developing protocols for annually processing several thousand zooplankton samples. In addition to the removal of fish larvae the zooplankton constituents will be separated, identified, enumerated, and the contribution of each of the abundant species to the total biomass will be determined. The Advisory Group of the Center, recognizing the need for establishing objective criteria for subsampling zooplankton, recommended that a workshop be convened to measure the variance associated with subsampling and to establish standard sorting protocols. The workshop was conducted at the Center in March 1976.

Methods

During the workshop counts of zooplankton were made at different aliquot levels and with three different splitting devices to measure the variances of subsamples.

Zooplankton collected during the ICNAF larval herring surveys from subarea five were used in the experiments. Sixteen fishery engineers and technicians on the staff of the Plankton Sorting Center aliquoted and enumerated the samples. To provide replication in the experiment four groups of four sorters each were assigned samples for processing. Each group was responsible for samples collected during one of the months of the larval survey (Sep.-Oct., Dec., Feb. Mar.). An effort was made in selecting samples for analysis to examine both large and small zooplankters. A total of seven taxa were enumerated - Amphipods, Chaetognaths, Copepods, Euphausids, Mysids, Pteropods, and Decapod larvae. Copepods were the most numerous taxon, representing $\geq$ 90 percent of the biomass in 15 of the 16 samples examined. In the Sept.-Oct. period, the samples were mostly copepodites; in Dec.-Feb. adult Copepods were the most numerous forms (Table 1).

Sample volumes ranged from 45 cc to 240 cc. Each sample was aliquoted five or more times beginning with a 1/64, 1/128, or 1/256 depending on the total volume. Four replicates at each aliquot level were examined. The end point for splitting was approximately 100 to 200 organisms. Sample volumes and species composition, however, varied in each of the months. Consequently the numbers of organisms in the final aliquot of a given set of four samples ranged widely (from about 20 to 200).

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[1] This number reassigned to this paper. It was previously used, in error, for paper by R. Schlitz which is Res.Doc. 76/VI/37 (also presented to ICNAF Environmental Working Group Meeting, Szczecin, Poland, April 1976, as Working Paper 76/IV/107).

[2] Revision of Working Paper 76/IV/114 presented at ICNAF Environmental Working Group Meeting, Szczecin, Poland, April 1976.

Table 1. Percentage composition of dominant zooplankters in each month by station.

Station	1	2	3	4
Month				
February				
Copepods	67.2	97.3	98.0	96.0
Chaetognaths	.5	.6	.9	1.2
Pteropods	32.0	2.0	1.0	2.8
Others	.3	.1	.1	.0
March				
Copepods	98.0	98.2	98.6	99.7
Chaetognaths	1.6	.6	1.1	.1
Pteropods	.0	.0	.0	.0
Others	.4	1.2	.3	.2
September				
Copepods	96.9	99.9	98.8	97.9
Chaetognaths	.0	.0	.8	.9
Pteropods	2.5	.0	.0	1.0
Others	.6	.1	.4	.2
December				
Copepods	98.0	99.4	99.4	90.8
Chaetognaths	1.1	.1	.3	1.8
Pteropods	.8	.5	.3	5.5
Others	.1	.0	.0	1.9

Generally the last aliquot was predominately Copepods; numbers of other taxa were greatly reduced, and in a large number of samples they were missing. The relationship between sample volumes and aliquot levels examined in the experiment is given in Figure 1.

There were 512 different aliquot examinations in which 550,000 organisms were sorted and enumerated. These data were subject to a two-way analysis of variance using log-transformed data to compare the variances of subsamples at the different aliquot levels for both sorter effect and sample effect. Probability values were obtained from F-ratios. Sorter effect was only rarely significant at the P.1000 level while sample effect was significant in all cases at the P.005 level.

Comparison of the means at each of the split levels for the more numerous taxa (copepods, chaetognaths and pteropods) demonstrated differences from the mean of no greater than eight percent between the highest and lowest estimates of the number of organisms in the entire sample.

Comparisons Among Aliquots of Zooplankters

An analysis of variance among aliquot means for each of the dominant taxa indicated that the optimum aliquot level for chaetognaths and pteropods was variable. In samples with comparatively high numbers of chaetognaths (ca 5,000) it was possible to split down to between a 1/256 and 1/512 aliquot; subsequent aliquots resulted in significant differences at the P.05 level. For samples with high densities of pteropods (ca 10,000) no significant difference among split levels was observed down to a 1/512 aliquot at the P.05 level.

The variation among split levels for copepods was relatively great. Differences were a function of sample volume. Using a one-way analysis of variance among split levels we found that in seven out of sixteen comparisons within the experiment significant differences were observed among different split levels. Within the range of low volumes (45 to 80 cc) variations found in three samples were thought to be the result of clumping from insufficient dilution of copepods with water. Variations in four of the samples are attributed to high volumes $\geq$ 180 cc/100m^3. In the nine samples within the range of moderate volumes (between 80 cc and 180 cc) it was possible to aliquot down to between a 1/2048 and 1/4096 or between 100 to 200 copepods.

Comparisons Among Splitters

Recently a number of modifications to the Folsom splitter have been adopted for use in studies of the zooplankters in coastal waters adjacent to the ICNAF area. Two were selected for comparison with the Folsom splitter. One a rectangular model with one-half liter capacity developed by Marine Research Corp (Falmouth, Mass.). The other is a larger rectangular splitter with a 1 liter capacity developed by Texas Instruments (Dallas, Texas).

Comparisons among the three splitters were based on a two-way analysis of variance following the same procedures used for zooplankton. The F ratios of the error-mean-squares between splitters for each of the three taxa counted (copepods, pteropods and chaetognaths) were not significantly different at the P.05 level. This comparison was based on early winter samples consisting of small volumes (45 cc to 85cc/100m^3). The dominant zooplankters in the comparisons were relatively large overwintering copepods.

Discussion

Initial estimates indicate that approximately 4,000 samples are required annually to adequately monitor changes in the distribution and abundance of the continental shelf zooplankton community from western Nova Scotia to Cape Hatteras. Through joint international surveys it is probable that sufficient vessel time can be made available in a proper time sequence and areal sampling patterns to achieve this monitoring level. What is less certain, however, is the ability of the scientific community to deal with this large number of samples in an efficient and timely manner.

As a first step in dealing with this problem we have attempted to establish a range of aliquot levels that will allow for minimal sorting time and maximum information return. Within the range of volumes (40 cc to 245cc/100m^3) and dominant zooplankton taxa examined (copepods, pteropods, chaetognaths), a number of problem areas were encountered that can probably only be overcome with a combination of "expert" on-the-spot decision making and general protocol outlines. In another document presented to this Working Group we have provided a general sorting protocol for consideration.

The results of the present experiment corroborate the conclusions reached by Longhurst and Seiburt (1967), namely that skill is required in the use of the Folsom sample splitter. We observed several instances where an operator did not stir the sample sufficiently rigorously to thoroughly mix the pteropod constituent. This was obvious from inspection of the numbers of pteropods on either side of the septum of the splitter. When the sample was realiquoted carefully this bias was eliminated.

The non-randomness introduced in the splitting of low volumes was also observed in previous aliquoting experiments with the Folsom splitter. To reduce operator bias, particularly in small volume samples it would be advisable to use an air-inlet manifold for mixing the sample (Longhurst and Siebert 1967). The number of aliquot levels required to reduce the size of large volume samples contributes to the non-randomness (Miller, 1975). Within our experiment we observed non-randomness with both high and low volumes. The causes are not entirely clear and need to be examined more fully. Reducing the number of splits from 13 to 11 is sufficient to reduce the variance between split levels. The numbers of copepods actually counted would then be increased from 170 to approximately 370 to reach a level of no significant differences among splits at the P.05 level. Other taxa which are present in fewer numbers are frequently not present in smaller aliquot levels that are at an acceptable probability level for copepods. It will be necessary, therefore to enumerate other taxa from larger aliquots. A series of spring and summer samples will be examined to determine optimal aliquot levels for other taxa.

References

Longhurst, A.R. and D.L.R. Seibert. 1967. Skill in the use of Folsom's plankton sample splitter. Limnol. Oceanogr. 12:334-335.

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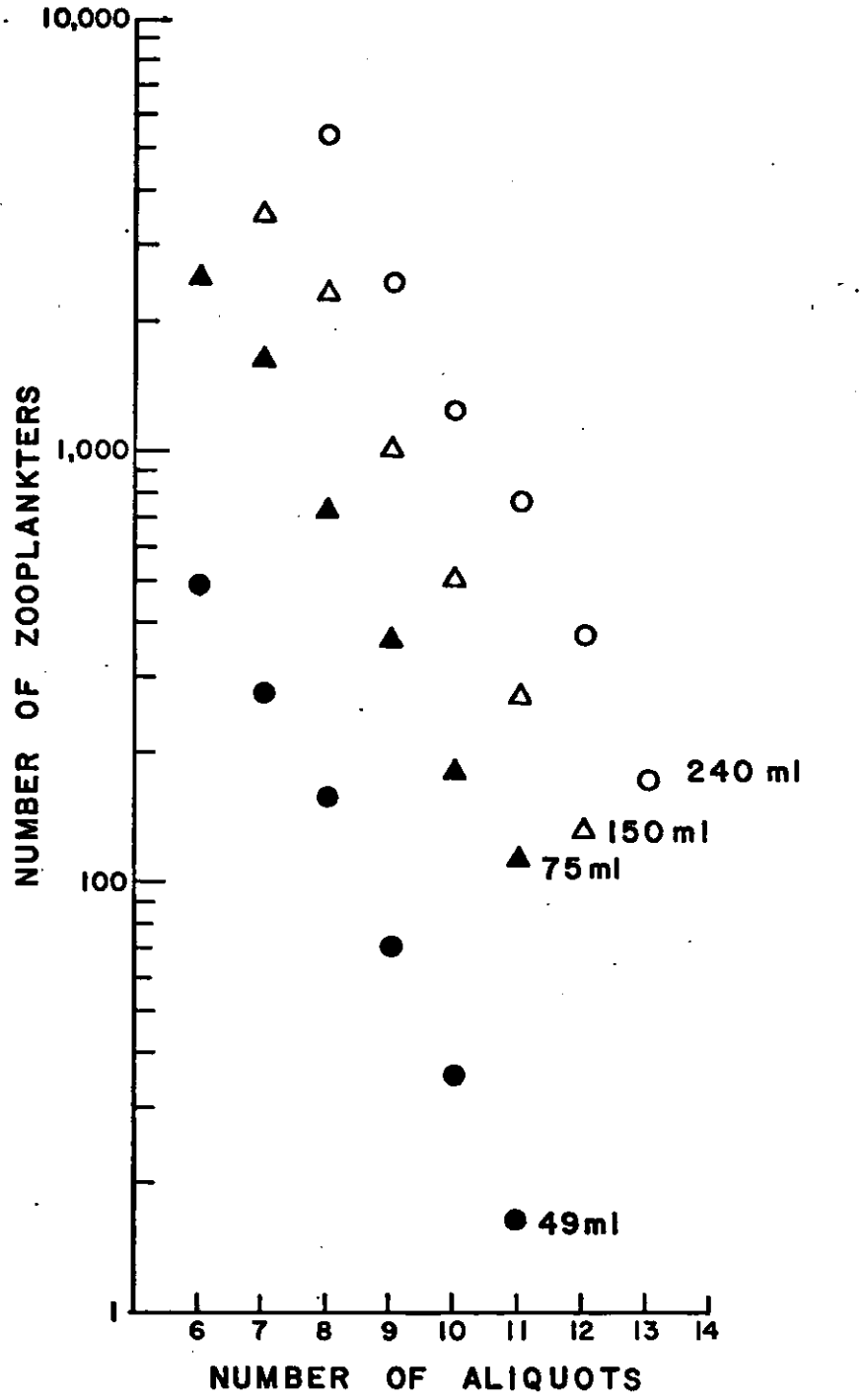

Figure 1. Graphical representation of number of copepods vs. aliquot level for each of four different samples representing the range of zooplankton volumes.