The 1998 - 1999 Split Sample Study for Chesapeake Bay Program Phytoplankton, Microzooplankton and Mesozooplankton Monitoring Components

June 8, 2000

Prepared by

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Executive Summary

The Chesapeake Bay Program (CBP) Monitoring Program has included plankton components since it began in 1984, but it has never carried out quality assurance comparisons of the laboratory methods employed in each jurisdiction. These comparisons are needed to confirm that the various plankton laboratories are producing high quality data useful to CBP managers. A split sample study was done in 1998 and 1999 to compare the Maryland and Virginia monitoring results for phytoplankton, microzooplankton and mesozooplankton. The study indicated generally good comparability between the phytoplankton monitoring programs while pointing out several important differences in the programs' abilities to identify and enumerate certain small cells with precision. Discrepancies were known to occur in the microzooplankton data because sample collections were limited to the >44 micron size fraction at some Maryland stations and the laboratories used different procedures. The Split Sample Study confirmed that laboratory counts for two important microzooplankton categories are comparable, indicated where analysis adjustments were need to make the third important category directly comparable, and reiterated the need for genus-species enumerations in one laboratory and the need for enhanced sample collection and analysis in the other laboratory. The phytoplankton and microzooplankton programs as they presently exist should be able to meet most of the management information needs for phyto- and microzooplankton listed in the Chesapeake Bay Basin-wide Monitoring Strategy (Draft 1999)

State managers and program staff were aware that laboratory method differences, implemented at the start of the Maryland and Virginia monitoring programs, were probably affecting the mesozooplankton monitoring results. While the existing monitoring data provide meaningful status and trend assessments *within* each state, the monitoring programs recognized the growing CBP information needs for mesozooplankton data that are comparable *bay-wide*. The programs modified their laboratory methods in 1998 in order to better estimate species richness in Maryland and eliminate laboratory sieving losses of smaller mesozooplankton taxa and life stages in Virginia. The 1998 - 1999 Split Sample Study indicates the desired outcomes of the modifications were only partially accomplished. A single method needs to be selected and implemented because the modified laboratory methods of the two programs do not produce comparable results. A single method will allow the programs to calculate and use a diverse suite of bay-wide mesozooplankton indicators and more effectively address the information needs of the Program.

Plankton indicators are proving to be useful tools in measuring overall ecosystem health, targeting restoration efforts in open water habitats, and tracking food web responses to management actions such as nutrient and sediment reductions. Ongoing data analyses indicate all of the plankton monitoring programs, including the mesozooplankton, can presently provide the monitoring data required to calculate many important plankton indicators. The monitoring data are able to distinctly characterize the various segments of Chesapeake Bay and its tidal tributaries. They are being used to confirm and track strong plankton linkages to water quality and other living resources. They appear to be sensitive to ecosystem change in tidal waters. Program improvements stemming from the Split Sample Study should serve to further enhance the existing usefulness of the plankton data.

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Introduction

Zooplankton Method Modifications Made Prior to the Split Sample Study After long-running discussions and several meetings, the Maryland and Virginia mesozooplankton laboratories agreed to modify their current laboratory methods in order to resolve the suspected discrepancies and improve data comparability. For mesozooplankton, the Virginia laboratory (Old Dominion University) continued to use its customary Controlled Variability Sampling (CVS) apparatus to obtain an "old method count" and added a 72 micron mesh sieve at the bottom of the CVS apparatus to capture smaller-sized mesozooplankton taxa. A "new method count" would be obtained by combining enumerations from the old method and the 72 micron sieve. The Maryland laboratory (Versar, Inc.) continued to use its usual subsample counting method and obtain an "old method count" for mesozooplankton. The laboratory then filtered the whole sample through a large-size screen to concentrate and enumerate the rarer, large-sized individuals. Versar obtained a "new method count" by combining enumerations from the old method and the large size sieve. The advantage of using the original method and producing "old" and "new" method counts is that–if the modifications both worked--future data would be both backward compatible with pre-1998 data within each state as well as directly comparable between states in the future.

For microzooplankton, the monitoring staffs agreed that additions to Maryland's sample collection method and modifications to Virginia's sampling counting method could make Maryland and Virginia results directly comparable. The Maryland laboratory (Academy of Natural Sciences) was at that time pumping water through a 44 micron net in the field to concentrate and collect a >44 micron sample fraction. This method gives good abundance measurements for rotifers and copepod nauplii - taxa most important to higher trophic levels. In the spring of 1998, ANS began collecting an additional, a whole water sample and expanded its laboratory analyses to count protozoans - the taxa which best reflect the extent of the microbial loop and the impacts of eutrophication. The net sample count and the whole water sample count, combined, was to become the "new ANS method count" for the Maryland microzooplankton program. *Abundances* calculated with the "new ANS method" were expected to be directly comparable to those of the existing Virginia microzooplankton program. The Virginia laboratory, Old Dominion University, agreed it could improve the level of taxonomy of its counts with some staff training. This improvement would make the state programs' indices of *diversity* directly comparable, as well.

Phytoplankton counting protocols in Maryland and Virginia are very similar and probably produce directly comparable data. However, this had never been confirmed with split sampling and both laboratories were interested in documenting the data's comparability. The one known discrepancy between the two programs was the fact that the Virginia program includes a picoplankton component (very small phytoplankton) whereas Maryland's program does not. The close linkages between picoplankton, bacteria and nutrients makes this component a very good one to monitor for early ecosystem responses to nutrient reductions.

1998 - 1999 Split Sample Study A split sampling study was done in the spring and summer of 1998 to compare results of the Maryland and Virginia plankton monitoring programs. The Virginia laboratory (Old Dominion University (ODU)) and Maryland laboratories (Versar, Inc.; Academy of Natural Sciences Estuarine Research Center (ANS)) used 24 phytoplankton, 12 microzooplankton and 24 mesozooplankton samples collected in April, May and June, 1998, during the regular monitoring cruises. The preserved samples were split in half. One split was be enumerated by the originating laboratory as part of its monitoring program, and the other was enumerated by the corresponding lab in the other state. The sites investigated included locations the length of the Bay, having a range of salinities, with exposure to different river basins and environmental conditions. The river sites also varied considerably regarding salinity regions, local ecological factors, and biota.

Two counts were produced by Versar and ODU for each Maryland and Virginia mesozooplankton split sample: one count generated with the laboratory's old method and one generated with their modified method. Specifically, Versar produced a count with its original method and a count which *included* enumerations of mesozooplankton caught on the added large-size sieve. ODU produced a count with its original CVS method and a count which *included* enumerations of mesozooplankton caught on the added 72 micron sieve.

The ANS collected whole water microzooplankton samples for the split sample study[1] and sent 12 splits to ODU. It counted the corresponding splits with its "new ANS method" (see above). The laboratories enumerated all microzooplankton taxa their customary taxonomic levels, and produced one count for each split sample. After the split samples had been counted, ODU staff Alicia LoGalbo traveled to ANS for 4 days and worked with ANS staff Stella Sellner to improve the level of taxonomy in the ODU counts and ensure comparable species identifications.

The ANS and ODU laboratories used their standard counting protocols to produce one count for each Maryland and Virginia phytoplankton split sample. Preserved water samples (1 liter) were thoroughly mixed and divided into equal splits (500 ml each). One split was analyzed by each laboratory. In addition to identifying areas of mutual strength, the split sampling effort also benefitted the program by identifying algal categories that needed more attention.

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[1] Because ANS's *collection* method differed from ODU, ODU would have to collect an additional, net (>44 micron) sample for ANS in order to create a real split sample. This was judged too much effort, so ODU and ANS only performed split samples on whole water samples collected in Maryland.

All split sample enumerations and the data analyses performed on them to-date were discussed at a "Plankton Summit" held on September 11-12, 1998 at Old Dominion University. Further analyses were done and additional meeting convened after the September 1998 Plankton Summit. The results and conclusions of the Plankton Split Sample Study are described in detail in the following three chapters.

A REVIEW OF SPLIT SAMPLE RESULTS REGARDING PHYTOPLANKTON COMPOSITION AND ABUNDANCE IN SAMPLES EXAMINED BY OLD DOMINION UNIVERSITY AND THE ACADEMY OF NATURAL SCIENCE ESTUARINE RESEARCH CENTER

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August 2000*

*This report replaces, with editing and content changes of a January/February 1999 copy previously released.

Participants and Purpose:

David Seaborn and Harold Marshall, Old Dominion University (ODU).
Richard Lacouture and Ann Marie Hartsig, Academy of Natural Science Estuarine Research Center (ANS).

The above participants met at the Phytoplankton Analysis Laboratory at Old Dominion University, Norfolk, Virginia on November 12, 1998. Both ANS and ODU representatives provided water samples that were previously examined in the Split Sampling study by the two laboratories. Sub-samples from these were then prepared for microscope analysis. Samples selected were those where differences in cell counts had been identified in the study. Side by side examinations of water samples by the participants were conducted. Results of the re-examination of these samples by those assembled are given below.

Differences Associated with Different Magnification Effects.

1. The identification of species above the cell size of 8 microns showed only minor taxonomic problems between the two laboratories. Little disagreement involving species categories or species identification was present. Identification questions were centered on only a few very small taxa (see #2 following).

RESOLUTION: None needed. The two laboratories will continue to work together on questions in the interpretation of species taxonomy in the future, as they have in the past.

2. Cells less than 8 microns in size. Several samples indicated the presence of 1 or 2 small algae, less than 6 microns in size, that were given different interpretations regarding their identity as either a diatom, a green cell (*Chlorella* sp.), or a cell placed in a general unidentified category of cells 3-5 microns in size. Microscopic analysis indicated some of these cells could be included in either one of these categories.

RESOLUTION: Differences in making calls of this type, of a very small cell with so few taxonomic features with light microscopy, is not uncommon. In an effort to resolve this particular question, ODU can conduct examinations of samples containing these cells with scanning electron microscopy which would clarify these identifications.

However, it should be noted that the present monitoring program does not support SEM analysis of cells within these small size categories, or where questionable identifications may be present. This is one reason a certain amount of lumping of cells into broader groups is often used for different levels of taxonomy, if essential identification characteristics are not discernable with light microscopy. In most cases, this lumping is found in cells belonging to one taxonomic category (e.g. pennate diatoms <10 microns in size), but it may also occur in mixed taxonomic categories (as in B-2 described below).

There are restraints that are imposed on monitoring phytoplankton populations as to the degree of species identification that can be expected. It should be understood that not every species can be identified using light microscopy alone.

COMMENT: There are differences in the initial amount of the water sample analyzed by the two laboratories and differences in the counting techniques between ODU and ANS regarding what magnifications are used. Both ODU and ANS identify taxa at 312x and 500x magnifications. In addition, ODU scans the entire sample at a lower magnification (125x) for species that were not noted at the other levels. The approaches vary in that ANS uses 500X as its primary magnification, while ODU uses 312X for the magnification containing the majority of species for its counting protocol. The combination of different sub-samples used in the analysis, the emphasis placed on the different magnifications, plus the additional lower magnification used by ODU will offer some bias between the results produced by the two laboratories. In spite of these differences, the two programs have mutual goals and overall a similar basis for species identification. There are also similar approaches used in the "lumping" of cells, within many of the specific taxonomic categories (e.g. pennate diatoms < 10 microns). Both of these laboratory approaches are well established in both programs, each with an extensive historical data base.

Taxonomic Evaluations

1. There is a difference in the nomenclature used by the two laboratories for species within the cyanobacteria genera *Merismopedia (ODU)* and *Agmenellum* (ANS). These genera are considered synonymous.

RESOLUTION: To be discussed within the two laboratoriers. Either one genus should be selected for use, or the taxonomic code numbers for similar species should be matched.

COMMENT: Both of the type species and genera for these two designations were established the same year (1839). The genus *Merismopedia* is used by Geitler (1932), Desikachary (1959), and in the revision of the cyanobacteria by Komarek and Anagnostidis (1986). We recommend this usage also.

2. The inclusion (lumping) of more than one generic group in the "small microflagellates" category was discussed. ANS counts all small flagellated cells noted within this size category, whereas, ODU will include small unidentifiable flagellated cells only if they contain an autotrophic (phytoplankton) characteristic (e.g. scales such as in coccolithophores, stained plastids). These differences result in higher counts in this category from ANS. The question raised is the lack of comparability in the counts in this group by the two labs, because past ANS records of this group would (may) include both heterotrophic and autotrophic cells.

Another factor in the discrepancy of microflagellates in the split samples counted by the two laboratories, is the inclusion of six different phyla and six taxa within the Chlorophyta in the microflagellate category by ANS. In contrast, ODU only includes two taxa into this category, placing other taxa included by ANS in specific phylogenetic categories, thereby creating a discrepancy simply based upon the different definitions of 'microflagellates' coined by the two laboratories.

RESOLUTION: Both ANS and ODU agree this category should not be included in the counts for the Bay Monitoring Program analysis for the Baywide indicators. However, both ODU and ANS will continue using their individual protocols for recording cells in this category.

3. *Microcystis* and the autotrophic picoplankton cell counts were discussed. The majority of the picoplankton cells are also cyanobacteria. Cells within these groups may be similar in appearance. Differences occur in many of the samples where Maryland's *Microcystis* cell counts are higher than ODU. During side by side comparisons of *Microcystis* colonies by personnel from the two labs, there were no differences in their identification. A possible variable in these counts is that ODU records the concentrations of the autotrophic picoplankton cells under a separate classification listing. These include clutches of cells that may not be identified as *Microcystis* by ODU under that category. ANS indicates they count small bluegreen spheres as *Microcystis* only when there is a colonial assemblage of cells. Both labs have the opinion that they have been calling the *Microcystis* colonies in the same way.

RESOLUTION: The laboratories concur on how they identify *Microcystis*. However, in the presence of these past differences in cell counts for *Microcystis*, it is not recommended to use cyanophyte densities as a Baywide indicator.

4. Maryland category #221 Blue Green Trichomes. The split samples indicated high concentrations of these trichomes reported by ANS in the Maryland samples, but that they were not reported by ODU in their examination of these samples. The original split water samples in which these were reported by ANS were re-examined at this time. These cells were not found in either the original Maryland or the ODU sample sets.

Comment: ANS believes there are optical resolution differences in the microscopes used by ODU and ANS that would explain the differences in counts of the thin filamentous cyanobacteria (1-2 um) and the interpretation differences in the identification of the small diatoms or chlorophyte cells. However, at ODU, in the search for these filamentous cells, 3 different Zeiss inverted plankton microscopes were used with the same negative results, with one microscope having higher magnification capabilities than that used at ANS.

RESOLUTION: Unresolved at this time, but further cell comparisons in this category are recommended. ANS indicated when these cells are noted again, they will provide samples to ODU. In addition, ANS has also invited an ODU representative to their lab to examine these at their facilities using their microscopes. (See Addendum)

Cell Count Differences Associated With Laboratory Protocols

1. Counts associated with cyanobacteria trichomes. ANS provides total cell counts for the cells in a cyanobacterium trichome. ODU records each trichome as 1 trichome, without cell number. This produces higher cell counts for the filamentous cyanobacteria in the Maryland data. For instance, this value may represent 35-40 cells per trichome for a particular cyanophyte, and be reported as such by Maryland, whereas, Virginia would record this as a single unit (trichome).

RESOLUTION: ANS will indicate the mean cell counts per trichome they have used for the filamentous cyanobacteria to ODU (Michael Lane, AMRL) . These cell values may be used to revise the past ODU Bay data set records for these species, and be used in future data entry by ODU.

2. Species Diversity. A comparison of the split samples indicated ODU includes a greater amount of species identified (44%) than ANS. Within the split samples analyzed, the range of

taxa identified was 10 to 47 for ANS, and 20 to 68 for ODU. There are two differences in the protocols used. One is in the sub-samples taken by the 2 labs to analyze, and the other is that ODU uses a 3rd level of lower magnification to scan the field for cells that are not included in the other magnification counts. There is an inherent difference between taxonomists in the degree of comfort that is felt in classifying organisms to the genus and species level. This variability occurs within some laboratories and between labs and could account for a certain degree of the differences within this parameter, in addition to the different protocols that are used by the two laboratories.

RESOLUTION: A third level of magnification (125x magnification scan) may increase the number of species recorded in the ANS analysis.

3. Autotrophic picoplankton analysis. This category represents one of the most important components and indicators of water quality in the Chesapeake Bay plankton community. Virginia has a long term data set for this category, yet it is lacking in the Maryland program. The incorporation of this component in the ANS analysis data set would be a valuable asset in the interpretation of health status and trends in the Bay estuarine system.

RESOLUTION: It is recommended that the analysis for the autotrophic picoplankton component be included in the Maryland plankton monitoring program.

Taxa Where Counts And Identifications Are Comparable For Indicator Purposes

Comparable results were found among the following taxonomic categories in both laboratories and which can be used for Chesapeake Bay-wide indicator purposes:

Diatom biomass
Dinoflagellate biomass
Chlorophyll a
Productivity

This does not mean the other taxonomic categories identified by both laboratories are not comparable, only that these categories mentioned above are considered to be the most useful in the development of a phytoplankton indicator system. ODU and ANS will examine the analysis results provided by this set in each of the salinity regimes, and make decisions if additional categories would be necessary.

Taxon Categories Not Considered Comparable Or Useful For Indicator Purposes

The following categories are not considered comparable for Bay wide analysis purposes:

Autotrophic Picoplankton *
Small microflagellates **
Cyanobacteria biomass***
Cyanobacteria cell concentrations***

* conducted only in Virginia
** different protocols used by the 2 labs
***After changes are made regarding cell counts/trichomes in the ODU data set.

Conclusions and Summary

1. The joint examination of the previously collected split samples took place by representatives of the two laboratories. Side by side comparisons were made of various taxa and their identifications.

2. Although there were a few differences in several calls of the very small taxa, there were suggestions as to how these differences would be resolved in future analyses by the two laboratories. There were no major differences noted in any of the other taxonomic categories examined. For instance, there were very close comparisons within the samples for diatoms and dinoflagellates.

3. Based on our discussions and the review of the data sets, ODU and ANS have made recommendations as to which components within the phytoplankton data set would be most suitable, and comparable across the Bay, for incorporation in the bio-indicator analysis program, in addition to those we do not recommend.

4. In addition, in order to provide closer, and continued agreement in phytoplankton identification between the two laboratories, it is recommended that: 1. Future discourse on matters of species identification between the two laboratories (ODU and ANS) be incorporated as an annual component of the Bay Monitoring Program, and this would include regular visitations by personnel to both laboratories; and 2. When needed, additional SEM analysis, or other protocols be incorporated to clarify any questions regarding the identification of major species within the Bay ecosystem.

5. The two laboratories (ODU and ANS) express their appreciation for the support of this project. The project was a worthwhile activity and the results of this interaction will enhance conformity in the analysis of the phytoplankton community within the Bay ecosystem.

ADDENDUM*

August 2000

*This addendum replaces, with editing and content changes, a previously modified release of the original February 1999 report.

Introduction

At the conclusion of the initial review of the split sample analysis between the two laboratories in November 1998, it was recommended by the participants that Old Dominion University (ODU) representatives meet at the Academy of Natural Sciences (ANS) to continue this review process. Scanning electron microscopy (SEM) examination was also suggested.

On February 4, 1999, the two ODU representatives traveled to the ANS laboratory to work with the ANS representatives to continue the split samples analysis. ODU also brought with them one of their laboratory microscopes.

Participants

David Seaborn and Harold Marshall, Old Dominion University

Richard Lacouture and Anne-Marie Hartsig, Academy of Natural Sciences.

Specific questions to be resolved were as follows:

Item 1. Clarify the status of the small size cells less than 6 microns in size. It was suggested that these cells be examined with SEM, and with the samples and microscopes at the ANS laboratory.

Item 2. Can the small #221 blue green (cyanobacteria) trichome category be identified with microscopes used in the different laboratories. Is there an optical resolution problem to be considered.

Results

Item 1. ODU conducted SEM analysis of the plankton samples originally examined in this study. The SEM micrographs indicated the size and occurrence of small centric diatoms, with cell diameters of 4 to 5 microns, and the presence of spherical, soft-bodied cells approximately 2 to 3 microns in size. These results indicated the presence of two categories of cells in the samples. The smaller soft-bodied cell could be classified as either a chlorophyte (e.g. *Chlorella* sp.) or placed in a size category of cells. Distinctions between these two groups were reviewed at this time with light microscopy.

Item 2. These blue green (cyanobacteria) trichomes were observed and identified with microscopes from both laboratories. Optical resolution using the different microscopes was not

an issue. The characteristics of these cells were reviewed and both groups agreed these cells should continue to be in the blue green trichome category. Its species identification will require further study. However, there was concern expressed by ANS that these trichomes were difficult to discern with the ODU microscope and that this may have been a factor in why the trichomes were not present as sub-dominants in the examination of samples at ODU (and were a common sub-dominant in the ANS analysis), and accounted for the discrepancies noted in comparing the split sample results for these blue green trichomes between the two labs. In response, the ODU laboratory staff has been made aware of the concern by ANS in regard to the differences in the counts of these blue green trichomes between our two labs, and although not finding any inaccuracies in their previous counts of this taxon, will take special attention in the future counts of this trichome . This is a positive and cooperative response exhibited by the laboratories to address either separate or mutual concerns by the laboratories regarding findings concerning taxon identification, or abundance, etc.

Further Activities

1. The ODU and ANS participants believe this past experience was very worthwhile and we plan to continue sample review and exchange practice this summer (1999). We will compare at least one set of water samples for phytoplankton at two mainstem stations, CB5.2 and CB6.1.

2. The two laboratories will continue to work closely on any future events related to the phytoplankton dynamics in the Bay, in addition to questions of species identifications, etc.

Recommendations

1. ODU and ANS recommend the continuation and financial support of future annual exchange visits by the laboratory participants to both the ODU and ANS phytoplankton laboratories.

2. ODU and ANS recommend further discussions between the two laboratories are essential regarding a continuous dialogue regarding species identifications, factors associated with algal bloom events, the presence of exotic and potentially toxic species in Chesapeake Bay, and ways in which our combined data sets and specific taxon groups will have broader application to the goals of the Chesapeake Bay Monitoring Program.

Analysis Protocols Followed by the Two Laboratories

I. Academy of Natural Science Estuarine Research Center

An appropriate subsample (generally 1 -10 ml) is pipetted from the 500 ml sample and placed in a one or two piece settling chamber (depending on the volume; 1-2 ml in a one piece chamber, > 2 ml in a two piece chamber). This subsample is allowed to settle for an appropriate amount of time (2-48 hrs.) If necessary, the upper settling column is slid from the bottom plate and placed on the microscope. The sample is initially analyzed at 500X, whereby > 200 individual cells are enumerated in at least 20 'randomly' selected fields. Additional fields are inspected, if necessary, until a minimum of 200 cells have been counted. Upon completion of this magnification, 20 random fields are inspected at 312.5X and any taxa not enumerated at 500X are done so at this lower magnification.

II. Old Dominion University Phytoplankton Analysis Lab

Two composite replicate 500 ml samples fixed in Lugol's solution are mixed (1000 ml) and a 500 ml sub-sample is obtained and preserved with buffered formalin. A procedure of settling (72 hours) followed by siphoning is repeated 3 times to reduce the original volume and its contents to a 40 ml concentrate of the original 500 ml sub-sample. A known volume of this concentrated 500 ml sample (e.g. 1.25, 2.5, etc. ml; determined by concentration of phytoplankton and silt) is transferred to an Utermöhl settling chamber and allowed to settle for 24-48 hours. At 312X magnification, a combined examination of at least10 random fields plus a minimum cell count of 200 is followed. If cell counts do not reach 200 cells from 10 random fields, additional fields are counted until that number is reached. The species counts are continued at 500X magnification for 10 additional fields. Species counted at this magnification are those not counted at 312X. The entire field of the counting chamber is then examined at 125X magnification for other species not counted with the other 2 magnifications.

Appendix: Summary of results of the phytoplankton split sample comparison between Old Dominion University and the Academy of Natural Sciences

Summary of results of the phytoplankton split sample comparison between Old Dominion University and the Academy of Natural Sciences

Elgin Perry

Using the dataset PHYTSUM.SD2 prepared by Jackie, the nodc codes that appeared to be used consistently between laboratories were selected. There are:

```
if nodccode = '03'      Blue Greens
or nodccode = '0701'    Diatoms
or nodccode = '1201'    Dinoflagellates
or nodccode = '0801';   Greens
```

The data from the two labs were then merged by date,station, layer, and nodccode. If a density for a taxanomic group appeared for on Lab and not the other, the Lab which did not have a density for that group was assigned a density of zero.

After matching the records, the difference between labs was computed as the density for ANS minus the density for ODU.

```
difdens = ansdens - odudens;
```

The percent difference is computed as the difference between the labs divided by the mean of the labs and scaled to percent.

```
difpct = 200 * difdens / (ansdens+odudens);
```

In addition, in an effort to achieve distributional properties more like the normal distribution, a difference variable was also computed in a logarithm metric.

```
lnoduden = log10(odudens+1);
lnansden = log10(ansdens+1);
lndifden = lnansden - lnoduden;
```

To compare the labs, this difference variable was subjected to the following statistical tests:

1. Shapiro-Wilks test for normality,
2. Paired t-test, and
3. Wilcoxon signed-rank test.

When it appeared that the normality assumption required by the paired t-test was not met, the results of the signed-rank test

are reported.

Other summary statistics as shown in the results were also computed.

Because problems remain in the data - I've not spent any time on interpretation. The departure from normality in these data is due to heavy tailed distributions in the difference scores which I think will diminish when the mismatching due to layer is fixed.

Results:

TAXA=Blue Greens

OBS	DATE	STATION	NODC	LAYER	ANSDENS	ODUDENS	DIFDENS	DIFPCT
1	05/01/97	TF4.2	03	BP	566499	0	566499	200.000
2	04/06/98	CB6.4	03	BP	5533248	256	5532992	199.981
3	04/06/98	CB7.4	03	BP	4775720	24902	4750818	197.925
4	04/10/98	RET4.3	03	AP	368883	658048	-289165	-56.316
5	04/13/98	TF5.5	03	AP	396128	262400	133728	40.614
6	04/20/98	MLE2.2	03		0	54784	-54784	-200.000
7	04/20/98	MLE2.2	03	AP	5268107	0	5268107	200.000
8	04/21/98	MCB4.3C	03		0	424576	-424576	-200.000
9	04/21/98	MCB4.3C	03	AP	483360	0	483360	200.000
10	04/21/98	MWT5.1	03	AP	2915360	0	2915360	200.000
11	04/27/98	XDE5339	03	AP	5370400	128	5370272	199.990
12	05/01/98	RET3.1	03	BP	0	0	0	.
13	05/06/98	RET3.1	03	BP	0	12962120	-12962120	-200.000
14	05/06/98	TF4.2	03	BP	0	1757184	-1757184	-200.000
15	05/08/98	LE3.6	03	AP	31453880	0	31453880	200.000
16	05/18/98	CB6.1	03	AP	9749056	2367780	7381276	121.835
17	05/18/98	LE3.6	03	AP	0	507648	-507648	-200.000
18	05/19/98	MCB3.3C	03		0	315392	-315392	-200.000
19	05/19/98	MCB3.3C	03	AP	1994720	0	1994720	200.000
20	05/19/98	MET5.2	03		0	2304	-2304	-200.000
21	05/19/98	MET5.2	03	AP	3426827	0	3426827	200.000
22	05/19/98	MWT5.1	03	AP	147916160	768	147915392	199.998
23	05/26/98	PXT0402	03		0	740352	-740352	-200.000
24	05/26/98	PXT0402	03	AP	14730240	0	14730240	200.000
25	06/01/98	MCB5.2	03		0	232832	-232832	-200.000
26	06/01/98	MCB5.2	03	AP	4040587	0	4040587	200.000
27	06/01/98	WE4.2	03	BP	5269760	27520	5242240	197.922
28	06/01/98	XEA6596	03		0	11374848	-11374848	-200.000
29	06/01/98	XEA6596	03	AP	60915680	0	60915680	200.000
30	06/03/98	CB7.3	03	AP	0	1152	-1152	-200.000
31	06/03/98	CB7.3C	03	AP	1152760	0	1152760	200.000
32	06/08/98	XDE5339	03	AP	1315200	640	1314560	199.805
33	06/08/98	XED4892	03		0	113920	-113920	-200.000
34	06/08/98	XED4892	03	WC	613760	0	613760	200.000
35	06/23/98	RET5.2	03	AP	145659460	58754560	86904900	85.028
36	06/25/98	SBE5	03	BP	3557088	378096	3178992	161.568

TAXA=Diatoms

OBS	DATE	STATION	NODC	LAYER	ANSDENS	ODUDENS	DIFDENS	DIFPCT
37	05/01/97	TF4.2	0701	BP	424699	0	424699	200.000
38	04/06/98	CB6.4	0701	BP	3428462	6445834	-3017372	-61.116
39	04/06/98	CB7.4	0701	BP	1965050	2117190	-152140	-7.454
40	04/10/98	RET4.3	0701	AP	1744010	1297536	446474	29.358
41	04/13/98	TF5.5	0701	AP	6815530	6250752	564778	8.645
42	04/20/98	MLE2.2	0701		0	2642176	-2642176	-200.000
43	04/20/98	MLE2.2	0701	AP	24775235	0	24775235	200.000
44	04/21/98	MCB4.3C	0701		0	1974528	-1974528	-200.000
45	04/21/98	MCB4.3C	0701	AP	41505520	0	41505520	200.000
46	04/21/98	MWT5.1	0701	AP	10664080	880384	9783696	169.496
47	04/27/98	XDE5339	0701	AP	79665732	1649792	78015940	191.884
48	05/01/98	RET3.1	0701	BP	42305020	0	42305020	200.000
49	05/06/98	RET3.1	0701	BP	0	36889147	-36889147	-200.000
50	05/06/98	TF4.2	0701	BP	0	769664	-769664	-200.000
51	05/08/98	LE3.6	0701	AP	10097709	0	10097709	200.000
52	05/18/98	CB6.1	0701	AP	5536366	4745800	790566	15.377
53	05/18/98	LE3.6	0701	AP	0	3194112	-3194112	-200.000
54	05/19/98	MCB3.3C	0701		0	4866432	-4866432	-200.000
55	05/19/98	MCB3.3C	0701	AP	11891600	0	11891600	200.000
56	05/19/98	MET5.2	0701		0	1137152	-1137152	-200.000
57	05/19/98	MET5.2	0701	AP	25982507	0	25982507	200.000
58	05/19/98	MWT5.1	0701	AP	24611145	10274048	14337097	82.196
59	05/26/98	PXT0402	0701		0	4813568	-4813568	-200.000
60	05/26/98	PXT0402	0701	AP	23466855	0	23466855	200.000
61	06/01/98	MCB5.2	0701		0	8043648	-8043648	-200.000
62	06/01/98	MCB5.2	0701	AP	9769013	0	9769013	200.000
63	06/01/98	WE4.2	0701	BP	7062335	6910720	151615	2.170
64	06/01/98	XEA6596	0701		0	13241984	-13241984	-200.000
65	06/01/98	XEA6596	0701	AP	20589755	0	20589755	200.000
66	06/03/98	CB7.3	0701	AP	0	9192594	-9192594	-200.000
67	06/03/98	CB7.3C	0701	AP	5707282	0	5707282	200.000
68	06/08/98	XDE5339	0701	AP	5699200	1343488	4355712	123.695
69	06/08/98	XED4892	0701		0	4616960	-4616960	-200.000
70	06/08/98	XED4892	0701	WC	14474507	0	14474507	200.000
71	06/23/98	RET5.2	0701	AP	7001140	13938688	-6937548	-66.262
72	06/25/98	SBE5	0701	BP	2454180	2742214	-288034	-11.086

TAXA=Dinoflagellates

OBS	DATE	STATION	NODC	LAYER	ANSDENS	ODUDENS	DIFDENS	DIFPCT
73	05/01/97	TF4.2	1201	BP	0	0	0	.
74	04/06/98	CB6.4	1201	BP	236788	291403	-54615	-20.680
75	04/06/98	CB7.4	1201	BP	96586	28102	68484	109.849
76	04/10/98	RET4.3	1201	AP	0	41216	-41216	-200.000
77	04/13/98	TF5.5	1201	AP	0	0	0	.
78	04/20/98	MLE2.2	1201		0	208384	-208384	-200.000
79	04/20/98	MLE2.2	1201	AP	1380960	0	1380960	200.000
80	04/21/98	MCB4.3C	1201		0	140416	-140416	-200.000
81	04/21/98	MCB4.3C	1201	AP	460320	0	460320	200.000
82	04/21/98	MWT5.1	1201	AP	76720	27648	49072	94.036
83	04/27/98	XDE5339	1201	AP	4173252	1685504	2487748	84.924
84	05/01/98	RET3.1	1201	BP	0	0	0	.
85	05/06/98	TF4.2	1201	BP	0	128	-128	-200.000
86	05/08/98	LE3.6	1201	AP	1163058	0	1163058	200.000
87	05/18/98	CB6.1	1201	AP	433078	877716	-444638	-67.843
88	05/18/98	LE3.6	1201	AP	0	919168	-919168	-200.000
89	05/19/98	MCB3.3C	1201		0	55424	-55424	-200.000
90	05/19/98	MCB3.3C	1201	AP	337253	0	337253	200.000
91	05/19/98	MET5.2	1201		0	7724672	-7724672	-200.000
92	05/19/98	MET5.2	1201	AP	7927733	0	7927733	200.000
93	05/19/98	MWT5.1	1201	AP	153440	302592	-149152	-65.413
94	05/26/98	PXT0402	1201	AP	0	0	0	.
95	06/01/98	MCB5.2	1201		0	467072	-467072	-200.000
96	06/01/98	MCB5.2	1201	AP	255733	0	255733	200.000
97	06/01/98	WE4.2	1201	BP	392795	114816	277979	109.524
98	06/01/98	XEA6596	1201		0	15360	-15360	-200.000
99	06/01/98	XEA6596	1201	AP	0	0	0	.
100	06/03/98	CB7.3	1201	AP	0	228453	-228453	-200.000
101	06/03/98	CB7.3C	1201	AP	426842	0	426842	200.000
102	06/08/98	XDE5339	1201	AP	2149090	1851776	297314	14.862
103	06/08/98	XED4892	1201		0	256	-256	-200.000
104	06/08/98	XED4892	1201	WC	0	0	0	.
105	06/23/98	RET5.2	1201	AP	68990	64512	4478	6.709
106	06/25/98	SBE5	1201	BP	60532	48014	12518	23.065

TAXA=Greens

OBS	DATE	STATION	NODC	LAYER	ANSDENS	ODUDENS	DIFDENS	DIFPCT
107	05/01/97	TF4.2	0801	BP	92221	0	92221	200.000
108	04/06/98	CB6.4	0801	BP	32936	126924	-93988	-117.588
109	04/06/98	CB7.4	0801	BP	1600962	84415	1516547	179.965
110	04/10/98	RET4.3	0801	AP	161834	213171	-51337	-27.379
111	04/13/98	TF5.5	0801	AP	434404	1055974	-621570	-83.411
112	04/20/98	MLE2.2	0801		0	1740621	-1740621	-200.000
113	04/20/98	MLE2.2	0801	AP	1022933	0	1022933	200.000
114	04/21/98	MCB4.3C	0801		0	4532096	-4532096	-200.000
115	04/21/98	MCB4.3C	0801	AP	460320	0	460320	200.000
116	04/21/98	MWT5.1	0801	AP	2608480	0	2608480	200.000
117	04/27/98	XDE5339	0801	AP	2576530	0	2576530	200.000
118	05/01/98	RET3.1	0801	BP	950270	0	950270	200.000
119	05/06/98	RET3.1	0801	BP	0	3094328	-3094328	-200.000
120	05/06/98	TF4.2	0801	BP	0	345318	-345318	-200.000
121	05/08/98	LE3.6	0801	AP	1646800	0	1646800	200.000
122	05/18/98	CB6.1	0801	AP	32936	7428458	-7395522	-198.234
123	05/18/98	LE3.6	0801	AP	0	1328512	-1328512	-200.000
124	05/19/98	MCB3.3C	0801		0	2131942	-2131942	-200.000
125	05/19/98	MCB3.3C	0801	AP	351650	0	351650	200.000
126	05/19/98	MET5.2	0801		0	2706074	-2706074	-200.000
127	05/19/98	MET5.2	0801	AP	153440	0	153440	200.000
128	05/19/98	MWT5.1	0801	AP	856740	0	856740	200.000
129	05/26/98	PXT0402	0801		0	2163763	-2163763	-200.000
130	05/26/98	PXT0402	0801	AP	1380960	0	1380960	200.000
131	06/01/98	MCB5.2	0801		0	1138253	-1138253	-200.000
132	06/01/98	MCB5.2	0801	AP	409173	0	409173	200.000
133	06/01/98	WE4.2	0801	BP	0	871552	-871552	-200.000
134	06/01/98	XEA6596	0801		0	4557875	-4557875	-200.000
135	06/01/98	XEA6596	0801	AP	2896430	0	2896430	200.000
136	06/03/98	CB7.3	0801	AP	0	169087	-169087	-200.000
137	06/03/98	CB7.3C	0801	AP	0	0	0	.
138	06/08/98	XDE5339	0801	AP	0	0	0	.
139	06/08/98	XED4892	0801		0	1565952	-1565952	-200.000
140	06/08/98	XED4892	0801	WC	1235015	0	1235015	200.000
141	06/23/98	RET5.2	0801	AP	9429140	9750323	-321183	-3.349
142	06/25/98	SBE5	0801	BP	0	831969	-831969	-200.000

TAXA=Blue Greens

Univariate Procedure

Variable=LNDIFDEN

Moments

N	36	Sum Wgts	36
Mean	1.046857	Sum	37.68684
Std Dev	5.166423	Variance	26.69192
Skewness	-0.28234	Kurtosis	-1.51058
T:Mean=0	1.215762	Pr>|T|	0.2322
Sgn Rank	86	Pr>=|S|	0.1620
W:Normal	0.872352	Pr<W	0.0004

Stem	Leaf	#	Boxplot
7	258	3	
6	135567	6	
5	3788	4	+-----+
4	36	2	| |
3	3	1	| |
2	33	2	| |
1	0	1	*--+--*
0	0246	4	| |
-0	3	1	| |
-1			| |
-2			| |
-3	41	2	| |
-4	7	1	+-----+
-5	976541	6	|
-6	2	1	|
-7	11	2	|

----+----+----+----+

TAXA=Diatoms

Univariate Procedure

Variable=LNDIFDEN

Moments

Std Dev	5.720108	Variance	32.71964
CV	2298.605	Std Mean	0.953351
T:Mean=0	0.261028	Pr>|T|	0.7956
Num ^= 0	36	Num > 0	20
M(Sign)	2	Pr>=|M|	0.6177
Sgn Rank	63	Pr>=|S|	0.3292
W:Normal	0.836075	Pr<W	0.0001

Stem	Leaf	#	Boxplot
7	0012344466	10	+-----+
6	8	1	| |
5	6	1	| |
4			| |
3			| |
2			| |
1	17	2	| |
0	001146	6	*--+--*
-0	3300	4	| |
-1			| |
-2			| |
-3			| |
-4			| |
-5	9	1	| |
-6	97775431	8	+-----+
-7	610	3	|

----+----+----+----+

TAXA=Dinoflagellates

Univariate Procedure

Variable=LNDIFDEN

Moments

N	34	Sum Wgts	34
Mean	-0.28434	Sum	-9.66757
Std Dev	3.947704	Variance	15.58437
Skewness	0.233647	Kurtosis	-0.70135
T:Mean=0	-0.41998	Pr>|T|	0.6772
Sgn Rank	-1	Pr>=|S|	0.9823
W:Normal	0.894571	Pr<W	0.0031

Stem	Leaf	#	Boxplot
6	119	3	
5	4567	4	
4			
3			
2			
1			
0	0000000114455	13	+------+
-0	331	3	| + |
-1			| |
-2	41	2	| |
-3			| |
-4	762	3	+------+
-5	7431	4	|
-6	90	2	|

----+----+----+----+

TAXA=Greens

Univariate Procedure

Variable=LNDIFDEN

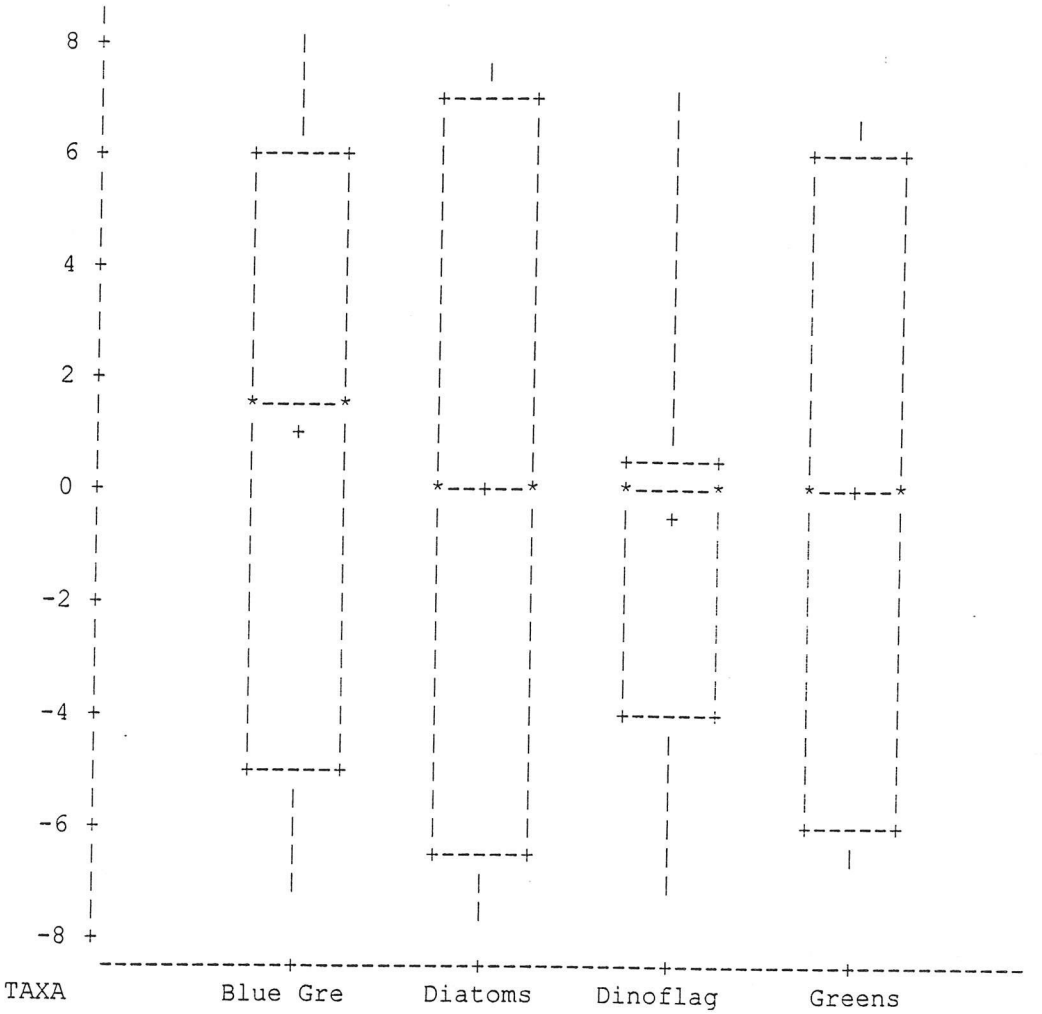
Moments

N	36	Sum Wgts	36
Mean	-0.15819	Sum	-5.69468
Std Dev	5.423099	Variance	29.41001
Skewness	0.019567	Kurtosis	-1.78465
T:Mean=0	-0.17501	Pr>|T|	0.8621
Sgn Rank	-37.5	Pr>=|S|	0.5295
W:Normal	0.797567	Pr<W	0.0001

Stem	Leaf	#	Boxplot
6	00112445	8	
5	025679	6	+------+
4			| |
3			| |
2			| |
1	3	1	| |
0	00	2	| |
-0	6410	4	*--+--*
-1			| |
-2	4	1	| |
-3			| |
-4			| |
-5	9952	4	| |
-6	7754332211	10	+------+

----+----+----+----+

Variable=LNDIFDEN



TAXA=Blue Greens

Variable	N	Mean	PCTDIF
ODUDENS	36	2526728.06	
ANSDENS	36	12707580.08	
DIFDENS	36	10180852.03	138

TAXA=Diatoms

Variable	N	Mean	PCTDIF
ODUDENS	36	4166066.97	
ANSDENS	36	10767692.56	
DIFDENS	36	6601625.58	88

TAXA=Dinoflagellates

Variable	N	Mean	PCTDIF
ODUDENS	34	443900.94	
ANSDENS	34	582152.06	
DIFDENS	34	138251.12	27

TAXA=Greens

Variable	N	Mean	PCTDIF
ODUDENS	36	1273239.08	
ANSDENS	36	787032.61	
DIFDENS	36	-486206.47	-47

ANS/ODU Microzooplankton Split Sampling Meeting Data Review Report

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ANS/ODU Microzooplankton Split Sampling Meeting
Data Review Report

The microzooplankton component of the MD Water Quality Monitoring Program began in 1984. VA added microzooplankton to their program in 1993. At that time, the differences in methodology between the 2 programs were discussed and preliminary data were examined. It was evident from the onset that there were some major differences in sampling and counting techniques. Recently, the importance of compatibility of data from both programs to establish Bay wide indicators has been discussed. It is from these discussions and a prior split sampling meeting that the need to make the programs more comparable has become a priority.

Purpose

The purpose of the meeting was to assemble the microzooplankton taxonomists from both ANSERC and ODU to review the results of the Z score analysis Elgin Perry had run on the original split sample counts, discuss the differences in collection and counting techniques between the MD and VA programs and, using the Z scores, reexamine samples which showed the greatest differences between the 2 labs. Listed below are the concerns, results of discussion, conclusions and recommendations that came from this meeting, held on January 20-22, 1999.

Concerns

1. ODU netting of samples leads to possible breakage of fragile ciliates. ODU did a series of counts comparing whole water and netted samples. From a 2 liter water sample, 50ml of sample were removed for a whole water count. The remaining sample was handled in the usual way with the larger organisms caught on a 73µm mesh net and the water passing through subsampled and a count done for the smaller organisms. The 2 methods compared well for ciliates.
Conclusion- Methods compared well for ciliates which occurred in large numbers but greater discrepancies existed for those organisms found in low numbers. Netting is not a problem once samples have been fixed.

2. Discrepancies in grouping of organisms.
The following is a table which lists the differences between MD and VA in defining various taxonomic groups of microzooplankton:

Group	ODU	ANSERC
Copepod nauplii	all, length <200µm	all
Rotifers	all, length <200µm	all
Sarcodinids	all	all
Tintinnids	all >20µm in width, length doesn't matter	all in mesohaline all > 44 µm in others
Non loric ciliates	all > 20µm in width, less than 200µm in length	all in mesohaline all > 44 µm in others

Barnacle nauplii	all < 200µm in length	none
Polychaete larvae	all < 200µm in length	none
Pelecypod larvae	all < 200µm in length (In other category)	all
Gastropod larvae	all < 200µm in length (In other category)	all
Cladocerans	all < 200µm in length	none

ODU uses the classical definition of microzooplankton being zooplankton 20-200µm in size. ANSERC also counts the non-loricate ciliates and tintinnids that are less than 20µm in size. ANSERC considers barnacle nauplii, polychaete larvae and cladocerans to be mesozooplankton and does not count any organisms within these groups. These organisms are enumerated in the MD mesozooplankton program. ODU size cutoffs for tintinnids and non-loricate ciliates are based on widths while ANSERC's size categories are based on length. Example- A ciliate that is 15µm wide and 60µm long is not counted by ODU. ANSERC counts it and puts it into a size category of 50-99µm in length.
Conclusion- Using current techniques, rotifers and copepod nauplii are the only groups that compare well between the 2 labs. Sarcodinids are too low in numbers to use. Ciliates and tintinnids cannot be used because of differences in counting techniques (based on size).
Recommendation- ODU adopts ANSERC's method of enumerating all ciliates and does not drop any ciliates from counts that are less than 20µm in width.

3. Calculating densities of organisms in subsamples using large multipliers. Both counting techniques use multipliers to convert the number of organisms counted in the subsample (raw count) to the number of organisms per ml (standardized count). Some of the multipliers are quite large and a low number or organisms seen in a subsample may appear to represent a very high density.
Recommendation- Have Elgin review this to determine the error involved in these methods.

4. Differences in magnification used by the 2 labs when examining smaller organisms. When doing whole water counts, ANSERC uses a magnification of 312.5X while ODU uses 200X as their highest magnification for their groups 2 and 3 which are predominately made up of the smallest organisms counted.
Recommendation- For ODU to be able to accurately count the smaller ciliates less than 20µm in width, they begin to use the same magnification as ANSERC.

5. Preservative differences. ODU uses Lugol's which stains darkly and shrinks soft bodied organisms but is necessary to preserve fragile ciliates. ANSERC uses formalin for net samples and Lugol's for whole water samples. Rotifers are easier to identify using formalin than Lugol's. This became apparent in one comparison count in which a ciliate fixed in Lugol's was identified as a rotifer because it's internal structures could not be seen.
Recommendation- ODU look into using a narcotizing agent such as neosynefrin prior to fixation in Lugol's to relax rotifers. The effect of this on fragile ciliates would have to be

carefully evaluated. Cross checking between the 2 labs when there is a questionable organism would eliminate some of the potential identification problems.

6. Degree of identification and method of grouping organisms. ODU doesn't speciate organisms, using only very broad categories. ANSERC takes rotifers and tintinnids to lowest possible level of identification. ANSERC categorizes ciliates based on size and general groupings.
Recommendation- Currently, ODU groups all their non-loricate ciliates as oligotrichs. This is not necessarily accurate as not all non-loricate ciliates are in this group. In their data sets, ODU should not use this group name. It should be changed to non-loricate ciliates.

7. Data dictionaries do not define exactly what is being counted and included in the data sets (such as ciliates>20 µm in width).
Recommendation- Both labs should review and edit their data dictionaries and make them more specific in regards to what is included in the data sets. It should be suggested to Jackie Johnson that somewhere in the documentation that is on the web, the differences between the labs regarding the counting techniques and grouping of the organisms in the counts be specified.

8. Continuation of comparison of counts between the 2 labs. The statistics that Elgin Perry ran on the split samples were invaluable for the comparison of the 2 counting techniques. The split sampling and meetings to compare results also proved to be very helpful in trying to make the monitoring data more comparable.
Recommendation- Split sampling between the labs be done annually and the results be compared with Elgin's guidance. There should also be a continuation of "ongoing technical collaboration" between the labs along with an annual meeting to discuss results. The idea of a formal basic training program for new microzooplankton taxonomists coming into the monitoring program along with the writing of a guide to microzooplankton in the Chesapeake Bay (which could ultimately be put on the web) were also proposed and needs to be discussed further.

Summary of Counting Differences Based on Z Scores and Actual Percent Differences

The following comments and tables summarize statistical and 'arithmetic' comparisons of the microzooplankton split samples. In the table at the end of the discussion, the actual Z scores are reported. A Z score less than -2 or greater than 2 indicates a difference between the labs.
Note- The comparison split samples taken from MWT5.1 in June has been omitted from discussion because of a discrepancy in sampling dates between the replicate samples sent to ODU.

1. Copepod nauplii- Z scores indicated no significant difference between the labs for this group of organisms.

2. Rotifers- Two major disagreements
MCB2.1- This appeared to be a taxonomic problem in which a ciliate was counted as a rotifer because it was difficult to identify after being fixed with Lugol's. When these were removed from ODU counts, the densities were ANSERC=85/liter and ODU=114/liter (rather than 1994 /liter). Need to rerun with corrected data.
MET5.1 in May- Samples examined for id problems and none could be found. There may have been a sampling problem, such as patchiness of the organisms, when the split samples were taken.

3. Tintinnids- Over half the samples had significant differences between the labs. Samples were rechecked for identification and 2 differences became obvious. There is a genus of tintinnid called *Tintinnidium* which is difficult to identify and may have been overlooked in ODU samples. Small tintinnids which are less than 20µm in width would not be counted by ODU, and this probably led to most of the differences between the labs.
Recommendation- When there is a question in identification of a dominant organism, cross checking between labs should be done. ODU should drop their cutoff of 20µm for the width of the tintinnids and include all of these organisms in their counts.

4. Sarcodinids- Sarcodinids usually occur in very low numbers. They can also be extremely difficult to identify in samples with debris. The sample taken at XEA6596 in June was reexamined because of extremely high numbers of sarcodinids found by ODU. When the subsamples were examined, the sarcodinids could not be found.
Recommendation- Sarcodinids should not be included when analyzing results of split sampling.

5. Non-loricate Ciliates- This group had the most discrepancies. Two major differences were found. ODU does not count the non-loricate ciliates less than 20µm in **width**. ANSERC counts all ciliates, grouping them by **length**. As mentioned previously, a ciliate which is 15µm wide by 60µm in length would be counted by ANSERC and not by ODU. There is no way to remove these from the ANSERC counts since they are not grouped by width. Because of drawings made when the count was done, we were able to do this for MET5.1 in May. ANSERC removed the ciliates less than 20µm in width from the final count. The ANSERC density was 1700 (instead of 9800)/liter and the

ODU density was 1800/liter. The second difference was due to the presence of the photosynthetic ciliate *Myrionecta (*or *Mesodinium) rubra* which can occur in very high numbers. ANSERC counts them and puts them in a separate category and ODU excludes them from their data sets. ANSERC only identified the obvious ones that were in side view and put the questionable ones in the ciliate category. ODU didn't report any.
Recommendation-ODU counts all ciliates regardless of size as well *as Myrionecta*. This would allow ciliates to be used as a Baywide indicator.

Overall Recommendation-For current Baywide comparisons, copepod nauplii and rotifers should be used. It is important to revise the counting protocol of the labs so that ciliates can be used as a Baywide indicator in the future.

ANS/ODU Microzooplankton Split Sampling Results

Z Scores

Statistical significance of split samples between ANS and ODU
Values greater than 2 or less than -2 indicate a difference between labs

STATION	MONTH	NAUPLII	ROTIFERS	TINTINNIDS	CILIATES	SARCODIN.
MCB5.2	MARCH	-0.57	-1.79	14.37	2.10	*
MET5.2	MARCH	1.68	-0.58	3.67	-1.86	0.36
MCB5.2	APRIL	0.69	-0.81	-6.06	-1.09	0.81
MET5.1	APRIL	-0.77	-1.44	1.20	-4.05	1.63
MCB2.1	MAY	1.89	-13.34	-1.10	27.87	*
MCB3.3C	MAY	-0.15	-1.82	8.66	36.60	*
MET5.1	MAY	0.51	6.10	6.06	37.02	6.41
MLE2.2	MAY	1.98	0.62	-3.12	48.52	*
MCB4.3C	JUNE	2.00	-1.02	18.45	62.84	*
PXT0402	JUNE	0.92	-2.48	3.17	4.52	4.62
XEA6596	JUNE	0.74	-3.36	-3.00	-26.71	-10.80

(*) INDICATES NOT PRESENT IN SAMPLE

ANS/ODU Microzooplankton Split Sampling Results

% DIFFERENCE BETWEEN SAMPLES

STATION	MONTH	NAUPLII	ROTIFERS	TINTINNIDS	CILIATES
MCB5.2	MARCH	29.82	24.26	47.12	12.05
MET5.2	MARCH	26.25	36.00	69.16	10.26
MCB5.2	APRIL	28.82	18.16	23.42	5.82
MET5.1	APRIL	11.93	36.76	30.79	11.15
MCB2.1	MAY	25.95	95.68	9.12	52.03
MCB3.3C	MAY	3.76	36.25	43.90	95.65
MET5.1	MAY	8.00	39.70	24.99	81.63
MLE2.2	MAY	21.64	16.62	35.46	99.20
MCB4.3C	JUNE	20.51	57.30	71.85	76.19
PXT0402	JUNE	14.52	25.33	25.41	27.44
XEA6596	JUNE	21.60	28.27	20.85	82.16

Appendix: Analyses of Microzooplankton 1998 Split Sample Data

November 13, 1998

To: Stella Sellner (ANS) and Alicia Logalbo (ODU)

Fr: Elgin Perry

Re: Microzooplankton split sample analyses

I've been working on a comparison of the micro zoo split sample data data that uses the sampling variance as a benchmark of difference between labs. My first job is to come up with some comparable taxanomic groups. The SAS code (I hope you can read it) below shows how I am reassigning the taxonomy that you report with your data into groups for comparison. Would you two please review this with the idea of what revisions are needed to make the data more comparable.

After the SAS code is a listing of the results of the first run. You might also look this over an note any problems that you see. The first table in the results shows how the taxonomy was reassigned for ANS. The next table shows the ANS data summed by taxanomic group. The next tables shows the Raw data for ODU. The next table shows the ODU data summed over size fractions. The last table show the two data sets merged by date, station, and taxanomic group with the z-score comparisons by sample and taxonomic group. A z-score > 2.0 indicates a difference between the labs.

The code that re-assigns the taxonomy of the ANS data.

```
if 6117 <= spec4 <= 6120                  then taxagrp = "COPEPODS";
if 4500 <= spec4 <= 4599                  then taxagrp = "ROTIFERS";
if 3512 <= spec4 <= 3539                  then taxagrp = "CILIATES";
if 3541 <= spec4 <= 3545                  then taxagrp = "CILIATES";
if taxa = "NON-LORICATE CILIATES <20 UM" then taxagrp = "CILIATES";
if taxa = "NON-LORICATE CILIATES >20 UM" then taxagrp = "CILIATES";
if taxa = "NON-LORICATE CILIATES <20UM"  then taxagrp = "CILIATES";
if taxa = "NON-LORICATE CILIATES >20UM"  then taxagrp = "CILIATES";
if taxa = "NON-LORICATE CILIATES"        then taxagrp = "CILIATES";
if taxa = "MYRIONECTA-LIKE CILIATES"     then taxagrp = "CILIATES";
if taxa = "DIDINIUM"                     then taxagrp = "CILIATES";
if taxa = "DIDINIUM SP."                 then taxagrp = "CILIATES";
if spec4 = 3540                          then taxagrp = "TINTINNI";
if taxa  = "TINTINNIDS <44UM"            then taxagrp = "TINTINNI";
if taxa  = "TINTINNIDS >44UM"            then taxagrp = "TINTINNI";
if taxa  = "OTHER TINTINNIDS <44UM"      then taxagrp = "TINTINNI";
if taxagrp = "" then taxagrp = "DROP;
```

Here is the code that re-assigns the ODU taxonomy.

```
if lbl = 'TINTINNINA'    then taxagrp = "TINTINNI";
if lbl = 'COPEPODA'      then taxagrp = "COPEPODS";
if lbl = 'ROTIFERA'      then taxagrp = "ROTIFERS";
if lbl = 'OLIGOTRICHIDA' then taxagrp = "CILIATES";
if taxagrp = "" then taxagrp = "DROP";
```

assignment of taxa groups

OBS	SPECCODE	SPEC4	TAXA	TAXAGRP	RAW_CNT	P	NHAT	NVAR
1	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	226.00000	0.0075	30133.33	3987644.44
2	3540020100030	3540	TINTINNOPSIS SUBACUTA-HUGE	TINTINNI	210.00000	0.0075	28000.00	3705333.33
3	3540010100050	3540	TINTINNIDIUM SP.-LARGE	TINTINNI	114.00000	0.0075	15200.00	2011466.67
4	4506130200002	4506	SYNCHAETA SPP. M-MEDIUM	ROTIFERS	54.00000	0.0075	7200.00	952800.00
5	4506070100000	4506	TRICHOCERCA SP.	ROTIFERS	29.00000	0.0075	3866.67	511688.89
6	4506130200001	4506	SYNCHAETA SPP. L-LARGE	ROTIFERS	29.00000	0.0075	3866.67	511688.89
7	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	5.00000	0.0075	666.67	88222.22
8	5100000000001	5100	GASTROPODA-LARVAE	DROP	1.00000	0.0075	133.33	17644.44
9		.	DIDINIUM	CILIATES	1.00000	1.0000	1.00	0.00
10		.	TINTINNIDS <44UM	TINTINNI	81.00000	1.0000	81.00	0.00
11		.	NON-LORICATE CILIATES <20 UM	CILIATES	21.00000	1.0000	21.00	0.00
12		.	NON-LORICATE CILIATES >20 UM	CILIATES	45.00000	1.0000	45.00	0.00
13	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	352.00000	0.0440	8000.00	173818.18
14	4506130200001	4506	SYNCHAETA SPP. L-LARGE	ROTIFERS	17.00000	0.0660	257.58	3645.09
15	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	6.00000	0.0660	90.91	1286.50
16	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	1.00000	0.0660	15.15	214.42
17	3442010000000	3442	DIFFLUGIIDAE	DROP	1.00000	0.0660	15.15	214.42
18	3540020100030	3540	TINTINNOPSIS SUBACUTA-HUGE	TINTINNI	1.00000	0.0660	15.15	214.42
19	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	1.00000	0.0660	15.15	214.42
20		.	TINTINNIDS <44UM	TINTINNI	2.00000	1.0000	2.00	0.00
21		.	NON-LORICATE CILIATES <20 UM	CILIATES	82.00000	1.0000	82.00	0.00
22		.	NON-LORICATE CILIATES >20 UM	CILIATES	63.00000	1.0000	63.00	0.00
23		.	MYRIONECTA-LIKE CILIATES	CILIATES	47.00000	1.0000	47.00	0.00
24	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	47.00000	0.0054	8703.70	1603093.28
25	4506070100000	4506	TRICHOCERCA SP.	ROTIFERS	40.00000	0.0054	7407.41	1364334.71
26	3540020100030	3540	TINTINNOPSIS SUBACUTA-HUGE	TINTINNI	37.00000	0.0054	6851.85	1262009.60
27	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	6.00000	0.0054	1111.11	204650.21
28	6118290100001	6118	ACARTIA SP.-NAUPLII	COPEPODS	5.00000	0.0054	925.93	170541.84
29	4506130200020	4506	SYNCHAETA BALTICA	ROTIFERS	5.00000	0.0054	925.93	170541.84
30	4506130200002	4506	SYNCHAETA SPP. M-MEDIUM	ROTIFERS	4.00000	0.0054	740.74	136433.47
31	4506130200001	4506	SYNCHAETA SPP. L-LARGE	ROTIFERS	3.00000	0.0054	555.56	102325.10
32	3445040100000	3445	CYPODERIA SP.	DROP	1.00000	0.0054	185.19	34108.37
33	3540010100050	3540	TINTINNIDIUM SP.-LARGE	TINTINNI	25.00000	1.0000	25.00	0.00
34		.	OTHER TINTINNIDS <44UM	TINTINNI	117.00000	1.0000	117.00	0.00
35		.	NON-LORICATE CILIATES <20UM	CILIATES	627.00000	1.0000	627.00	0.00
36		.	NON-LORICATE CILIATES >20UM	CILIATES	94.00000	1.0000	94.00	0.00
37	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	127.00000	0.0140	9071.43	638887.76
38	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	74.00000	0.0280	2642.86	91744.90
39	4506010406000	4506	BRACHIONUS ANGULARIS	ROTIFERS	23.00000	0.0280	821.43	28515.31
40	4506010100000	4506	KERATELLA SP.	ROTIFERS	18.00000	0.0280	642.86	22316.33

OBS	SPECCODE	SPEC4	TAXA	TAXAGRP	RAW_CNT	P	NHAT	NVAR
41	4507050100000	4507	FILINIA SP.	ROTIFERS	17.00000	0.0280	607.14	21076.53
42	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	11.00000	0.0280	392.86	13637.76
43	4506130300000	4506	POLYARTHRA SP.	ROTIFERS	9.00000	0.0280	321.43	11158.16
44	3540010100050	3540	TINTINNIDIUM SP.-LARGE	TINTINNI	6.00000	0.0280	214.29	7438.78
45	4506130200001	4506	SYNCHAETA SPP. L-LARGE	ROTIFERS	5.00000	0.0280	178.57	6198.98
46	3442010000000	3442	DIFFLUGIIDAE	DROP	5.00000	0.0280	178.57	6198.98
47	3445040100000	3445	CYPHODERIA SP.	DROP	5.00000	0.0280	178.57	6198.98
48	4506010103020	4506	KERATELLA COCHLEARIS COCHLEARIS	ROTIFERS	4.00000	0.0280	142.86	4959.18
49	4506010203000	4506	NOTHOLCA ACUMINATA	ROTIFERS	3.00000	0.0280	107.14	3719.39
50	3540020100030	3540	TINTINNOPSIS SUBACUTA-HUGE	TINTINNI	1.00000	0.0280	35.71	1239.80
51	4506010402000	4506	BRACHIONUS CALYCIFLORUS	ROTIFERS	1.00000	0.0280	35.71	1239.80
52	4507040200000	4507	CONOCHILUS SP.	ROTIFERS	1.00000	0.0280	35.71	1239.80
53		.	TINTINNIDS <44UM	TINTINNI	33.00000	1.0000	33.00	0.00
54		.	NON-LORICATE CILIATES >20UM	CILIATES	38.00000	1.0000	38.00	0.00
55		.	NON-LORICATE CILIATES <20UM	CILIATES	28.00000	1.0000	28.00	0.00
56	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	289.00000	0.0110	26272.73	2362157.02
57	6118290100001	6118	ACARTIA SP.-NAUPLII	COPEPODS	224.00000	0.0110	20363.64	1830876.03
58	4506130200001	4506	SYNCHAETA SPP. L-LARGE	ROTIFERS	37.00000	0.0210	1761.90	82138.32
59	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	36.00000	0.0210	1714.29	79918.37
60	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	25.00000	0.0210	1190.48	55498.87
61	4506130200002	4506	SYNCHAETA SPP. M-MEDIUM	ROTIFERS	4.00000	0.0210	190.48	8879.82
62	5500000000001	5500	PELECYPODA-LARVAE	DROP	1.00000	0.0210	47.62	2219.95
63	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	1.00000	0.0210	47.62	2219.95
64	4506070100000	4506	TRICHOCERCA SP.	ROTIFERS	1.00000	0.0210	47.62	2219.95
65		.	TINTINNIDS <44UM	TINTINNI	8.00000	1.0000	8.00	0.00
66		.	MYRIONECTA-LIKE CILIATES	CILIATES	89.00000	0.5000	178.00	178.00
67		.	NON-LORICATE CILIATES	CILIATES	1210.00000	0.5000	2420.00	2420.00
68	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	534.00000	0.0190	28105.26	1451119.11
69	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	300.00000	0.0190	15789.47	815235.46
70	6118290100001	6118	ACARTIA SP.-NAUPLII	COPEPODS	196.00000	0.0370	5297.30	137872.90
71	4506130200001	4506	SYNCHAETA SPP. L-LARGE	ROTIFERS	31.00000	0.0370	837.84	21806.43
72	4506130200002	4506	SYNCHAETA SPP. M-MEDIUM	ROTIFERS	23.00000	0.0370	621.62	16178.96
73	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	23.00000	0.0370	621.62	16178.96
74	3540010100050	3540	TINTINNIDIUM SP.-LARGE	TINTINNI	3.00000	0.0370	81.08	2110.30
75	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	1.00000	0.0370	27.03	703.43
76		.	TINTINNIDS <44UM	TINTINNI	24.00000	1.0000	24.00	0.00
77		.	DIDINIUM	CILIATES	12.00000	1.0000	12.00	0.00
78		.	MYRIONECTA-LIKE CILIATES	CILIATES	99.00000	0.5000	198.00	198.00
79		.	NON-LORICATE CILIATES	CILIATES	731.00000	0.5000	1462.00	1462.00
80	4506010100000	4506	KERATELLA SP.	ROTIFERS	429.00000	0.0023	186521.74	80909886.58

assignment of taxa groups

OBS	SPECCODE	SPEC4	TAXA	TAXAGRP	RAW_CNT	P	NHAT	NVAR
81	4506010406000	4506	BRACHIONUS ANGULARIS	ROTIFERS	118.00000	0.0046	25652.17	5550907.37
82	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	77.00000	0.0046	16739.13	3622202.27
83	3442010000000	3442	DIFFLUGIIDAE	DROP	60.00000	0.0046	13043.48	2822495.27
84	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	57.00000	0.0046	12391.30	2681370.51
85	4507050100000	4507	FILINIA SP.	ROTIFERS	44.00000	0.0046	9565.22	2069829.87
86	4506130300000	4506	POLYARTHRA SP.	ROTIFERS	43.00000	0.0046	9347.83	2022788.28
87	4507040200000	4507	CONOCHILUS SP.	ROTIFERS	38.00000	0.0046	8260.87	1787580.34
88	4506010203000	4506	NOTHOLCA ACUMINATA	ROTIFERS	36.00000	0.0046	7826.09	1693497.16
89	4506070100000	4506	TRICHOCERCA SP.	ROTIFERS	10.00000	0.0046	2173.91	470415.88
90	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	6.00000	0.0046	1304.35	282249.53
91	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	5.00000	0.0046	1086.96	235207.94
92	4506130400000	4506	PLOESOMA SP.	ROTIFERS	5.00000	0.0046	1086.96	235207.94
93	4506120100000	4506	ASPLANCHNA SP.	ROTIFERS	4.00000	0.0046	869.57	188166.35
94	4506010402000	4506	BRACHIONUS CALYCIFLORUS	ROTIFERS	3.00000	0.0046	652.17	141124.76
95	4506010103060	4506	KERATELLA COCHLEARIS TECTA	ROTIFERS	2.00000	0.0046	434.78	94083.18
96	3445040100000	3445	CYPHODERIA SP.	DROP	2.00000	0.0046	434.78	94083.18
97	4508010100000	4508	COLLOTHECA SP.	ROTIFERS	2.00000	0.0046	434.78	94083.18
98	4506010106000	4506	KERATELLA VALGA	ROTIFERS	1.00000	0.0046	217.39	47041.59
99	4504000000000	4504	BDELLOIDA- UNIDED BDELLIOD ROTIFER	ROTIFERS	1.00000	0.0046	217.39	47041.59
100		.	TINTINNIDS <44UM	TINTINNI	95.00000	1.0000	95.00	0.00
101		.	NON-LORICATE CILIATES >20UM	CILIATES	98.00000	1.0000	98.00	0.00
102		.	NON-LORICATE CILIATES <20UM	CILIATES	2.00000	1.0000	2.00	0.00
103	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	158.00000	0.0120	13166.67	1084055.56
104	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	64.00000	0.0230	2782.61	118200.38
105	3534030700000	3534	STAUROPHRYA SP.	CILIATES	58.00000	0.0230	2521.74	107119.09
106	3442010000000	3442	DIFFLUGIIDAE	DROP	43.00000	0.0230	1869.57	79415.88
107	4506130300000	4506	POLYARTHRA SP.	ROTIFERS	32.00000	0.0230	1391.30	59100.19
108	4506010103020	4506	KERATELLA COCHLEARIS COCHLEARIS	ROTIFERS	28.00000	0.0230	1217.39	51712.67
109	4507040200000	4507	CONOCHILUS SP.	ROTIFERS	23.00000	0.0230	1000.00	42478.26
110	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	15.00000	0.0230	652.17	27703.21
111	4506010100000	4506	KERATELLA SP.	ROTIFERS	12.00000	0.0230	521.74	22162.57
112	4506010103060	4506	KERATELLA COCHLEARIS TECTA	ROTIFERS	9.00000	0.0230	391.30	16621.93
113	4506010402000	4506	BRACHIONUS CALYCIFLORUS	ROTIFERS	8.00000	0.0230	347.83	14775.05
114	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	3.00000	0.0230	130.43	5540.64
115	4506130200001	4506	SYNCHAETA SPP. L-LARGE	ROTIFERS	2.00000	0.0230	86.96	3693.76
116	4507050100000	4507	FILINIA SP.	ROTIFERS	2.00000	0.0230	86.96	3693.76
117	3442020100000	3442	ARCELLA SP.	DROP	2.00000	0.0230	86.96	3693.76
118	4506010203000	4506	NOTHOLCA ACUMINATA	ROTIFERS	1.00000	0.0230	43.48	1846.88
119	4506010403000	4506	BRACHIONUS HAVANAENSIS	ROTIFERS	1.00000	0.0230	43.48	1846.88
120	4507040100000	4507	CONOCCHILOIDES SP.	ROTIFERS	1.00000	0.0230	43.48	1846.88

OBS	SPECCODE	SPEC4	TAXA	TAXAGRP	RAW_CNT	P	NHAT	NVAR
121		.	TINTINNIDS <44UM	TINTINNI	15.00000	1.0000	15.00	0.00
122		.	NON-LORICATE CILIATES <20UM	CILIATES	143.00000	1.0000	143.00	0.00
123		.	NON-LORICATE CILIATES >20UM	CILIATES	173.00000	1.0000	173.00	0.00
124	4506010103060	4506	KERATELLA COCHLEARIS TECTA	ROTIFERS	724.00000	0.0045	160888.89	35592197.53
125	4506070100000	4506	TRICHOCERCA SP.	ROTIFERS	133.00000	0.0090	14777.78	1627197.53
126	4506130300000	4506	POLYARTHRA SP.	ROTIFERS	110.00000	0.0090	12222.22	1345802.47
127	4506010403000	4506	BRACHIONUS HAVANAENSIS	ROTIFERS	39.00000	0.0090	4333.33	477148.15
128	4506010103020	4506	KERATELLA COCHLEARIS COCHLEARIS	ROTIFERS	33.00000	0.0090	3666.67	403740.74
129	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	31.00000	0.0090	3444.44	379271.60
130	4507050100000	4507	FILINIA SP.	ROTIFERS	31.00000	0.0090	3444.44	379271.60
131	4506010409000	4506	BRACHIONUS CAUDATUS	ROTIFERS	14.00000	0.0090	1555.56	171283.95
132	3442010000000	3442	DIFFLUGIIDAE	DROP	14.00000	0.0090	1555.56	171283.95
133	4506010402000	4506	BRACHIONUS CALYCIFLORUS	ROTIFERS	11.00000	0.0090	1222.22	134580.25
134	4506130400000	4506	PLOESOMA SP.	ROTIFERS	9.00000	0.0090	1000.00	110111.11
135	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	8.00000	0.0090	888.89	97876.54
136	4506010406000	4506	BRACHIONUS ANGULARIS	ROTIFERS	7.00000	0.0090	777.78	85641.98
137	4506010500000	4506	KELLICOTTIA SP.	ROTIFERS	4.00000	0.0090	444.44	48938.27
138	4506130200002	4506	SYNCHAETA SPP. M-MEDIUM	ROTIFERS	4.00000	0.0090	444.44	48938.27
139	4508010100000	4508	COLLOTHECA SP.	ROTIFERS	2.00000	0.0090	222.22	24469.14
140	4506010400000	4506	BRACHIONUS SP.	ROTIFERS	1.00000	0.0090	111.11	12234.57
141	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	1.00000	0.0090	111.11	12234.57
142	4507040100000	4507	CONOCHILOIDES SP.	ROTIFERS	1.00000	0.0090	111.11	12234.57
143		.	TINTINNIDS <44UM	TINTINNI	35.00000	1.0000	35.00	0.00
144		.	NON-LORICATE CILIATES <20UM	CILIATES	899.00000	1.0000	899.00	0.00
145		.	NON-LORICATE CILIATES >20UM	CILIATES	39.00000	1.0000	39.00	0.00
146	6118290100001	6118	ACARTIA SP.-NAUPLII	COPEPODS	548.00000	0.0220	24909.09	1107322.31
147	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	30.00000	0.0220	1363.64	60619.83
148	5500000000001	5500	PELECYPODA-LARVAE	DROP	5.00000	0.0220	227.27	10103.31
149	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	4.00000	0.0220	181.82	8082.64
150	0000000000002	0	UNIDED. TROCHOPHORE LARVAE	DROP	3.00000	0.0220	136.36	6061.98
151	4506130200010	4506	SYNCHAETA BICORNIS	ROTIFERS	1.00000	0.0220	45.45	2020.66
152		.	TINTINNIDS <44UM	TINTINNI	13.00000	1.0000	13.00	0.00
153		.	DIDINIUM SP.	CILIATES	5.00000	1.0000	5.00	0.00
154		.	NON-LORICATE CILIATES <20UM	CILIATES	434.00000	1.0000	434.00	0.00
155		.	NON-LORICATE CILIATES >20UM	CILIATES	114.00000	1.0000	114.00	0.00
156	6118290100001	6118	ACARTIA SP.-NAUPLII	COPEPODS	385.00000	0.0110	35000.00	3146818.18
157	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	322.00000	0.0220	14636.36	650652.89
158	4506010401000	4506	BRACHIONUS PLICATILIS	ROTIFERS	115.00000	0.0220	5227.27	232376.03
159	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	66.00000	0.0220	3000.00	133363.64
160	5500000000001	5500	PLELECYPODA-LARVAE	DROP	24.00000	0.0220	1090.91	48495.87

OBS	SPECCODE	SPEC4	TAXA	TAXAGRP	RAW_CNT	P	NHAT	NVAR
161	4506130200003	4506	SYNCHAETA SPP. S-SMALL	ROTIFERS	21.00000	0.022	954.55	42433.88
162	4506010402000	4506	BRACHIONUS CALYCIFLORUS	ROTIFERS	8.00000	0.022	363.64	16165.29
163	4506010100000	4506	KERATELLA SP.	ROTIFERS	6.00000	0.022	272.73	12123.97
164	4506130200002	4506	SYNCHAETA SPP. M-MEDIUM	ROTIFERS	2.00000	0.022	90.91	4041.32
165	4506070100000	4506	TRICHOCERCA SP.	ROTIFERS	2.00000	0.022	90.91	4041.32
166	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	2.00000	0.022	90.91	4041.32
167	5100000000001	5100	GASTROPODA-LARVAE	DROP	1.00000	0.022	45.45	2020.66
168		.	TINTINNIDS <44UM	TINTINNI	37.00000	1.000	37.00	0.00
169		.	DIDINIUM SP.	CILIATES	1.00000	1.000	1.00	0.00
170		.	NON-LORICATE CILIATES <20UM	CILIATES	49.00000	1.000	49.00	0.00
171		.	NON-LORICATE CILIATES >20UM	CILIATES	250.00000	1.000	250.00	0.00
172		.	MYRIONECTA-LIKE CILIATES	CILIATES	69.00000	1.000	69.00	0.00
173	6117000000001	6117	COPEPOD NAUPLII	COPEPODS	264.00000	0.014	18857.14	1328081.63
174	4507050100000	4507	FILINIA SP.	ROTIFERS	92.00000	0.014	6571.43	462816.33
175	3442010000000	3442	DIFFLUGIIDAE	DROP	48.00000	0.014	3428.57	241469.39
176	3540020123003	3540	TINTINNOPSIS FIMBRIATA-MEUNIERI GRP	TINTINNI	36.00000	0.014	2571.43	181102.04
177	4506010400000	4506	BRACHIONUS SP.	ROTIFERS	12.00000	0.014	857.14	60367.35
178	4506010409000	4506	BRACHIONUS CAUDATUS	ROTIFERS	11.00000	0.014	785.71	55336.73
179	4506010406000	4506	BRACHIONUS ANGULARIS	ROTIFERS	7.00000	0.014	500.00	35214.29
180	4507040200000	4507	CONOCHILUS SP.	ROTIFERS	6.00000	0.014	428.57	30183.67
181	4506010203020	4506	KERATELLA COCHLEARIS COCHLEARIS	ROTIFERS	4.00000	0.014	285.71	20122.45
182	4506010100000	4506	KERATELLA SP.	ROTIFERS	2.00000	0.014	142.86	10061.22
183	4507040100000	4507	CONOCHILOIDES SP.	ROTIFERS	2.00000	0.014	142.86	10061.22
184	3442020100000	3442	ARCELLA SP.	DROP	2.00000	0.014	142.86	10061.22
185	4506070100000	4506	TRICHOCERCA SP.	ROTIFERS	1.00000	0.014	71.43	5030.61
186	4500000000000	4500	ROTIFERA- UNIDED ROTIFER	ROTIFERS	1.00000	0.014	71.43	5030.61
187	3445040100000	3445	CYPHODERIA SP.	DROP	1.00000	0.014	71.43	5030.61
188		.	TINTINNIDS <44UM	TINTINNI	25.00000	1.000	25.00	0.00
189		.	NON-LORICATE CILIATES <20UM	CILIATES	87.00000	1.000	87.00	0.00
190		.	NON-LORICATE CILIATES >20 UM	CILIATES	43.00000	1.000	43.00	0.00

Data after summing over taxa groups

TAXAGRP=CILIATES

OBS	CNT_LAB	DATE	STATION	AESTCNT	AESTSVAR
1	ANS	03/23/98	MCB5.2	67.00	0.00
2	ANS	03/24/98	MET5.2	192.00	0.00
3	ANS	04/06/98	MCB5.2	721.00	0.00
4	ANS	04/07/98	MET5.1	66.00	0.00
5	ANS	05/04/98	MLE2.2	2598.00	2598.00
6	ANS	05/05/98	MCB3.3C	1672.00	1660.00
7	ANS	05/05/98	MET5.1	100.00	0.00
8	ANS	05/06/98	MCB2.1	2837.74	107119.09
9	ANS	06/01/98	XEA6596	938.00	0.00
10	ANS	06/02/98	MCB4.3C	553.00	0.00
11	ANS	06/03/98	MWT5.1	369.00	0.00
12	ANS	06/08/98	PXT0402	130.00	0.00

TAXAGRP=COPEPODS

OBS	CNT_LAB	DATE	STATION	AESTCNT	AESTSVAR
13	ANS	03/23/98	MCB5.2	666.67	88222.22
14	ANS	03/24/98	MET5.2	8000.00	173818.18
15	ANS	04/06/98	MCB5.2	2037.04	375192.04
16	ANS	04/07/98	MET5.1	9071.43	638887.76
17	ANS	05/04/98	MLE2.2	22077.92	1910794.40
18	ANS	05/05/98	MCB3.3C	5918.92	154051.86
19	ANS	05/05/98	MET5.1	16739.13	3622202.27
20	ANS	05/06/98	MCB2.1	13166.67	1084055.56
21	ANS	06/01/98	XEA6596	3444.44	379271.60
22	ANS	06/02/98	MCB4.3C	24909.09	1107322.31
23	ANS	06/03/98	MWT5.1	38000.00	3280181.82
24	ANS	06/08/98	PXT0402	18857.14	1328081.63

TAXAGRP=ROTIFERS

OBS	CNT_LAB	DATE	STATION	AESTCNT	AESTSVAR
25	ANS	03/23/98	MCB5.2	45066.67	5963822.22
26	ANS	03/24/98	MET5.2	287.88	4073.92
27	ANS	04/06/98	MCB5.2	18333.33	3376728.40
28	ANS	04/07/98	MET5.1	3285.71	114061.22
29	ANS	05/04/98	MLE2.2	3238.10	150956.92
30	ANS	05/05/98	MCB3.3C	17275.96	853924.28
31	ANS	05/05/98	MET5.1	255652.17	95869111.53
32	ANS	05/06/98	MCB2.1	8608.70	365682.42
33	ANS	06/01/98	XEA6596	206222.22	40583901.23
34	ANS	06/02/98	MCB4.3C	1409.09	62640.50
35	ANS	06/03/98	MWT5.1	7090.91	315223.14
36	ANS	06/08/98	PXT0402	9857.14	694224.49

Data after summing over taxa groups

TAXAGRP=TINTINNI

OBS	CNT_LAB	DATE	STATION	AESTCNT	AESTSVAR
37	ANS	03/23/98	MCB5.2	43281.00	5716800.00

38	ANS	03/24/98	MET5.2	108.06	1500.92
39	ANS	04/06/98	MCB5.2	6993.85	1262009.60
40	ANS	04/07/98	MET5.1	2925.86	100423.47
41	ANS	05/04/98	MLE2.2	26280.73	2362157.02
42	ANS	05/05/98	MCB3.3C	28210.34	1453229.41
43	ANS	05/05/98	MET5.1	12486.30	2681370.51
44	ANS	05/06/98	MCB2.1	145.43	5540.64
45	ANS	06/01/98	XEA6596	35.00	0.00
46	ANS	06/02/98	MCB4.3C	194.82	8082.64
47	ANS	06/03/98	MWT5.1	14673.36	650652.89
48	ANS	06/08/98	PXT0402	2596.43	181102.04

OBS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
1	03/23/98	MCB5.2	OLIGOTRICHIDA	CILIATES	31 TO 73 U	54.00000	0.050	1080	20520
2	03/23/98	MCB5.2	OLIGOTRICHIDA	CILIATES	< 30 U	212.00000	0.050	4240	80560
3	03/23/98	MCB5.2	OLIGOTRICHIDA	CILIATES	>73 U	74.00000	1.000	74	0
4	03/23/98	MCB5.2	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.050	0	0
5	03/23/98	MCB5.2	COPEPODA	COPEPODS	< 30 U	0.00000	0.050	0	0
6	03/23/98	MCB5.2	COPEPODA	COPEPODS	>73 U	19.00000	1.000	19	0
7	03/23/98	MCB5.2	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.050	0	0
8	03/23/98	MCB5.2	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.050	0	0
9	03/23/98	MCB5.2	SARCODINA	DROP	31 TO 73 U	0.00000	0.050	0	0
10	03/23/98	MCB5.2	CLADOCERA	DROP	31 TO 73 U	0.00000	0.050	0	0
11	03/23/98	MCB5.2	BALANOMORPHA	DROP	< 30 U	0.00000	0.050	0	0
12	03/23/98	MCB5.2	POLYCHAETA	DROP	< 30 U	0.00000	0.050	0	0
13	03/23/98	MCB5.2	SARCODINA	DROP	< 30 U	0.00000	0.050	0	0
14	03/23/98	MCB5.2	CLADOCERA	DROP	< 30 U	0.00000	0.050	0	0
15	03/23/98	MCB5.2	BALANOMORPHA	DROP	>73 U	4.00000	1.000	4	0
16	03/23/98	MCB5.2	POLYCHAETA	DROP	>73 U	8.00000	1.000	8	0
17	03/23/98	MCB5.2	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
18	03/23/98	MCB5.2	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
19	03/23/98	MCB5.2	ROTIFERA	ROTIFERS	31 TO 73 U	51.00000	0.050	1020	19380
20	03/23/98	MCB5.2	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.050	0	0
21	03/23/98	MCB5.2	ROTIFERA	ROTIFERS	>73 U	170.00000	1.000	170	0
22	03/23/98	MCB5.2	TINTINNINA	TINTINNI	31 TO 73 U	19.00000	0.050	380	7220
23	03/23/98	MCB5.2	TINTINNINA	TINTINNI	< 30 U	266.00000	0.050	5320	101080
24	03/23/98	MCB5.2	TINTINNINA	TINTINNI	>73 U	468.00000	1.000	468	0
25	03/24/98	MET5.2	OLIGOTRICHIDA	CILIATES	31 TO 73 U	49.00000	0.025	1960	76440
26	03/24/98	MET5.2	OLIGOTRICHIDA	CILIATES	< 30 U	302.00000	0.025	12080	471120
27	03/24/98	MET5.2	OLIGOTRICHIDA	CILIATES	>73 U	1.00000	1.000	1	0
28	03/24/98	MET5.2	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.025	0	0
29	03/24/98	MET5.2	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
30	03/24/98	MET5.2	COPEPODA	COPEPODS	>73 U	118.00000	1.000	118	0
31	03/24/98	MET5.2	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
32	03/24/98	MET5.2	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0
33	03/24/98	MET5.2	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
34	03/24/98	MET5.2	CLADOCERA	DROP	31 TO 73 U	0.00000	0.025	0	0
35	03/24/98	MET5.2	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0
36	03/24/98	MET5.2	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
37	03/24/98	MET5.2	SARCODINA	DROP	< 30 U	0.00000	0.025	0	0
38	03/24/98	MET5.2	CLADOCERA	DROP	< 30 U	0.00000	0.025	0	0
39	03/24/98	MET5.2	BALANOMORPHA	DROP	>73 U	4.00000	1.000	4	0
40	03/24/98	MET5.2	POLYCHAETA	DROP	>73 U	0.00000	1.000	0	0

assignment of taxa groups

OBS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
41	03/24/98	MET5.2	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
42	03/24/98	MET5.2	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
43	03/24/98	MET5.2	ROTIFERA	ROTIFERS	31 TO 73 U	0.00000	0.025	0	0
44	03/24/98	MET5.2	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
45	03/24/98	MET5.2	ROTIFERA	ROTIFERS	>73 U	9.00000	1.000	9	0
46	03/24/98	MET5.2	TINTINNINA	TINTINNI	31 TO 73 U	0.00000	0.025	0	0
47	03/24/98	MET5.2	TINTINNINA	TINTINNI	< 30 U	3.00000	0.025	120	4680
48	03/24/98	MET5.2	TINTINNINA	TINTINNI	>73 U	4.00000	1.000	4	0
49	04/06/98	MCB5.2	OLIGOTRICHIDA	CILIATES	31 TO 73 U	27.00000	0.050	540	10260
50	04/06/98	MCB5.2	OLIGOTRICHIDA	CILIATES	< 30 U	372.00000	0.050	7440	141360
51	04/06/98	MCB5.2	OLIGOTRICHIDA	CILIATES	>73 U	5.00000	1.000	5	0
52	04/06/98	MCB5.2	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.050	0	0
53	04/06/98	MCB5.2	COPEPODA	COPEPODS	< 30 U	0.00000	0.050	0	0
54	04/06/98	MCB5.2	COPEPODA	COPEPODS	>73 U	29.00000	1.000	29	0
55	04/06/98	MCB5.2	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.050	0	0
56	04/06/98	MCB5.2	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.050	0	0
57	04/06/98	MCB5.2	SARCODINA	DROP	31 TO 73 U	0.00000	0.050	0	0
58	04/06/98	MCB5.2	CLADOCERA	DROP	31 TO 73 U	0.00000	0.050	0	0
59	04/06/98	MCB5.2	BALANOMORPHA	DROP	< 30 U	0.00000	0.050	0	0
60	04/06/98	MCB5.2	POLYCHAETA	DROP	< 30 U	0.00000	0.050	0	0
61	04/06/98	MCB5.2	SARCODINA	DROP	< 30 U	0.00000	0.050	0	0
62	04/06/98	MCB5.2	CLADOCERA	DROP	< 30 U	0.00000	0.050	0	0
63	04/06/98	MCB5.2	BALANOMORPHA	DROP	>73 U	4.00000	1.000	4	0
64	04/06/98	MCB5.2	POLYCHAETA	DROP	>73 U	10.00000	1.000	10	0
65	04/06/98	MCB5.2	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
66	04/06/98	MCB5.2	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
67	04/06/98	MCB5.2	ROTIFERA	ROTIFERS	31 TO 73 U	19.00000	0.050	380	7220
68	04/06/98	MCB5.2	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.050	0	0
69	04/06/98	MCB5.2	ROTIFERA	ROTIFERS	>73 U	68.00000	1.000	68	0
70	04/06/98	MCB5.2	TINTINNINA	TINTINNI	31 TO 73 U	27.00000	0.050	540	10260
71	04/06/98	MCB5.2	TINTINNINA	TINTINNI	< 30 U	719.00000	0.050	14380	273220
72	04/06/98	MCB5.2	TINTINNINA	TINTINNI	>73 U	93.00000	1.000	93	0
73	04/07/98	MET5.1	OLIGOTRICHIDA	CILIATES	31 TO 73 U	48.00000	0.025	1920	74880
74	04/07/98	MET5.1	OLIGOTRICHIDA	CILIATES	< 30 U	135.00000	0.025	5400	210600
75	04/07/98	MET5.1	OLIGOTRICHIDA	CILIATES	>73 U	1.00000	1.000	1	0
76	04/07/98	MET5.1	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.025	0	0
77	04/07/98	MET5.1	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
78	04/07/98	MET5.1	COPEPODA	COPEPODS	>73 U	206.00000	1.000	206	0
79	04/07/98	MET5.1	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
80	04/07/98	MET5.1	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0

OBS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
81	04/07/98	MET5.1	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
82	04/07/98	MET5.1	CLADOCERA	DROP	31 TO 73 U	0.00000	0.025	0	0
83	04/07/98	MET5.1	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0
84	04/07/98	MET5.1	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
85	04/07/98	MET5.1	SARCODINA	DROP	< 30 U	0.00000	0.025	0	0
86	04/07/98	MET5.1	CLADOCERA	DROP	< 30 U	0.00000	0.025	0	0
87	04/07/98	MET5.1	BALANOMORPHA	DROP	>73 U	0.00000	1.000	0	0
88	04/07/98	MET5.1	POLYCHAETA	DROP	>73 U	0.00000	1.000	0	0
89	04/07/98	MET5.1	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
90	04/07/98	MET5.1	CLADOCERA	DROP	>73 U	59.00000	1.000	59	0
91	04/07/98	MET5.1	ROTIFERA	ROTIFERS	31 TO 73 U	2.00000	0.025	80	3120
92	04/07/98	MET5.1	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
93	04/07/98	MET5.1	ROTIFERA	ROTIFERS	>73 U	72.00000	1.000	72	0
94	04/07/98	MET5.1	TINTINNINA	TINTINNI	31 TO 73 U	9.00000	0.025	360	14040
95	04/07/98	MET5.1	TINTINNINA	TINTINNI	< 30 U	90.00000	0.025	3600	140400
96	04/07/98	MET5.1	TINTINNINA	TINTINNI	>73 U	1.00000	1.000	1	0
97	04/13/98	PXT0402	OLIGOTRICHIDA	CILIATES	31 TO 73 U	2.00000	0.025	80	3120
98	04/13/98	PXT0402	OLIGOTRICHIDA	CILIATES	< 30 U	22.00000	0.025	880	34320
99	04/13/98	PXT0402	OLIGOTRICHIDA	CILIATES	>73 U	0.00000	1.000	0	0
100	04/13/98	PXT0402	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.025	0	0
101	04/13/98	PXT0402	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
102	04/13/98	PXT0402	COPEPODA	COPEPODS	>73 U	517.00000	1.000	517	0
103	04/13/98	PXT0402	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
104	04/13/98	PXT0402	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0
105	04/13/98	PXT0402	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
106	04/13/98	PXT0402	CLADOCERA	DROP	31 TO 73 U	0.00000	0.025	0	0
107	04/13/98	PXT0402	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0
108	04/13/98	PXT0402	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
109	04/13/98	PXT0402	SARCODINA	DROP	< 30 U	0.00000	0.025	0	0
110	04/13/98	PXT0402	CLADOCERA	DROP	< 30 U	0.00000	0.025	0	0
111	04/13/98	PXT0402	BALANOMORPHA	DROP	>73 U	0.00000	1.000	0	0
112	04/13/98	PXT0402	POLYCHAETA	DROP	>73 U	0.00000	1.000	0	0
113	04/13/98	PXT0402	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
114	04/13/98	PXT0402	CLADOCERA	DROP	>73 U	4.00000	1.000	4	0
115	04/13/98	PXT0402	ROTIFERA	ROTIFERS	31 TO 73 U	4.00000	0.025	160	6240
116	04/13/98	PXT0402	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
117	04/13/98	PXT0402	ROTIFERA	ROTIFERS	>73 U	105.00000	1.000	105	0
118	04/13/98	PXT0402	TINTINNINA	TINTINNI	31 TO 73 U	2.00000	0.025	80	3120
119	04/13/98	PXT0402	TINTINNINA	TINTINNI	< 30 U	7.00000	0.025	280	10920
120	04/13/98	PXT0402	TINTINNINA	TINTINNI	>73 U	0.00000	1.000	0	0

ssignment of taxa groups

BS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
21	04/13/98	XDE5339	OLIGOTRICHIDA	CILIATES	31 TO 73 U	5.00000	0.025	200	7800
22	04/13/98	XDE5339	OLIGOTRICHIDA	CILIATES	< 30 U	136.00000	0.025	5440	212160
23	04/13/98	XDE5339	OLIGOTRICHIDA	CILIATES	>73 U	0.00000	1.000	0	0
24	04/13/98	XDE5339	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.025	0	0
25	04/13/98	XDE5339	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
26	04/13/98	XDE5339	COPEPODA	COPEPODS	>73 U	107.00000	1.000	107	0
27	04/13/98	XDE5339	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
28	04/13/98	XDE5339	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0
29	04/13/98	XDE5339	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
30	04/13/98	XDE5339	CLADOCERA	DROP	31 TO 73 U	0.00000	0.025	0	0
31	04/13/98	XDE5339	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0
32	04/13/98	XDE5339	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
33	04/13/98	XDE5339	SARCODINA	DROP	< 30 U	0.00000	0.025	0	0
34	04/13/98	XDE5339	CLADOCERA	DROP	< 30 U	0.00000	0.025	0	0
35	04/13/98	XDE5339	BALANOMORPHA	DROP	>73 U	2.00000	1.000	2	0
36	04/13/98	XDE5339	POLYCHAETA	DROP	>73 U	4.00000	1.000	4	0
37	04/13/98	XDE5339	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
38	04/13/98	XDE5339	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
39	04/13/98	XDE5339	ROTIFERA	ROTIFERS	31 TO 73 U	3.00000	0.025	120	4680
40	04/13/98	XDE5339	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
41	04/13/98	XDE5339	ROTIFERA	ROTIFERS	>73 U	17.00000	1.000	17	0
42	04/13/98	XDE5339	TINTINNINA	TINTINNI	31 TO 73 U	1.00000	0.025	40	1560
43	04/13/98	XDE5339	TINTINNINA	TINTINNI	< 30 U	70.00000	0.025	2800	109200
44	04/13/98	XDE5339	TINTINNINA	TINTINNI	>73 U	5.00000	1.000	5	0
45	05/04/98	MLE2.2	OLIGOTRICHIDA	CILIATES	31 TO 73 U	2.00000	0.025	80	3120
46	05/04/98	MLE2.2	OLIGOTRICHIDA	CILIATES	< 30 U	93.00000	0.025	3720	145080
47	05/04/98	MLE2.2	OLIGOTRICHIDA	CILIATES	>73 U	95.00000	1.000	95	0
48	05/04/98	MLE2.2	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.025	0	0
49	05/04/98	MLE2.2	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
50	05/04/98	MLE2.2	COPEPODA	COPEPODS	>73 U	346.00000	1.000	346	0
51	05/04/98	MLE2.2	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
52	05/04/98	MLE2.2	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0
53	05/04/98	MLE2.2	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
54	05/04/98	MLE2.2	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0
55	05/04/98	MLE2.2	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
56	05/04/98	MLE2.2	SARCODINA	DROP	< 30 U	0.00000	0.025	0	0
57	05/04/98	MLE2.2	BALANOMORPHA	DROP	>73 U	20.00000	1.000	20	0
58	05/04/98	MLE2.2	POLYCHAETA	DROP	>73 U	2.00000	1.000	2	0
59	05/04/98	MLE2.2	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
60	05/04/98	MLE2.2	ROTIFERA	ROTIFERS	31 TO 73 U	0.00000	0.025	0	0

OBS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
161	05/04/98	MLE2.2	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
162	05/04/98	MLE2.2	ROTIFERA	ROTIFERS	>73 U	54.00000	1.000	54	0
163	05/04/98	MLE2.2	TINTINNINA	TINTINNI	31 TO 73 U	9.00000	0.025	360	14040
164	05/04/98	MLE2.2	TINTINNINA	TINTINNI	< 30 U	73.00000	0.025	2920	113880
165	05/04/98	MLE2.2	TINTINNINA	TINTINNI	>73 U	13.00000	1.000	13	0
166	05/05/98	MCB3.3C	OLIGOTRICHIDA	CILIATES	31 TO 73 U	147.00000	0.050	2940	55860
167	05/05/98	MCB3.3C	OLIGOTRICHIDA	CILIATES	< 30 U	493.00000	0.050	9860	187340
168	05/05/98	MCB3.3C	OLIGOTRICHIDA	CILIATES	>73 U	33.00000	1.000	33	0
169	05/05/98	MCB3.3C	COPEPODA	COPEPODS	31 TO 73 U	1.00000	0.050	20	380
170	05/05/98	MCB3.3C	COPEPODA	COPEPODS	< 30 U	0.00000	0.050	0	0
171	05/05/98	MCB3.3C	COPEPODA	COPEPODS	>73 U	103.00000	1.000	103	0
172	05/05/98	MCB3.3C	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.050	0	0
173	05/05/98	MCB3.3C	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.050	0	0
174	05/05/98	MCB3.3C	SARCODINA	DROP	31 TO 73 U	0.00000	0.050	0	0
175	05/05/98	MCB3.3C	CLADOCERA	DROP	31 TO 73 U	0.00000	0.050	0	0
176	05/05/98	MCB3.3C	BALANOMORPHA	DROP	< 30 U	0.00000	0.050	0	0
177	05/05/98	MCB3.3C	POLYCHAETA	DROP	< 30 U	0.00000	0.050	0	0
178	05/05/98	MCB3.3C	SARCODINA	DROP	< 30 U	0.00000	0.050	0	0
179	05/05/98	MCB3.3C	CLADOCERA	DROP	< 30 U	0.00000	0.050	0	0
180	05/05/98	MCB3.3C	BALANOMORPHA	DROP	>73 U	10.00000	1.000	10	0
181	05/05/98	MCB3.3C	POLYCHAETA	DROP	>73 U	25.00000	1.000	25	0
182	05/05/98	MCB3.3C	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
183	05/05/98	MCB3.3C	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
184	05/05/98	MCB3.3C	ROTIFERA	ROTIFERS	31 TO 73 U	25.00000	0.050	500	9500
185	05/05/98	MCB3.3C	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.050	0	0
186	05/05/98	MCB3.3C	ROTIFERA	ROTIFERS	>73 U	42.00000	1.000	42	0
187	05/05/98	MCB3.3C	TINTINNINA	TINTINNI	31 TO 73 U	30.00000	0.050	600	11400
188	05/05/98	MCB3.3C	TINTINNINA	TINTINNI	< 30 U	119.00000	0.050	2380	45220
189	05/05/98	MCB3.3C	TINTINNINA	TINTINNI	>73 U	29.00000	1.000	29	0
190	05/05/98	MET5.1	OLIGOTRICHIDA	CILIATES	31 TO 73 U	0.00000	0.025	0	0
191	05/05/98	MET5.1	OLIGOTRICHIDA	CILIATES	< 30 U	90.00000	0.025	3600	140400
192	05/05/98	MET5.1	OLIGOTRICHIDA	CILIATES	>73 U	1.00000	1.000	1	0
193	05/05/98	MET5.1	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.025	0	0
194	05/05/98	MET5.1	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
195	05/05/98	MET5.1	COPEPODA	COPEPODS	>73 U	308.00000	1.000	308	0
196	05/05/98	MET5.1	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
197	05/05/98	MET5.1	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0
198	05/05/98	MET5.1	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
199	05/05/98	MET5.1	CLADOCERA	DROP	31 TO 73 U	0.00000	0.025	0	0
200	05/05/98	MET5.1	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0

S	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
1	05/05/98	MET5.1	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
2	05/05/98	MET5.1	SARCODINA	DROP	< 30 U	0.00000	0.025	0	0
3	05/05/98	MET5.1	CLADOCERA	DROP	< 30 U	0.00000	0.025	0	0
4	05/05/98	MET5.1	BALANOMORPHA	DROP	>73 U	23.00000	1.000	23	0
5	05/05/98	MET5.1	POLYCHAETA	DROP	>73 U	0.00000	1.000	0	0
6	05/05/98	MET5.1	SARCODINA	DROP	>73 U	4.00000	1.000	4	0
7	05/05/98	MET5.1	CLADOCERA	DROP	>73 U	64.00000	1.000	64	0
8	05/05/98	MET5.1	ROTIFERA	ROTIFERS	31 TO 73 U	36.00000	0.025	1440	56160
9	05/05/98	MET5.1	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
0	05/05/98	MET5.1	ROTIFERA	ROTIFERS	>73 U	1643.00000	1.000	1643	0
1	05/05/98	MET5.1	TINTINNINA	TINTINNI	31 TO 73 U	203.00000	0.025	8120	316680
2	05/05/98	MET5.1	TINTINNINA	TINTINNI	< 30 U	157.00000	0.025	6280	244920
3	05/05/98	MET5.1	TINTINNINA	TINTINNI	>73 U	38.00000	1.000	38	0
4	05/06/98	MCB2.1	OLIGOTRICHIDA	CILIATES	31 TO 73 U	22.00000	0.050	440	8360
5	05/06/98	MCB2.1	OLIGOTRICHIDA	CILIATES	< 30 U	809.00000	0.050	16180	307420
6	05/06/98	MCB2.1	OLIGOTRICHIDA	CILIATES	>73 U	3.00000	1.000	3	0
7	05/06/98	MCB2.1	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.050	0	0
8	05/06/98	MCB2.1	COPEPODA	COPEPODS	< 30 U	0.00000	0.050	0	0
9	05/06/98	MCB2.1	COPEPODA	COPEPODS	>73 U	194.00000	1.000	194	0
0	05/06/98	MCB2.1	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.050	0	0
1	05/06/98	MCB2.1	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.050	0	0
2	05/06/98	MCB2.1	SARCODINA	DROP	31 TO 73 U	0.00000	0.050	0	0
3	05/06/98	MCB2.1	CLADOCERA	DROP	31 TO 73 U	0.00000	0.050	0	0
4	05/06/98	MCB2.1	BALANOMORPHA	DROP	< 30 U	0.00000	0.050	0	0
5	05/06/98	MCB2.1	POLYCHAETA	DROP	< 30 U	0.00000	0.050	0	0
6	05/06/98	MCB2.1	SARCODINA	DROP	< 30 U	0.00000	0.050	0	0
7	05/06/98	MCB2.1	CLADOCERA	DROP	< 30 U	0.00000	0.050	0	0
8	05/06/98	MCB2.1	BALANOMORPHA	DROP	>73 U	0.00000	1.000	0	0
9	05/06/98	MCB2.1	POLYCHAETA	DROP	>73 U	0.00000	1.000	0	0
0	05/06/98	MCB2.1	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
1	05/06/98	MCB2.1	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
2	05/06/98	MCB2.1	ROTIFERA	ROTIFERS	31 TO 73 U	178.00000	0.050	3560	67640
3	05/06/98	MCB2.1	ROTIFERA	ROTIFERS	< 30 U	15.00000	0.050	300	5700
4	05/06/98	MCB2.1	ROTIFERA	ROTIFERS	>73 U	128.00000	1.000	128	0
5	05/06/98	MCB2.1	TINTINNINA	TINTINNI	31 TO 73 U	18.00000	0.050	360	6840
6	05/06/98	MCB2.1	TINTINNINA	TINTINNI	< 30 U	147.00000	0.050	2940	55860
7	05/06/98	MCB2.1	TINTINNINA	TINTINNI	>73 U	4.00000	1.000	4	0
8	06/01/98	XEA6595	OLIGOTRICHIDA	CILIATES	31 TO 73 U	20.00000	0.025	800	31200
9	06/01/98	XEA6595	OLIGOTRICHIDA	CILIATES	< 30 U	1073.00000	0.025	42920	1673880
0	06/01/98	XEA6595	OLIGOTRICHIDA	CILIATES	>73 U	7.00000	1.000	7	0

assignment of taxa groups

OBS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
241	06/01/98	XEA6595	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.025	0	0
242	06/01/98	XEA6595	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
243	06/01/98	XEA6595	COPEPODA	COPEPODS	>73 U	54.00000	1.000	54	0
244	06/01/98	XEA6595	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
245	06/01/98	XEA6595	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0
246	06/01/98	XEA6595	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
247	06/01/98	XEA6595	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0
248	06/01/98	XEA6595	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
249	06/01/98	XEA6595	SARCODINA	DROP	< 30 U	123.00000	0.025	4920	191880
250	06/01/98	XEA6595	BALANOMORPHA	DROP	>73 U	0.00000	1.000	0	0
251	06/01/98	XEA6595	POLYCHAETA	DROP	>73 U	0.00000	1.000	0	0
252	06/01/98	XEA6595	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
253	06/01/98	XEA6595	ROTIFERA	ROTIFERS	31 TO 73 U	127.00000	0.025	5080	198120
254	06/01/98	XEA6595	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
255	06/01/98	XEA6595	ROTIFERA	ROTIFERS	>73 U	671.00000	1.000	671	0
256	06/01/98	XEA6595	TINTINNINA	TINTINNI	31 TO 73 U	7.00000	0.025	280	10920
257	06/01/98	XEA6595	TINTINNINA	TINTINNI	< 30 U	214.00000	0.025	8560	333840
258	06/01/98	XEA6595	TINTINNINA	TINTINNI	>73 U	4.00000	1.000	4	0
259	06/02/98	MCB4.3	OLIGOTRICHIDA	CILIATES	31 TO 73 U	64.00000	0.050	1280	24320
260	06/02/98	MCB4.3	OLIGOTRICHIDA	CILIATES	< 30 U	502.00000	0.050	10040	190760
261	06/02/98	MCB4.3	OLIGOTRICHIDA	CILIATES	>73 U	15.00000	1.000	15	0
262	06/02/98	MCB4.3	COPEPODA	COPEPODS	31 TO 73 U	1.00000	0.050	20	380
263	06/02/98	MCB4.3	COPEPODA	COPEPODS	< 30 U	0.00000	0.050	0	0
264	06/02/98	MCB4.3	COPEPODA	COPEPODS	>73 U	376.00000	1.000	376	0
265	06/02/98	MCB4.3	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.050	0	0
266	06/02/98	MCB4.3	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.050	0	0
267	06/02/98	MCB4.3	SARCODINA	DROP	31 TO 73 U	0.00000	0.050	0	0
268	06/02/98	MCB4.3	CLADOCERA	DROP	31 TO 73 U	0.00000	0.050	0	0
269	06/02/98	MCB4.3	BALANOMORPHA	DROP	< 30 U	0.00000	0.050	0	0
270	06/02/98	MCB4.3	POLYCHAETA	DROP	< 30 U	0.00000	0.050	0	0
271	06/02/98	MCB4.3	SARCODINA	DROP	< 30 U	0.00000	0.050	0	0
272	06/02/98	MCB4.3	CLADOCERA	DROP	< 30 U	0.00000	0.050	0	0
273	06/02/98	MCB4.3	BALANOMORPHA	DROP	>73 U	0.00000	1.000	0	0
274	06/02/98	MCB4.3	POLYCHAETA	DROP	>73 U	8.00000	1.000	8	0
275	06/02/98	MCB4.3	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
276	06/02/98	MCB4.3	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
277	06/02/98	MCB4.3	ROTIFERA	ROTIFERS	31 TO 73 U	3.00000	0.050	60	1140
278	06/02/98	MCB4.3	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.050	0	0
279	06/02/98	MCB4.3	ROTIFERA	ROTIFERS	>73 U	6.00000	1.000	6	0
280	06/02/98	MCB4.3	TINTINNINA	TINTINNI	31 TO 73 U	2.00000	0.050	40	760

BS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
31	06/02/98	MCB4.3	TINTINNINA	TINTINNI	< 30 U	71.00000	0.050	1420	26980
32	06/02/98	MCB4.3	TINTINNINA	TINTINNI	>73 U	5.00000	1.000	5	0
33	06/02/98	MWT5.1	OLIGOTRICHIDA	CILIATES	31 TO 73 U	181.00000	0.050	3620	68780
34	06/02/98	MWT5.1	OLIGOTRICHIDA	CILIATES	< 30 U	931.00000	0.050	18620	353780
35	06/02/98	MWT5.1	OLIGOTRICHIDA	CILIATES	>73 U	24.00000	1.000	24	0
36	06/02/98	MWT5.1	COPEPODA	COPEPODS	31 TO 73 U	0.00000	0.050	0	0
37	06/02/98	MWT5.1	COPEPODA	COPEPODS	< 30 U	0.00000	0.050	0	0
38	06/02/98	MWT5.1	COPEPODA	COPEPODS	>73 U	74.00000	1.000	74	0
39	06/02/98	MWT5.1	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.050	0	0
90	06/02/98	MWT5.1	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.050	0	0
91	06/02/98	MWT5.1	SARCODINA	DROP	31 TO 73 U	0.00000	0.050	0	0
92	06/02/98	MWT5.1	CLADOCERA	DROP	31 TO 73 U	0.00000	0.050	0	0
93	06/02/98	MWT5.1	BALANOMORPHA	DROP	< 30 U	0.00000	0.050	0	0
94	06/02/98	MWT5.1	POLYCHAETA	DROP	< 30 U	0.00000	0.050	0	0
95	06/02/98	MWT5.1	SARCODINA	DROP	< 30 U	0.00000	0.050	0	0
96	06/02/98	MWT5.1	CLADOCERA	DROP	< 30 U	0.00000	0.050	0	0
97	06/02/98	MWT5.1	BALANOMORPHA	DROP	>73 U	12.00000	1.000	12	0
98	06/02/98	MWT5.1	POLYCHAETA	DROP	>73 U	3.00000	1.000	3	0
99	06/02/98	MWT5.1	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
00	06/02/98	MWT5.1	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
01	06/02/98	MWT5.1	ROTIFERA	ROTIFERS	31 TO 73 U	6.00000	0.050	120	2280
02	06/02/98	MWT5.1	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.050	0	0
03	06/02/98	MWT5.1	ROTIFERA	ROTIFERS	>73 U	515.00000	1.000	515	0
04	06/02/98	MWT5.1	TINTINNINA	TINTINNI	31 TO 73 U	16.00000	0.050	320	6080
05	06/02/98	MWT5.1	TINTINNINA	TINTINNI	< 30 U	416.00000	0.050	8320	158080
06	06/02/98	MWT5.1	TINTINNINA	TINTINNI	>73 U	65.00000	1.000	65	0
07	06/04/98	MLE2.2	CLADOCERA	DROP	31 TO 73 U	0.00000	0.025	0	0
08	06/04/98	MLE2.2	CLADOCERA	DROP	< 30 U	0.00000	0.025	0	0
09	06/04/98	MLE2.2	CLADOCERA	DROP	>73 U	0.00000	1.000	0	0
10	06/08/98	PXT0402	OLIGOTRICHIDA	CILIATES	31 TO 73 U	4.00000	0.025	160	6240
11	06/08/98	PXT0402	OLIGOTRICHIDA	CILIATES	< 30 U	152.00000	0.025	6080	237120
12	06/08/98	PXT0402	OLIGOTRICHIDA	CILIATES	>73 U	0.00000	1.000	0	0
13	06/08/98	PXT0402	COPEPODA	COPEPODS	31 TO 73 U	1.00000	0.025	40	1560
14	06/08/98	PXT0402	COPEPODA	COPEPODS	< 30 U	0.00000	0.025	0	0
15	06/08/98	PXT0402	COPEPODA	COPEPODS	>73 U	283.00000	1.000	283	0
16	06/08/98	PXT0402	BALANOMORPHA	DROP	31 TO 73 U	0.00000	0.025	0	0
17	06/08/98	PXT0402	POLYCHAETA	DROP	31 TO 73 U	0.00000	0.025	0	0
18	06/08/98	PXT0402	SARCODINA	DROP	31 TO 73 U	0.00000	0.025	0	0
19	06/08/98	PXT0402	CLADOCERA	DROP	31 TO 73 U	0.00000	0.025	0	0
20	06/08/98	PXT0402	BALANOMORPHA	DROP	< 30 U	0.00000	0.025	0	0

OBS	DATE	STATION	TAXA	TAXAGRP	SIZE_FRA	RAW_CNT	P	NHAT	NVAR
321	06/08/98	PXT0402	POLYCHAETA	DROP	< 30 U	0.00000	0.025	0	0
322	06/08/98	PXT0402	SARCODINA	DROP	< 30 U	0.00000	0.025	0	0
323	06/08/98	PXT0402	CLADOCERA	DROP	< 30 U	0.00000	0.025	0	0
324	06/08/98	PXT0402	BALANOMORPHA	DROP	>73 U	0.00000	1.000	0	0
325	06/08/98	PXT0402	POLYCHAETA	DROP	>73 U	0.00000	1.000	0	0
326	06/08/98	PXT0402	SARCODINA	DROP	>73 U	0.00000	1.000	0	0
327	06/08/98	PXT0402	CLADOCERA	DROP	>73 U	60.00000	1.000	60	0
328	06/08/98	PXT0402	ROTIFERA	ROTIFERS	31 TO 73 U	3.00000	0.025	120	4680
329	06/08/98	PXT0402	ROTIFERA	ROTIFERS	< 30 U	0.00000	0.025	0	0
330	06/08/98	PXT0402	ROTIFERA	ROTIFERS	>73 U	271.00000	1.000	271	0
331	06/08/98	PXT0402	TINTINNINA	TINTINNI	31 TO 73 U	33.00000	0.025	1320	51480
332	06/08/98	PXT0402	TINTINNINA	TINTINNI	< 30 U	61.00000	0.025	2440	95160
333	06/08/98	PXT0402	TINTINNINA	TINTINNI	>73 U	8.00000	1.000	8	0

summed over size fractions

OBS	DATE	STATION	TAXAGRP	OESTCNT	OESTSVAR
1	03/23/98	MCB5.2	CILIATES	5394	101080
2	03/24/98	MET5.2	CILIATES	14041	547560
3	04/06/98	MCB5.2	CILIATES	7985	151620
4	04/07/98	MET5.1	CILIATES	7321	285480
5	04/13/98	PXT0402	CILIATES	960	37440
6	04/13/98	XDE5339	CILIATES	5640	219960
7	05/04/98	MLE2.2	CILIATES	3895	148200
8	05/05/98	MCB3.3C	CILIATES	12833	243200
9	05/05/98	MET5.1	CILIATES	3601	140400
10	05/06/98	MCB2.1	CILIATES	16623	315780
11	06/01/98	XEA6595	CILIATES	43727	1705080
12	06/02/98	MCB4.3	CILIATES	11335	215080
13	06/02/98	MWT5.1	CILIATES	22264	422560
14	06/08/98	PXT0402	CILIATES	6240	243360
15	03/23/98	MCB5.2	COPEPODS	19	0
16	03/24/98	MET5.2	COPEPODS	118	0
17	04/06/98	MCB5.2	COPEPODS	29	0
18	04/07/98	MET5.1	COPEPODS	206	0
19	04/13/98	PXT0402	COPEPODS	517	0
20	04/13/98	XDE5339	COPEPODS	107	0
21	05/04/98	MLE2.2	COPEPODS	346	0
22	05/05/98	MCB3.3C	COPEPODS	123	380
23	05/05/98	MET5.1	COPEPODS	308	0
24	05/06/98	MCB2.1	COPEPODS	194	0
25	06/01/98	XEA6595	COPEPODS	54	0
26	06/02/98	MCB4.3	COPEPODS	396	380
27	06/02/98	MWT5.1	COPEPODS	74	0
28	06/08/98	PXT0402	COPEPODS	323	1560
29	03/23/98	MCB5.2	ROTIFERS	1190	19380
30	03/24/98	MET5.2	ROTIFERS	9	0
31	04/06/98	MCB5.2	ROTIFERS	448	7220
32	04/07/98	MET5.1	ROTIFERS	152	3120
33	04/13/98	PXT0402	ROTIFERS	265	6240
34	04/13/98	XDE5339	ROTIFERS	137	4680
35	05/04/98	MLE2.2	ROTIFERS	54	0
36	05/05/98	MCB3.3C	ROTIFERS	542	9500
37	05/05/98	MET5.1	ROTIFERS	3083	56160
38	05/06/98	MCB2.1	ROTIFERS	3988	73340
39	06/01/98	XEA6595	ROTIFERS	5751	198120
40	06/02/98	MCB4.3	ROTIFERS	66	1140
41	06/02/98	MWT5.1	ROTIFERS	635	2280
42	06/08/98	PXT0402	ROTIFERS	391	4680
43	03/23/98	MCB5.2	TINTINNI	6168	108300
44	03/24/98	MET5.2	TINTINNI	124	4680
45	04/06/98	MCB5.2	TINTINNI	15013	283480
46	04/07/98	MET5.1	TINTINNI	3961	154440
47	04/13/98	PXT0402	TINTINNI	360	14040
48	04/13/98	XDE5339	TINTINNI	2845	110760
49	05/04/98	MLE2.2	TINTINNI	3293	127920
50	05/05/98	MCB3.3C	TINTINNI	3009	56620

summed over size fractions

OBS	DATE	STATION	TAXAGRP	OESTCNT	OESTSVAR
51	05/05/98	MET5.1	TINTINNI	14438	561600
52	05/06/98	MCB2.1	TINTINNI	3304	62700
53	06/01/98	XEA6595	TINTINNI	8844	344760
54	06/02/98	MCB4.3	TINTINNI	1465	27740
55	06/02/98	MWT5.1	TINTINNI	8705	164160
56	06/08/98	PXT0402	TINTINNI	3768	146640

TAXAGRP=CILIATES

OBS	STATION	DATE	AESTCNT	AESTSVAR	OESTCNT	OESTSVAR	DIFF	VARDIFF	Z_SCORE
1	MCB2.1	05/06/98	2837.74	107119.09	16623	315780	-13785.26	442359.83	-20.7266
2	MCB3.3C	05/05/98	1672.00	1660.00	12833	243200	-11161.00	259365.00	-21.9153
3	MCB4.3	06/02/98	.	.	11335	215080	.	.	.
4	MCB4.3C	06/02/98	553.00	0.00	.	.	.	.	.
5	MCB5.2	03/23/98	67.00	0.00	5394	101080	-5327.00	106541.00	-16.3202
6	MCB5.2	04/06/98	721.00	0.00	7985	151620	-7264.00	160326.00	-18.1415
7	MET5.1	04/07/98	66.00	0.00	7321	285480	-7255.00	292867.00	-13.4061
8	MET5.1	05/05/98	100.00	0.00	3601	140400	-3501.00	144101.00	-9.2227
9	MET5.2	03/24/98	192.00	0.00	14041	547560	-13849.00	561793.00	-18.4769
10	MLE2.2	05/04/98	2598.00	2598.00	3895	148200	-1297.00	157291.00	-3.2703
11	MWT5.1	06/02/98	.	.	22264	422560	.	.	.
12	MWT5.1	06/03/98	369.00	0.00	.	.	.	.	.
13	PXT0402	04/13/98	.	.	960	37440	.	.	.
14	PXT0402	06/08/98	130.00	0.00	6240	243360	-6110.00	249730.00	-12.2266
15	XDE5339	04/13/98	.	.	5640	219960	.	.	.
16	XEA6595	06/01/98	.	.	43727	1705080	.	.	.
17	XEA6596	06/01/98	938.00	0.00	.	.	.	.	.

TAXAGRP=COPEPOD NAUPLII

OBS	STATION	DATE	AESTCNT	AESTSVAR	OESTCNT	OESTSVAR	DIFF	VARDIFF	Z_SCORE
18	MCB2.1	05/06/98	13166.67	1084055.56	194	0	12972.67	1097416.22	12.3835
19	MCB3.3C	05/05/98	5918.92	154051.86	123	380	5795.92	160473.78	14.4684
20	MCB4.3	06/02/98	.	.	396	380	.	.	.
21	MCB4.3C	06/02/98	24909.09	1107322.31	.	.	.	.	.
22	MCB5.2	03/23/98	666.67	88222.22	19	0	647.67	88907.89	2.1721
23	MCB5.2	04/06/98	2037.04	375192.04	29	0	2008.04	377258.08	3.2693
24	MET5.1	04/07/98	9071.43	638887.76	206	0	8865.43	648165.18	11.0118
25	MET5.1	05/05/98	16739.13	3622202.27	308	0	16431.13	3639249.40	8.6131
26	MET5.2	03/24/98	8000.00	173818.18	118	0	7882.00	181936.18	18.4789
27	MLE2.2	05/04/98	22077.92	1910794.40	346	0	21731.92	1933218.32	15.6300
28	MWT5.1	06/02/98	.	.	74	0	.	.	.
29	MWT5.1	06/03/98	38000.00	3280181.82	.	.	.	.	.
30	PXT0402	04/13/98	.	.	517	0	.	.	.
31	PXT0402	06/08/98	18857.14	1328081.63	323	1560	18534.14	1348821.78	15.9586
32	XDE5339	04/13/98	.	.	107	0	.	.	.
33	XEA6595	06/01/98	.	.	54	0	.	.	.
34	XEA6596	06/01/98	3444.44	379271.60	.	.	.	.	.

TAXAGRP=ROTIFERS

OBS	STATION	DATE	AESTCNT	AESTSVAR	OESTCNT	OESTSVAR	DIFF	VARDIFF	Z_SCORE
35	MCB2.1	05/06/98	8608.70	365682.42	3988	73340	4620.70	451619.12	6.8758
36	MCB3.3C	05/05/98	17275.96	853924.28	542	9500	16733.96	881242.24	17.8259
37	MCB4.3	06/02/98	.	.	66	1140	.	.	.
38	MCB4.3C	06/02/98	1409.09	62640.50	.	.	.	.	.
39	MCB5.2	03/23/98	45066.67	5963822.22	1190	19380	43876.67	6029458.89	17.8688
40	MCB5.2	04/06/98	18333.33	3376728.40	448	7220	17885.33	3402729.73	9.6958
41	MET5.1	04/07/98	3285.71	114061.22	152	3120	3133.71	120618.94	9.0230
42	MET5.1	05/05/98	255652.17	95869111.53	3083	56160	252569.17	96184006.71	25.7531
43	MET5.2	03/24/98	287.88	4073.92	9	0	278.88	4370.80	4.2183
44	MLE2.2	05/04/98	3238.10	150956.92	54	0	3184.10	154249.01	8.1073
45	MWT5.1	06/02/98	.	.	635	2280	.	.	.
46	MWT5.1	06/03/98	7090.91	315223.14	.	.	.	.	.
47	PXT0402	04/13/98	.	.	265	6240	.	.	.
48	PXT0402	06/08/98	9857.14	694224.49	391	4680	9466.14	709152.63	11.2410
49	XDE5339	04/13/98	.	.	137	4680	.	.	.
50	XEA6595	06/01/98	.	.	5751	198120	.	.	.
51	XEA6596	06/01/98	206222.22	40583901.23	.	.	.	.	.

TAXAGRP=TINTINNI

OBS	STATION	DATE	AESTCNT	AESTSVAR	OESTCNT	OESTSVAR	DIFF	VARDIFF	Z_SCORE
52	MCB2.1	05/06/98	145.43	5540.64	3304	62700	-3158.57	71690.08	-11.7967
53	MCB3.3C	05/05/98	28210.34	1453229.41	3009	56620	25201.34	1541068.76	20.3008
54	MCB4.3	06/02/98	.	.	1465	27740	.	.	.
55	MCB4.3C	06/02/98	194.82	8082.64	.	.	.	.	.
56	MCB5.2	03/23/98	43281.00	5716800.00	6168	108300	37113.00	5874549.00	15.3122
57	MCB5.2	04/06/98	6993.85	1262009.60	15013	283480	-8019.15	1567496.45	-6.4051
58	MET5.1	04/07/98	2925.86	100423.47	3961	154440	-1035.14	261750.33	-2.0233
59	MET5.1	05/05/98	12486.30	2681370.51	14438	561600	-1951.70	3269894.81	-1.0793
60	MET5.2	03/24/98	108.06	1500.92	124	4680	-15.94	6412.98	-0.1990
61	MLE2.2	05/04/98	26280.73	2362157.02	3293	127920	22987.73	2519650.75	14.4819
62	MWT5.1	06/02/98	.	.	8705	164160	.	.	.
63	MWT5.1	06/03/98	14673.36	650652.89	.	.	.	.	.
64	PXT0402	04/13/98	.	.	360	14040	.	.	.
65	PXT0402	06/08/98	2596.43	181102.04	3768	146640	-1171.57	334106.47	-2.0269
66	XDE5339	04/13/98	.	.	2845	110760	.	.	.
67	XEA6595	06/01/98	.	.	8844	344760	.	.	.
68	XEA6596	06/01/98	35.00	0.00	.	.	.	.	.

TAXAGRP=CILIATES
Univariate Procedure
Variable=DIFF

Moments

N	9	Sum Wgts	9
Mean	-7727.7	Sum	-69549.3
Std Dev	4387.334	Variance	19248697
Skewness	-0.25196	Kurtosis	-0.98646
USS	6.9145E8	CSS	1.5399E8
CV	-56.7742	Std Mean	1462.445
T:Mean=0	-5.28409	Pr>|T|	0.0007
Num ^= 0	9	Num > 0	0
M(Sign)	-4.5	Pr>=|M|	0.0039
Sgn Rank	-22.5	Pr>=|S|	0.0039
W:Normal	0.935927	Pr<W	0.5332

Quantiles(Def=5)

100% Max	-1297	99%	-1297
75% Q3	-5327	95%	-1297
50% Med	-7255	90%	-1297
25% Q1	-11161	10%	-13849
0% Min	-13849	5%	-13849
		1%	-13849

Range	12552	Q3-Q1	5834	Mode	-13849

Extremes

Lowest	Obs	Highest	Obs
-13849(	9)	-7255(	7)
-13785.3(	1)	-6110(	14)
-11161(	2)	-5327(	5)
-7264(	6)	-3501(	8)
-7255(	7)	-1297(	10)

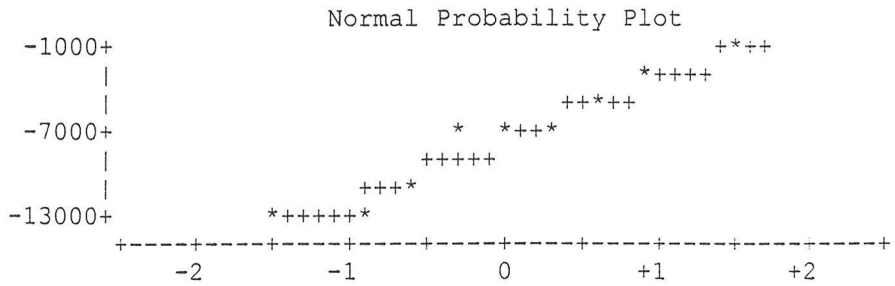
Missing Value	.
Count	8
% Count/Nobs	47.06

TAXAGRP=CILIATES

Univariate Procedure

Variable=DIFF

```
   Stem Leaf                     #  Boxplot
     -0 3                        1     |
     -2 5                        1     |
     -4 3                        1  +-----+
     -6 331                      3  *--+--*
     -8                             |     |
    -10 2                        1  +-----+
    -12 88                       2     |
        ----+----+----+----+
    Multiply Stem.Leaf by 10**+3
```



TAXAGRP=COPEPODS

Univariate Procedure

Variable=DIFF

Moments

N	9	Sum Wgts	9
Mean	10540.99	Sum	94868.91
Std Dev	7355.413	Variance	54102103
Skewness	0.165971	Kurtosis	-1.23572
USS	1.4328E9	CSS	4.3282E8
CV	69.77915	Std Mean	2451.804
T:Mean=0	4.299279	Pr>|T|	0.0026
Num ^= 0	9	Num > 0	9
M(Sign)	4.5	Pr>=|M|	0.0039
Sgn Rank	22.5	Pr>=|S|	0.0039
W:Normal	0.960806	Pr<W	0.8008

Quantiles(Def=5)

100% Max	21731.92	99%	21731.92
75% Q3	16431.13	95%	21731.92
50% Med	8865.429	90%	21731.92
25% Q1	5795.919	10%	647.6667
0% Min	647.6667	5%	647.6667
		1%	647.6667
Range	21084.26		
Q3-Q1	10635.21		
Mode	647.6667		

Extremes

Lowest	Obs	Highest	Obs
647.6667(	5)	8865.429(	7)
2008.037(	6)	12972.67(	1)
5795.919(	2)	16431.13(	8)
7882(	9)	18534.14(	14)
8865.429(	7)	21731.92(	10)

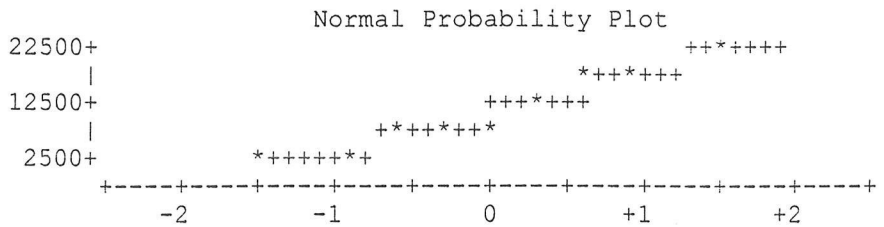
Missing Value	.
Count	8
% Count/Nobs	47.06

TAXAGRP=COPEPODS

Univariate Procedure

Variable=DIFF

```
   Stem Leaf                     #  Boxplot
      2 2                        1      |
      1 69                       2  +------+
      1 3                        1  |   +  |
      0 689                      3  *------*
      0 12                       2      |
        ----+----+----+----+
    Multiply Stem.Leaf by 10**+4
```



TAXAGRP=ROTIFERS

Univariate Procedure

Variable=DIFF

Moments

N	9	Sum Wgts	9
Mean	39083.18	Sum	351748.7
Std Dev	81163.26	Variance	6.5875E9
Skewness	2.852985	Kurtosis	8.300991
USS	6.645E10	CSS	5.27E10
CV	207.668	Std Mean	27054.42
T:Mean=0	1.444614	Pr>|T|	0.1866
Num ^= 0	9	Num > 0	9
M(Sign)	4.5	Pr>=|M|	0.0039
Sgn Rank	22.5	Pr>=|S|	0.0039
W:Normal	0.517725	Pr<W	0.0001

Quantiles(Def=5)

100% Max	252569.2	99%	252569.2
75% Q3	17885.33	95%	252569.2
50% Med	9466.143	90%	252569.2
25% Q1	3184.095	10%	278.8788
0% Min	278.8788	5%	278.8788
		1%	278.8788
Range	252290.3		
Q3-Q1	14701.24		
Mode	278.8788		

Extremes

Lowest	Obs	Highest	Obs
278.8788(	9)	9466.143(	14)
3133.714(	7)	16733.96(	2)
3184.095(	10)	17885.33(	6)
4620.696(	1)	43876.67(	5)
9466.143(	14)	252569.2(	8)

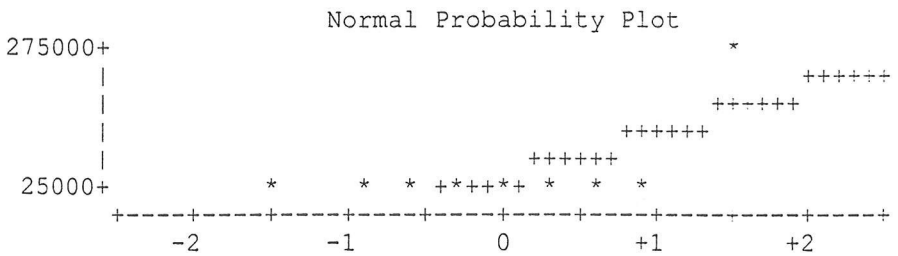
Missing Value	.
Count	8
% Count/Nobs	47.06

TAXAGRP=ROTIFERS

Univariate Procedure

Variable=DIFF

```
   Stem Leaf                     #  Boxplot
      2 5                        1     *
      2
      1
      1
      0
      0 00001224                 8  +--0--+
        ----+----+----+----+
    Multiply Stem.Leaf by 10**+5
```



TAXAGRP=TINTINNI

Univariate Procedure

Variable=DIFF

Moments

N	9	Sum Wgts	9
Mean	7772.223	Sum	69950.01
Std Dev	16115.94	Variance	2.5972E8
Skewness	0.9999	Kurtosis	-0.64631
USS	2.6215E9	CSS	2.0778E9
CV	207.353	Std Mean	5371.979
T:Mean=0	1.446808	Pr>|T|	0.1860
Num ^= 0	9	Num > 0	3
M(Sign)	-1.5	Pr>=|M|	0.5078
Sgn Rank	1.5	Pr>=|S|	0.9102
W:Normal	0.803049	Pr<W	0.0226

Quantiles(Def=5)

100% Max	37113	99%	37113
75% Q3	22987.73	95%	37113
50% Med	-1035.14	90%	37113
25% Q1	-1951.7	10%	-8019.15
0% Min	-8019.15	5%	-8019.15
		1%	-8019.15
Range	45132.15		
Q3-Q1	24939.42		
Mode	-8019.15		

Extremes

Lowest	Obs	Highest	Obs
-8019.15(	6)	-1035.14(	7)
-3158.57(	1)	-15.9394(	9)
-1951.7(	8)	22987.73(	10)
-1171.57(	14)	25201.34(	2)
-1035.14(	7)	37113(	5)

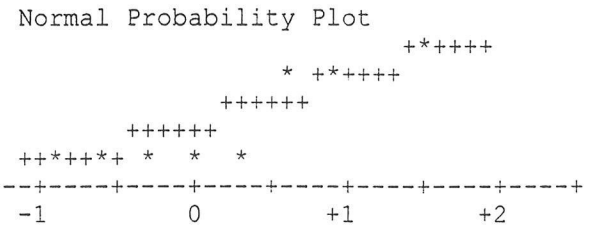
Missing Value	.
Count	8
% Count/Nobs	47.06

TAXAGRP=TINTINNI

Univariate Procedure

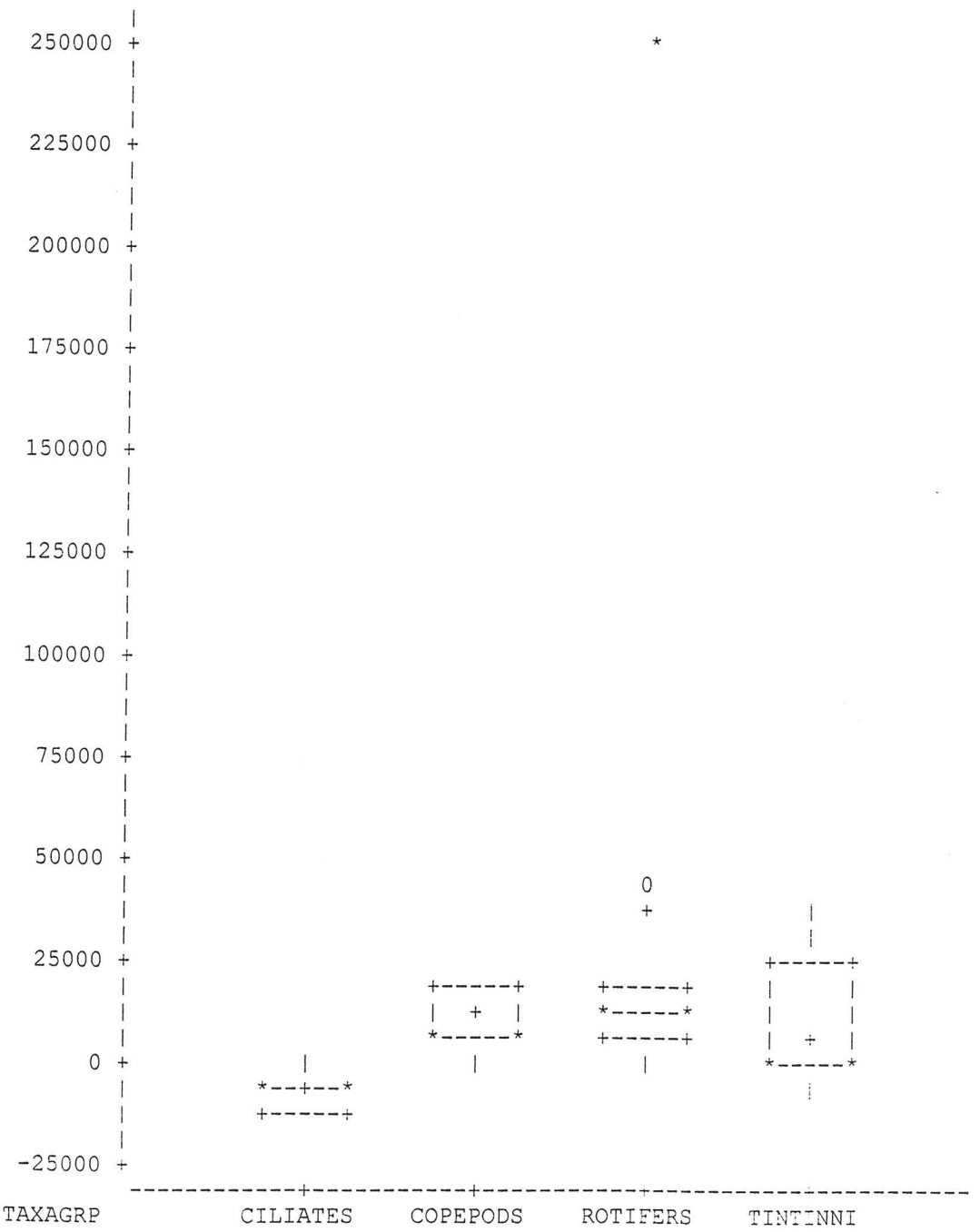
Variable=DIFF

```
Stem Leaf                     #  Boxplot
   3 7                        1     |
   2 35                       2  +------+
   1                             |      |
   0                             |  +   |
  -0 832110                   6  *------*
     ----+----+----+----+
  Multiply Stem.Leaf by 10**+4
```



Univariate Procedure
Schematic Plots

Variable=DIFF



Split Sampling Study for the Maryland and Virginia Mesozooplankton Monitoring Programs

Final Report, June 2000

Prepared by

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for

United States Environmental Protection Agency
Chesapeake Bay Program
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Forward

A draft report of the 1998-1999 Mesozooplankton Split Sample Study was compiled by the Interstate Commission on the Potomac River Basin in April, 2000. The draft report was reviewed by the state monitoring program managers, principal investigators and staff of the zooplankton monitoring programs, and representatives from the Chesapeake Bay Program Monitoring and Living Resources subcommittees. Comments and recommended changes from reviewers were documented in a tracking sheet and specific changes to the draft report ("actions") were proposed. The tracking sheet and proposed changes were submitted for review and approval to the Monitoring Subcommittee Coordinator and the program managers in the Virginia Department of Environmental Quality and Maryland Department of Natural Resources. Approved changes were then implemented in this final report. Uncontested sections were also edited to condense or clarify text.

This is a chapter of ICPRB Report 00-3

To receive additional copies of the report please call or write:
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301-984-1908

Funds to support this effort came from the US Environmental Protection Agency Grant CB-993067-01.

Executive Summary

Laboratory methods of the Maryland and Virginia mesozooplankton monitoring programs had not been compared before this 1998-1999 Split Sampling Study, however state managers and laboratory staff were aware that method differences were affecting the monitoring results. The programs implemented modifications to their laboratory counting protocols in 1998 in order to better estimate species richness in Maryland and eliminate laboratory sieving losses of smaller mesozooplankton taxa and life stages in Virginia. The goal was to make Chesapeake Bay mesozooplankton counts in the two states directly comparable. The 1998 - 1999 Split Sample Study indicates the desired outcomes of the modifications were only partially accomplished. The "new" Versar counting method (Maryland program) has improved Versar's ability to measure species richness, an important Bay-wide indicator, and the "new" ODU counting method (Virginia program) has increased ODU's taxa counts per sample. However, the "new" ODU method still produces significantly lower total counts than the Versar method. The method consistently counts less of certain taxa, particularly the immature (copepodite) life stage of calanoid copepods which are a common and frequently dominant taxonomic group. Sample variances in counts produced with the "new" ODU method are higher than sample variances in counts produced with the Versar method, hence the ODU estimates of precision are lower. Finally, the number of taxa identified per sample was on average lower in the "new" ODU counts.

A single method needs to be selected and implemented because the modified laboratory methods of the two programs do not produce comparable results. While program principal investigators feel the existing monitoring data provide meaningful status and trend assessments *within* each state, a single method will ensure that Maryland and Virginia results are comparable *bay-wide*. It will allow the CBP monitoring programs to calculate and use a diverse suite of bay-wide mesozooplankton indicators and more effectively address the information needs of the Program. Bay-wide zooplankton community indicators are needed because they are useful tools in measuring overall ecosystem health, targeting restoration efforts in open water habitats, and tracking food web responses to management actions such as nutrient and sediment reductions.

The Split Sample Study identified other procedural problems that need to be resolved. There appears to be within laboratory and between laboratory differences in taxonomic identifications. These differences could be reconciled with side-by-side comparisons and the assembly of a photographic or archival specimen collection for Chesapeake Bay mesozooplankton. Quality assurance procedures should be maintained in each laboratory to ensure adequate taxonomic training of new technical staff. Quality assurance (repeated) counts for each laboratory should be regularly submitted to the states, the Chesapeake Bay Program or their designees for independent analysis. Regular site visits between the two states' technical staffs should be carried out to ensure comparable interstate taxonomy. A split sample study should be done annually for at least the next few years to ensure interstate count comparability.

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Split Sampling Study for the Maryland and Virginia Mesozooplankton Monitoring Programs

Final Report, June 2000

Introduction

The Chesapeake Bay Monitoring Program has included a plankton component since it began in 1984. The current Maryland and Virginia zooplankton programs are partially or mostly funded through the CBP Living Resources Subcommittee. Old Dominion University (ODU) collects and counts mesozooplankton for Virginia Department of Environmental Quality (VADEQ); Versar, Inc. collects and counts mesozooplankton for Maryland Department of Natural Resources (MDDNR). While sample collection methods in the field are reasonably comparable, discrepancies were suspected in the mesozooplankton data from the start because the laboratories began their monitoring programs using different laboratory analysis methods (see Appendix A and documentation on-line at http://www.chesapeakebay.net/). The ODU and Versar laboratory methods had not been directly compared before the 1998-1999 Split Sampling Study.

Versar employs a variation of a commonly used counting technique of subsampling using the Stempel pipette method. The Versar method dilutes samples to a standard volume (e.g. 800 mls) and counts subsamples until the requisite number of organisms has been counted to attain $\pm$20% precision for the total count. This method is known to less accurately count the rarer species of zooplankton. Early in the program, Versar also scanned the entire sample at low magnification and counted all larger, rarer forms. The laboratory dropped this effort in 1989 due to budget constraints, but instituted a hierarchical counting modification which produces better counts of subdominant species. An error level of at least 25% is presently obtained for the dominant and subdominant taxa while a level of $\leq$20% is maintained for the total count.

ODU employs a modification of the innovative Controlled Variability Sampling (CVS) method which is intended to reduce the variance in counts of the larger, rarer forms (Alden et al. 1982). Samples are filtered through the CVS apparatus which consists of a stack of differently sized sieves that sort zooplankton individuals by size ranges. Organisms on each sieve are washed off the sieve and repeatedly split with a Folsom Plankton Splitter until the number of organisms has been reduced to a level where an entire split can be counted. This method is designed to more accurately count the larger, rarer forms. An error level of 35% was chosen for both common and rare species of interest. The CVS method used in the ODU monitoring program was different from the method originally described in Alden et al. (1982) in an important way: the monitoring program used a series of 2000, 850, 600, 300, and 200 micron sieves while the original method used a series of 2000, 850, 600, 300, 150, and 75 micron sieves. A percentage of mesozooplankton taxa was suspected of being lost by the monitoring program CVS apparatus because its bottom sieve (200 microns) was the same mesh size as that of the ODU plankton nets used to collect mesozooplankton samples in the field. Long, narrow mesozooplankton such as copepods are know to pass through 200 micron mesh plankton nets and sieves (e.g. Edmondson and Winberg 1981, Harris et al. 2000).

Recent efforts to develop and apply bay-wide zooplankton indicators of ecosystem health highlighted the data discrepancies. Program principal investigators felt that status and trend analyses of the monitoring data *within* each state were valid and provided good information in spite of the methodology biases. However, the application and use of many potential bay-wide indicators were suspect because Maryland and Virginia data sets did not appear to be comparable. The Chesapeake Bay Program needs bay-wide zooplankton community indicators because they are useful tools in measuring overall ecosystem health and targeting restoration efforts in open water habitats (status), and tracking food web responses to management actions such as nutrient and sediment reductions (trends and linkages). Mesozooplankton indicators will soon be used to measure CBP progress in attaining plankton restoration goals. Before bay-wide indicators can calculated and used with any confidence, the Maryland and Virginia mesozooplankton monitoring data must be made comparable.

After long-running discussions and several meetings, the ODU and Versar mesozooplankton monitoring program staffs met in January 1998 at ODU for a side-by-side comparison of counting techniques. The comparisons showed that Maryland protocols insufficiently measured mesozooplankton species richness because they were not counting large, rare taxa (e.g. *Neomysis americana*, *Rithropanopeus harrissii*). Virginia protocols counted significantly lower abundances of major mesozooplankton species (Table 1), especially for the sole tidal freshwater sample where the ODU total count was less than 1% of the Versar total count.

The Maryland and Virginia programs agreed that modifications to their current laboratory methods might resolve the discrepancies. The laboratories recommended specific changes to improve comparability. The "new" method modifications would give the programs both backwards and forwards compatibility in both states. This was the desired outcome from the management and data analysis perspectives. The states would not lose data for long-term trend analyses (backward comparability), and they would have direct comparability in the future (forward comparability). Regular split sampling would be used to document that this "performance-based approach" was successful, i.e. different methods were producing the same results.

The proposed modification were as follows:

- ODU staff would continue to use the customary Controlled Variability Sampling (CVS) apparatus. They would attach a 72 micron sieve to the bottom of the CVS apparatus in order to capture smaller-sized individuals which had previously been washed through the CVS system into the sink. ODU would obtain an "old method count" using data collected from the CVS original sieves and a "new method count" by combining enumerations from the old method and the 72 micron sieve.
- Versar would add a step to its usual subsample counting method. After completing its standard protocol and obtaining an "old method count," Versar would filter the whole sample through a large-size (1 mm) screen to concentrate and enumerate the rarer, large-sized individuals. Versar would obtained a "new method count" by combining enumerations from the old method and the large-size sieve.

Calculations of species densities that *include* the additional "patch" counts are intended to make the mesozooplankton results from the two laboratories directly comparable. If split sampling shows that they were, the "new method counts" would be used in the future to calculate bay-wide

indicators. Calculations of species densities that do not include the additional "patch" counts would allow both laboratories to maintain backward compatibility with the historical data in each state and continue to determine long-term trends.

In July 1998, ODU ended efforts to measure mesozooplankton biomass (dry weights and ash-free dry weights) and began counting the additional, 72 micron sample fraction. Versar had already dropped its laboratory measurements of biomass and had begun counts of the sample fraction caught in the large-sized sieve. A split sample study was needed to confirm that the new methods were working as intended.

Split Sample Project - Round 1

A split sample project was proposed to the Monitoring Subcommittee in the Spring of 1998, and funds were made available to the contractors to enumerate split samples. The "new method counts" for mesozooplankton were intended to demonstrate the new methods' comparability. Split samples were collected in April, May and June of 1998. The Virginia and Maryland laboratories each collected 12 mesozooplankton samples during their regular monitoring cruises. The preserved samples were split in half. One split was enumerated by the originating laboratory as part of its monitoring program, and the other was enumerated by the corresponding lab in the other state. Unless otherwise noted, the counts produced for each split sample were enumerations of all taxa in the sample, identified to the usual taxonomic level. The sites investigated included locations in a range of salinities, with exposure to different river basins and environmental conditions. Two sets of counts were produced by Versar and ODU for each split sample: one count generated with the laboratory's old method and one generated with their modified method. Specifically, Versar produced a count with its original method and a count which included enumerations of mesozooplankton caught on the added 850 micron sieve. ODU produced a count with its original CVS method and a count which included enumerations of mesozooplankton caught on the added 72 micron sieve. Mr.Mateja, Mr. Crock, and Mr. Miebert, the three ODU mesozooplankton laboratory staff, all participated in counting the 24 Virginia split samples.[1] Mr. Craig Bruce of the Versar staff counted the 24 Maryland split samples. All split sample enumerations were completed in the late summer of 1998, and the results were forwarded to the CBP Quality Assurance Officer and to the Interstate Commission on the Potomac River Basin for analysis. Commission staff sent the raw data to Elgin Perry (statistician) for analysis and also calculated a suite of mesozooplankton indicators (Table 2, 3).

The results of this first set of mesozooplankton split sample counts ("Round 1") were discussed at the "Plankton Summit" meeting[2] and in a subsequent conference call. It was concluded that the Round 1 mesozooplankton results were mostly invalid due to a malfunction of the modified

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[1] The author of this report was under the impression that the ODU laboratory supervisor, George Mateja, was the sole counter of the Virginia split samples in Round 1 and listed him as such in the minutes of various conference calls and in the report's draft version. The ODU Principal Investigator indicated in his review that all three of the ODU mesozooplankton laboratory staff participated in counting the Round 1 split samples.

[2] A meeting of the plankton monitoring program principal investigators, staff, managers and data analysts was held at Old Dominion University on September 11-12, 1998. Dubbed the "Plankton Summit" by participants, the purpose of the two-day meeting was to review the initial results of the phytoplankton, microzooplankton and mesozooplankton split sample studies. Participants were also given a tour of the ODU plankton laboratories.

CVS method at ODU and other problems. The motorized siever of the CVS method originally had a stack of 2000, 850, 600, 300, and 200 micron sieves, and an additional sieve chamber with a size of around 75 microns was added to the bottom of the sieve array in order to capture and count the smaller mesozooplankton taxa. The modified apparatus appeared to function normally while the first round of splits was carried out. However, after the Plankton Summit, ODU staff realized that zooplankton were being forced out of the sides of the smallest, added sieve chamber. The normal tolerances that worked for the other sieve chambers were not working between the 75 micron and 200 micron chamber because of increased water pressure in the 75 micron sieve chamber. The problem could be fixed by removing the 75 micron chamber and adding a 63 micron passive sieve placed underneath as a catch basin for discharge water. There were additional problems with Round 1 of the split sample study that cast doubt on the validity of the results. Six splits counted by ODU (five collected by Versar, one by ODU) were in a state of decomposition when they were processed for sampling by ODU. The laboratory sheets note "poorly preserved" on the samples. Also, ODU's original electronic data submittal contained many data entry problems, so a number of iterations of the data developed as these errors were caught and corrected. The processed data (e.g. indicators) produced by ICPRB are probably accurate for the most part however they were never closely checked against the raw data sheets to confirm that all the errors were caught.

Despite the CVS method malfunction, the poorly preserved samples, and the data entry errors, there are a few general conclusions that can be drawn from some of the data:

- Species richness was higher in the modified Versar method, indicating the Versar modified method, or "patch," was working.
- Percent differences between the Versar and ODU counts were often greater than $\pm$ 20%, suggesting a high degree of variability is occurring in one or both laboratories.
- The QA/QC counts of the two laboratories could not be directly compared because ODU does replicate counts on only the splits of a single size fraction of the sample while Versar counts an additional subsample of the entire sample.
- There appear to be taxonomic differences in the counts produced by the two laboratories. Specifically:
  - *Temora longicornis* vs. *T. turbinata*
  - *Cyclops vernalis* vs. *Anthocyclops vernalis*
  - *Eurytemora affinis* vs. *Eurytemora hurinoides*
  - Polyhaline species *Temora longicornis* identified at tidal fresh and oligohaline stations by ODU
  - RET3.1 differences in Cladocera

The differences in the ODU and Versar "new" method counts continue to prevent bay-wide application of most of the indicators developed for mesozooplankton to characterize health of the zooplankton community in Chesapeake Bay.

The following recommendations were made during the "Plankton Summit" before the flaws in the ODU data were realized:

- Recommendation: A more thorough statistical analysis of the split sample data should be performed.
- Recommendation: The laboratories should institute a regular split sample program.
- Recommendation: If future split sample counts aren't comparable, a microspheres (beads) experiment could be done to compare the lab methods using known quantities of

different sized beads. Alternatively, the method could be tested on a prepared sample of known quantity and species composition.

- Recommendation: The laboratories should resolve taxonomic issues that seem to be occurring between laboratories.
- Recommendation: The historical data should be corrected to reflect the taxonomic regrouping, changes, etc. made in resolving the above taxonomic issues. This will involve resubmittal of the data.
- Recommendation: Several taxa are counted by both the micro- and mesozooplankton programs. The group made the following decision:
  - *Bosmina*, barnacle nauplii and polychaete larvae counts from the mesozooplankton program data should be used for purposes of calculating bay-wide indicators.
  - Pelecypod larvae, rotifer, and copepod nauplii counts from the microzooplankton program data should be used for the bay-wide indicators.
  - Individual programs should count whatever is in their samples if they want to and put those numbers in their own databases, but should include only the appropriate taxa in the databases they submit to the CBP Data Center.
- Recommendation: Provided there are funds, ODU should start to do complete replicate sample counts as part of an additional QA/QC procedure to check precision. The method will be similar to the procedure currently used by Versar. This would involve an additional sample count per month. ODU's old QA/QC method of counting both pairs of a split for one of the seives may or may not be continued.

Split Sample Project - Round 2

The ODU mesozooplankton monitoring program proposed redoing the split sample counts after further modifying the CVS method to overcome the leakage problem caused by the addition of the 75 micron sieve. They would: a) return to using the originally sieves (i.e. 2000, 850, 600, 300, and 200 u mesh) and approach, b) funnel the water washing through the CVS apparatus into a large diameter, 64 u mesh sieve as it drains into the sink, and c) count all of the original sieve fractions and the additional 64 u sieve fraction. The ODU laboratory could perform recounts on some or all of their original split samples (12 from Versar, 12 from ODU) because a) Versar archived its 12 split samples after counting them and could provide them to ODU, and b) ODU archived splits of its original split samples and could recount them. Measures could be taken to ensure the counts are done "blind."

Funds within an existing grant to the Interstate Commission on the Potomac River Basin (ICPRB) were reallocated to provide funds for ODU to recount splits. The planned statistical analysis of the questionable Round 1 split samples was discarded, and Dr. Elgin Perry (statistician) agreed to analyze the new split sample results ("Round 2") as they were produced. A contract was set up between ICPRB and Mr. Forrest Crock, the ODU technical staff member designated by ODU Principal Investigator Kent Carpenter to do the recounts. Assurances were made that Mr. Crock had been trained by Mr. Mateja, the ODU laboratory supervisor, and produced comparable counts.

In Round 2, ten archived split samples from Round 1 were recounted by ODU, then a side-by-side taxonomic comparison was performed by the staff of the two programs, and finally ten new split samples were counted by both Versar and ODU. Round 2 results were reviewed after the

first five splits, the second five splits, and the final ten splits. Adjustments were made to the original Round 2 scope of work at these times as issues developed or were resolved. The results of the first ten (archived) split samples are not directly comparable to the second ten (new) split samples because the laboratories made corrections in taxonomic identifications midway. Furthermore, ODU made changes in life stage identification procedures between the first five and second five splits which affected comparisons of adult and copepodite life stage abundances of certain copepods. Attention therefore is focused on the last ten split sample results in this report section, although major points from the first ten split samples are presented.

Round 2 - First Ten

Results of the ODU recounts of ten archived split samples ("first ten") were matched by ICPRB with their Versar counterparts (enumerated earlier as part of Round 1). In order to remove analysis differences caused by laboratory differences in level of taxonomic identification, ICPRB staff reviewed the species list and inserted an additional, adjusted NODC code for each count ("newNODC"). Species names or NODC codes that were closely related but not identical could then be matched on this new field. In many cases this involved backing species identifications to a higher taxonomic level (e.g. making "*Acartia* sp." and "*Acartia tonsa*"equivalent to "*Acartia*"). The data and data documentation were forwarded to Elgin Perry for statistical analysis. The following steps were taken by Dr. Perry in analyzing the split sample results. The procedures are described in more detail in Appendix B.

- Process the data: All raw counts within sample that have the same NEWNODC code were summed. The estimated taxon totals were recomputed from the raw data (this confirmed that Perry and ICPRB handling of the taxonomic data produced identical results). The estimated total count and sampling variance for each sample-taxa-lifestage (and sieve in the case of ODU) was computed. (For ODU, estimated counts and their variances were summed across sieves.)
- Coefficient of Variance: Univariate analysis was done on the difference between the Versar coefficient of variation and the ODU coefficient of variation for taxa-lifestages identified in both split samples. Variable = CVDIFF = Versar Coefficient of Variation minus ODU Coefficient of Variation. (Table 4).
- Z-Score: A z-score was computed using the variances in order to compare the labs on a sample by sample/taxa by taxa basis.
- Wilcoxon Signed Rank: A Wilcoxon signed rank analysis was done on all samples for each taxa. (Table 5).

The results were discussed by the zooplankton monitoring program staff in two conference calls. A summary of the observations and decisions is presented below:

- *Copepod life stage enumerations* Differences in Versar and ODU life stage counting procedures in laboratory were apparent in the original Round 1 counts and the first five recounts. They prevented direct comparisons of copepod results. Specifically, ODU enumerated copepodite life stages of just of *Acartia*, *Eurytemora*, and *Mesocyclops* while Versar enumerated copepodites of all copepods. Copepodites that are not identified as such in the ODU samples are automatically grouped with the "adult" category when the ICPRB indicator calculations are run. Therefore, the "adult copepod" category inadvertently contained copepodite numbers in the ODU results. ODU enumerated copepodites of all copepod species in counts after the first five split samples recounts.

- *Taxonomic identifications* There appear to be recurring differences between Versar and ODU identifications of some species.
- *Individual taxa abundances* The results of the Wilcoxon Sign Rank statistical test (Table 5) seem consistent with the sample by sample comparisons in that when the p-value for the signed rank analysis is small, the individual sample analyses show a preponderance of differences in one direction with at least some of them significant. As Dr. Perry pointed out at the Plankton Summit, small raw counts can have a big and sometimes arbitrary impact on split sample outcomes, and often do not accurately represent sample contents. Taxa comparisons that include small raw counts should probably be ignored in these ten split samples.
- *Total copepod and total mesozooplankton count comparisons* Counts for total mesozooplankton and total copepods, two general groupings of the data, are not affected by the small raw count issues above. Versar counts for these taxonomic groupings are usually higher than the equivalent ODU counts (Figure 1).
- *Taxa richness and diversity* On average, Versar identified more unique taxa per sample than ODU (Table 6). Versar may be finding more small-sized mesozooplankton taxa such as ostracods, *Alona*, chydorids, *Saphirella*, and *Cyclops vernalis* but raw counts of these taxa are often low and are therefore not as reliable.
- *Coefficients of variation* The coefficients of variation for the ODU counts are noticeably higher than the Versar coefficients of variation (Figure 2, Table 4).

The results suggest the ODU "patch" (i.e. addition of small mesh screen positioned below the CVS stacked sieves) is partially correcting the original loss problem but the Versar-ODU counts are not directly comparable and other issues–primarily taxonomic--need to be addressed.

Side-by-side review of Chesapeake Bay mesozooplankton taxonomy

The monitoring programs agreed that before more split samples were counted, the technical staff of the monitoring programs should meet and resolve the taxonomic differences apparent in the ODU/Versar split samples. Versar staff Craig Bruce traveled to ODU on March 10, 1999 and met with ODU staff Forrest Crock for two days. On March 12, 1999, they were joined by the George Mateja and Conrad Miebert, ODU, and Claire Buchanan, ICPRB, to review their findings. These findings were also summarized by the Versar staff after the meeting (Appendix C). Briefly,

- The laboratories agreed that annual meetings of the technical staff to discuss taxonomy and laboratory counting techniques should be continued to ensure comparability and allow the continued development of bay-wide zooplankton indicators.
- The laboratories agreed to stop including counts of rotifers since the microzooplankton programs generate more accurate numbers for this microzooplankton group.
- The laboratories agreed to identify to the lowest taxonomic level (e.g. *Gammarus* instead of unidentified amphipod) when possible in order to avoid inter-laboratory differences related to level of taxonomic identification.
- Versar technical staff previously misidentified barnacle cypris as ostracods at high salinity stations.
- One ODU staff previously misidentifying *Eurytemora* as *Temora* at some freshwater stations.
- ODU previously misidentifying *Eurytemora affinis* as *E. americana*.
- It appears that *E. affinis* and *E. hirundoides* are now considered to be synonymous.

- Nomenclature changes such as *Cyclops vernalis* to *Acanthocyclops vernalis* and *Cyclops bicuspidatus* to *Diacyclops thomasi* were discussed.
- To maintain consistency between the laboratories, it was agreed that:
  - The most common species of *Daphnia* will be identified to species level.
  - The most common species of Harpacticoid will be identified to genus and/or species level.
  - The most common Diptera will be identified to family or genus.
  - The most common Amphipod will be identified to family or genus.
  - Crab zoea and megalops will be identified to species level.
  - Specific larval stages (e.g. trochophore and spionidae) will not be differentiated. Instead they will be reported as polychaete larvae.
- The absence of *Bosmina longirostris* in the ODU and Versar Round 1 WE4.2 counts and its strong presence in the ODU Round 2 WE4.2 count was discussed. *Bosmina*, a freshwater species, is not found at mesohaline stations such as WE4.2. ODU felt the *Bosmina* count may have come from sample contamination during ODU sample sieving/splitting procedures.

Concerns about possible contamination of the split samples remaining to be recounted by ODU lead the group to agree to finish the split sample study with ten new samples. These same concerns also raised the issue of whether or not the Round 2 First Ten results and conclusions were tainted by contaminated split samples.

Round 2 Last Ten

Ten samples from the regular Maryland monitoring program (five from March, five from April) were used to avoid additional costs to the study. The samples were sent to ODU for counting after they had been counted and reconstituted by Versar staff. The split sample results were received by ICPRB staff in June, 1999, merged and sent to Elgin Perry for statistical analysis. The results (Table 7, Appendix D) and additional analyses provided by ICPRB (Table 8) were discussed in an October 19, 1999, conference call and in subsequent phone calls and emails. Briefly,

- *Total mesozooplankton count comparison* Differences between the Versar and ODU total mesozooplankton counts for individual split samples were greater than +20% in 9 out of 10 (90%) split samples, indicating Versar usually counted larger numbers of organisms in the split samples (Figure 3).
- *Individual taxa abundances* Sample-by-sample comparisons of taxa identified by both laboratories show that nearly a quarter of the z-scores (23%) are greater than 2.0 (i.e. Versar counts are significantly larger than ODU counts) while 11% of the z-scores are less than 2.0 (i.e. ODU counts significantly larger than Versar counts) (Appendix D). When the p-value for the Wilcoxon Signed Rank analysis is small (Table 7), the individual sample analysis show a preponderance of differences in one direction with at least some of them significant (Appendix D). In some cases, large sign rank differences are the result of the laboratories still identifying taxa to different taxonomic levels (lumping-versus-splitting). For example:
  - "Balanidae" vs "Balanus"
  - "trochophore" (ODU) and "polychaete" (Versar)
  - harpacticoida (Versar) vs *Canuella elongata*, *Euterpina acutifrons* (ODU)
- *Pooled data* In order to circumvent the high variance and/or low counts for some taxa, count data from the ten split samples were pooled to obtain "number per 10 samples."

- The pooled data show that several species and taxonomic groups have similar counts (i.e. # per 10 samples) and the % difference for these pooled counts are less than ±20% (Table 8). These species include *Eurytemora* adults, *Acartia* adults, Podonidae, and *Bosmina*. The Wilcoxon Rank Sign test supports these results (Table 7) although it suggests that differences in the *Eurytemora* adults counts are borderline significant ($p<0.0840$).
- Versar has higher pooled counts for each of the three general taxonomic groupings (Table 8): total Cladocera +39.9%, total Copepods +69.5%, miscellaneous +38.6%.
- Examination of the pooled data (Table 8) suggest the smallest body sizes and the narrowest body shapes may be the most affected, i.e. they have the largest percent differences. These include a) all small, round-bodied mesozooplankton without large spines (i.e. chydorids, barnacle cypris, ostracods) +61.2%, b) barnacle nauplii (these are tri-cornered and spiny but can be very small) +77.8%, and *Acartia* copepodites (minus their antennae, these are small- to medium-sized, narrow taxa) +76.8%
- Counts of *Eurytemora affinis* copepodite, a medium-sized life stage of a common and important copepod, were significantly different in the split sample results, with Versar counting approximately 3.8 times more individuals that ODU, for a percent difference of 116% (Table 8).

  The possibility of both the ODU and Versar methods biasing counts of this species life stage was discussed and tentatively discounted (why would *Eurytemora affinis* copepodites be affected by a particular method but not the copepodites of other copepod species?).

- *Coefficient of variation* The coefficients of variation in the ODU taxa counts were again larger than those for the Versar counts, indicating ODU estimates of precision are lower than those of Versar (Figure 4).
- *Taxonomic identifications* Some differences that may be the result of conflicting taxa identifications. These possible identification differences are evident when ODU counts of taxa *within* a larger taxonomic group are higher than Versar counts while Versar counts for the *whole* group are higher than ODU counts (Table 8). For example,
  - ODU counts more "other Calanoid copepods" than Versar while Versar counts more total Calanoid copepods than ODU
  - ODU counts more "Cyclopoid" copepods than Versar while Versar counts more "total Copepods" than ODU
  - ODU counts more "other Cladocera" than Versar while Versar counts more "total Cladocera" than ODU

  Potential identification differences are also seen when species by species comparisons are made and non-rare species that are found by one laboratory are never found by the other. For example,

Taxon	ODU total/10 splits	Versar total/10 splits
Alona (cladocera)	17,408	0
Ilyocrptus spinifer (cladocera)	0	75,200
Pseudocalanus copepodites (copepod)	306,048	0

Although different in some ways from the Round 2 First Ten results, the Round 2 Second Ten results generally confirmed the earlier conclusions.

Round 2 Last Ten Followup

Several action items intended to complete the analyses or follow-up on the findings were recommended during the conference call and afterward:

- Check calculations. George Mateja, Forrest Crock, Craig Bruce and Claire Buchanan checked the various spreadsheets to determine if any correction factors, and especially those for *Eurytemora affinis* copepodites, were incorrect in the originally submitted results or the analyzed results. ODU found no errors in their split sample database while Versar staff found one error. The *Eurytemora affinis* copepodite count in one Versar split sample (Station TF1.5, 3-22-99) had a subsample volume of 2 where it should have been 1. Therefore, the Versar count for this split sample underestimated *Eurytemora affinis* copepodites, as well as total copepods, total mesozooplankton. This correction further accentuated the differences between ODU and Versar counts.
- Taxonomic groupings. Elgin Perry made comparisons of specific taxonomic groupings. These analyses were intended to circumvent taxonomic identification issues (i.e. level of taxonomy, different identifications, different life stage) and demonstrate whether or not the two methods are capturing and counting the same numbers of similar shaped/sized critters. Results are shown in Table 9.
  - Selected Copepods (all adults and copepodites minus *Eurytemora* copepodites). Nine out of the ten samples show significant ($-2.0 \leq z \geq +2.0$) differences in counts. While the large z-scores do *not* show a preponderance in the positive or negative direction, they indicate that variance is unusually high in this grouping. Note: z-scores are similarly high for the common *Eurytemora* copepodites (calanoid), the one taxa excluded from the "selected copepod" grouping (Appendix D). However, for *Eurytemora* copepodites there is a preponderance of positive signs meaning Versar consistently had higher counts. *Acartia* copepodites (calanoid), a common taxon that was included in the selected copepods comparison, also shows a preponderance of positive signs when analyzed separately (Appendix D).
  - Polychaetes AND Trochophores. Most z-scores were non-significant indicating that the Versar and ODU counts are similar. Differences observed earlier are apparently due to the use of different life stage codes/names.
  - Round organisms (all cladocerans plus some of the miscellaneous group, including ostracods and barnacle cypris but excluding barnacle nauplii). Most z-scores were non-significant indicating that the Versar and ODU counts are similar. This result juxtaposed on the sharp differences observed for individual taxa within this grouping such as chydorids, Daphnia, "other Cladocera," and ostracods (see Table 8) suggests that there are still taxonomic identification differences between some of the categories.
  - Barnacle nauplii. Half of the samples show count significant differences ($z > +2.0$) with a preponderance of positive signs meaning Versar consistently had higher counts.
- Taxonomic identifications.
  - Specimen Archive. Each laboratory is beginning to assemble a reference collection of all the species encountered during regular sample analyses. Versar, for example, is "picking" 2 or more individuals of each species (and sex if possible) and preserving them in sample vials. This could eventually become a long-term reference collection to be compared and shared by both laboratories.
  - Meeting. Representatives of both laboratories should at some point meet and do a side by side comparison of their reference collections. Species identifications that cannot be resolved or that are in question will be submitted to outside experts for analysis.

  - List of experts. Laboratories will send to Claire Buchanan a list of experts in taxonomic identifications.
- Correction factors. Claire Buchanan reviewed a selection of the split sample results to determine if conversion factors could be used on the older, "pre-patch" ODU and Versar data for the purpose of calculating Bay-wide indicators (Table 8). The usefulness of the conversion factors appears doubtful given a) the taxonomic discrepancies between the states, and b) analysis results of the actual monitoring data (see discussion).
- Implement regular split sample comparisons as approved CBP funds become available. Joe Macknis (EPA) has indicated that the Chesapeake Bay Program would like to see plankton split sample counts done as soon as possible and has orchestrated the monitoring funds to allow this to happen. A critical issue evident in the previous split sample results is the apparent differences in taxonomic identifications. A possible use of the split sample allotment this year would be for ODU and Versar monitoring staffs to focus solely on resolving taxonomic issues rather than performing standard split sample counts.

Discussion

Analysis of the Round 2 mesozooplankton split sample results indicated that the desired outcomes of the laboratory method modifications were only partially accomplished. The ODU total mesozooplankton counts are, on average, still lower than Versar's and the ODU method appears to selectively undercount key taxa, particularly the immature (copepodite) life stage of calanoid copepods and small-sized taxa. The study also raised several unexpected issues: taxa richness is lower in the ODU samples, and the species lists and level-of-taxonomy are not identical between the two laboratories.

Selecting a Method

A fundamental requirement of the mesozooplankton monitoring data is that the data be directly comparable in order to meet present and future management needs. Representatives of the CBP mesozooplankton monitoring programs all acknowledged that a "performance based" approach was not possible with the modified Versar Stempel pipette method and the ODU "new" CVS method. In other words, the two laboratories could not use their different methods to produce directly comparable results. A single enumeration method needs to be selected and implemented. A single method will ensure that Maryland and Virginia results are comparable *bay-wide*. It will allow the CBP monitoring programs to calculate and use a diverse suite of bay-wide mesozooplankton indicators. Bay-wide zooplankton community indicators are needed because they are useful tools in tracking food web responses to management actions such as nutrient and sediment reductions, targeting restoration efforts in open water habitats, and evaluating overall ecosystem health. They will soon be used to measure progress towards plankton restoration goals. The differences and similarities in the ODU and Versar data evident in the Split Sample Study results were discussed at length by the monitoring program staffs, in their efforts to select a common method. The major issues that were debated are summarized in the following discussion and in Appendix F: "Tracking Sheet for Reviews of the April 2000 Draft Report on the Mesozooplankton Split Sample Study."

What are Mesozooplankton Taxa?

The question of whether or not the taxa undercounted in the CVS method were truly "mesozooplankton" was discussed throughout the split sample study. There was disagreement

on whether the CBP mesozooplankton monitoring programs should be counting a) organisms retained on a 200 micron mesh sieve in the laboratory, or b) organisms belonging to specific taxonomic groups and/or trophic levels that are retained in the monitoring programs' 202 micron mesh plankton nets in the field. A literature check indicates the latter is the preferred definition of mesozooplankton. Plankton categories have been proposed and refined for over a century, and the categories, or "functional groups," defined by Sieburth et al. (1978) are now widely accepted (Harris et al. 2000). Mesozooplankton are identified on the basis of taxonomy and trophic level, and are comprised mainly of copepod adults and copepodites in ocean settings but include cladocera, ostracods, and meroplankton larvae in estuarine waters (e.g. Seiburth et al. 1978, Harris et al. 2000, Day et al. 1989). Zooplankton as a whole span a wide size spectrum (six orders of magnitude) which necessitates grouping them into size fractions that can be effectively collected. The upper and lower limits chosen for each size fraction were selected so that they encompass the bulk of an individual zooplankton category (Sieburth et al. 1978). Since nets were - and still are - the primary means of collected zooplankton greater than 20 micron, this meant that plankton nets with mesh openings equal to the lower size limit should collect the bulk of an individual zooplankton category when towed correctly in the water. A size range of 200 micron - 20 mm (body length) was selected for the mesozooplankton even though immature individuals of some species are smaller than 200 microns and hence not adequately sampled by a 200 micron mesh plankton net. A brief overview of the five zooplankton categories and size ranges is given in Appendix E, and discussed in more detail in Sieburth et al (1978) and Harris et al. (2000).

Counts of certain zooplankton commonly caught in the plankton net tows were not used in the split sample study for various reasons:

- *Large-sized copepod nauplii and rotifers*: Versar and ODU submit mesozooplankton data sets to the CBP Data Center that include counts of large-sized copepod nauplii and rotifers which are technically microzooplankton (Appendix E). The monitoring program principal investigators discussed taking these microzooplankton counts out of the data sets submitted to the CBP Data Center in 1995 but chose to leave them in. It was thought that these counts of nauplii captured in a 200 u mesh plankton net tow may some day provide useful information about the proportion of larger copepod nauplii in the population. These microzooplankton counts are not used in calculations of bay-wide indicators, and they were not analyzed in the mesozooplankton split sample study.
- *Fish eggs and larvae*: ODU includes counts of fish eggs and larvae in data sets submitted to the CBP Data Center while Versar, Inc. does not. Versar's chief reason for excluding counts of these mesozooplankton taxa is that the staff believe the plankton nets currently used in the Maryland program do not adequately sample fish eggs and larvae. These counts were not analyzed in the mesozooplankton split sample study.

Counts of all other mesozooplankton taxa, even those with body lengths approaching 200 microns (e.g. early copepodite life stages, immature cladocerans, *Bosmina*, small ostracods, small meroplankton larvae), were analyzed in the split sample study.

The effects of net clogging and extrusion on the taxonomic composition of the mesozooplankton samples were discussed several times during the split sample study. The limitations of using a 202 micron mesh plankton net *in the field* to collect mesozooplankton taxa are recognized by both the Maryland and Virginia laboratories, and were a factor in their original choices of plankton net and sampling protocols. An unknown percentage of mesozooplankton taxa with

lengths and/or widths less than 200 microns are probably extruded from both the Maryland and Virginia plankton nets during towing. On the other hand, the plankton nets are clogged by detritus and phytoplankton during towing which somewhat counters the extrusion losses. Once concentrated in the bottle at the cod-end of the plankton net, the Maryland mesozooplankton samples are further concentrated with a 110 micron sieve before they are rinsed into sample jars and preserved while the Virginia mesozooplankton samples are simply washed into a 1-liter sample container and preserved. The Maryland ship-board sieving step is supported in zooplankton methodology manuals (e.g. Edmondson and Winberg 1971, Harris et al. 2000) but there is a risk that some mesozooplankton individuals could be extruded though the sieve. Possible losses during plankton tows and ship-board sieving would not affect the split sample results of this study.

Counts from "Old" versus "New" CVS Methods

Count comparisons of the "old" and "new" CVS method used by ODU demonstrate that the "new" method counts for total mesozooplankton were approximately 1.50 times greater, or 50% larger, than the "old" method counts in the twenty-one, Round 2 split samples (Table10). Thus, the "old" CVS method appears to undercount total mesozooplankton abundances. This study result is supported by a recent analysis of the 1985 - 1998 monitoring data which found that Versar and ODU total mesozooplankton counts for two adjacent stations in the Chesapeake Bay mainstem were significantly different (C. Buchanan, unpublished).[3] The median abundance was 2.42 times higher in Versar samples collected at Maryland station CB5.2 as compared to ODU samples collected at Virginia station CB6.1 and counted with the "old" CVS method. Together, the split sample and field results indicate that the pre-1999 mesozooplankton monitoring results in the Virginian Chesapeake Bay are undercounted. Further examination of Table 8 indicates that copepod and cladoceran counts gained the most when the method was changed while total counts for the miscellaneous group did not change significantly. Several individual taxa showed no significant differences on average between the "old" and "new" CVS method counts: adult *Eurytemora affinis* (frequent common calanoid copepod species in tidal freshwaters), adult *Acartia* spp. (dominant calanoid copepod genus in mesohaline/polyhaline salinities), Podonidae (mesohaline/polyhaline cladoceran family), harpacticoid copepods, and barnacle cypris and nauplii life-stages (meroplankton). If the "new" CVS method is instituted at ODU, these five taxa could possibly be used for long-term trends, thereby maintaining some backward comparability in Virginia. Only one of them proved to be directly comparable to Versar taxa counts, however.

Versar vs ODU Taxa Counts Count comparisons of all Round 2 split samples indicate that Versar's Stempel pipette counts for total mesozooplankton were still higher than ODU's "new" CVS method counts, despite increases in the ODU counts after adding the 64 micron sieve. The pipette method counts were on average 2.05 times greater than the CVS method counts. This

[3] To reduce biases introduced by salinity-sensitive species, only 1985 - 1998 data points associated with salinities normally experienced by both stations (14.3 - 21.5 ppt) were used. The Versar median abundance was 7,639/m^3 (n=117) and the ODU median abundance was 3,147.8/m^3 (n=126). The Mann-Whitney test indicates the medians are significantly different ($z = 2.6859$, $p<0.01$). This degree of difference was not found between adjacent Maryland stations in the mesohaline waters (i.e. CB5.2 and CB4.3C) or adjacent Virginia stations (i.e. CB6.1 and CB6.4) in mesohaline/polyhaline waters.

translates to an *average* percent difference[4] of +42.9%, and a % difference for the *pooled* data (the sum of all Versar counts compared to the sum of all ODU counts) equal to +69% (Figure 5). Stempel pipette counts for all Round 2 counts of the three major mesozooplankton taxonomic groupings (copepods, cladocera, miscellaneous) and the dominant copepod order (calanoids) were also higher than the "new" CVS method counts (Figures 6, 7). The twenty-one Versar and ODU split sample counts were roughly the same for the less common cyclopoid copepods (-2.8%), and "new" CVS counts for the rarer harpacticoid copepods were larger than the pipette counts (Figure 6). This latter result is unexpected because split counts for copepods as a whole were higher with the pipette method.

When Stempel pipette counts are compared to "new" CVS method counts on a taxa by taxa basis, it appears as if four abundant taxa are primarily responsible for the observed differences between the Versar and ODU total counts: copepodite *Eurytemora affinis*, copepodite *Acartia tonsa*, barnacle nauplii and chydorids. Versar counts of copepodite *Eurytemora affinis*, the most abundant taxa in this split sample study, were 3.78x greater than the ODU counts (Table 8). Copepodite *Acartia tonsa*, barnacle nauplii, and chydorids were, respectively, 2.25x, 2.27x, and 4.76x more abundant in the Versar counts. The differences in total mesozooplankton counts caused by the higher Versar taxa counts are partially countered by taxa differences in the opposite direction caused by a few higher ODU taxa counts. These latter differences are unusual because they occur within taxonomic groupings where the Versar count is higher. For example, ODU counts for cyclopoid copepods and for "other calanoids" (excludes *Acartia* and *Eurytemora*) were higher than Versar's, yet ODU *total* copepod counts were lower than Versar's (Table 8). The countervailing differences in some taxa indicate laboratory inconsistencies in taxonomic identification are still occurring that need to be found and resolved.

Further comparisons of the split samples suggest Versar Stempel pipette counts and ODU "new" CVS method counts for four relatively abundant taxa might be directly comparable: adult *Eurytemora affinis*, adult *Acartia tonsa*, Podonidae, and *Bosmina* (Table 8). If the Chesapeake Bay Program decides to maintain two different mesozooplankton counting protocols for the sake of backward compatibility with the pre-1999 data (i.e. it accepts ODU counts produced by the "new" CVS method and continues to accept Versar counts produced by the Stempel pipette method), then these four taxa have the greatest potential for being directly comparable in post-1998 Virginia and Maryland monitoring data. Their direct comparability would need to be confirmed with additional split samples. While these four taxa are important constituents of the zooplankton community and seasonally abundant, bay-wide evaluations of zooplankton community health that are based solely on these four species will not be adequate for the Chesapeake Bay Program.

The possibility using the split sample results to develop correction factors to adjust mesozooplankton counts in the pre-1999 CBP monitoring data was discussed during the course

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[4] Percent difference is the difference of the Versar and ODU counts for a split sample divided by their mean, then multiplied by 100. Positive values indicate Versar counts are higher; negative values indicate ODU counts are higher. Values greater than $\pm$20% can be considered significantly different ($p<0.05$). The *average* % difference is the average of the % differences for several of samples. The % difference of the *pooled* sample data is obtained by calculating % difference on the sum of all Versar counts and the sum of all ODU counts being compared. By summing the Versar and ODU counts, arbitrary biases introduced by small raw counts in a few of the split samples is minimized.

of the study. Correction factors were calculated for abundant taxa in the "Last Ten" split samples (Table 8), however, sample by sample comparisons of taxa differences suggest that the variability experienced in taxa life-stage sizes will result in unstable correction factors and the attempt to develop the factors was discarded. Three taxa might not need correction factors to be directly comparable in the pre-1999 CBP monitoring data: adult *Eurytemora affinis*, adult *Acartia tonsa*, and Podonidae. All three appear to be minimally affected by the "old" to "new" CVS method change (see above), and the Split Sample Study indicates their "new" CVS method counts and Stempel pipette counts are directly comparable. Analysis of the monitoring data warns against this conclusion for *Acartia*, however (Figure 8). Actual Stempel pipette counts of *Acartia* in the Maryland samples were 4.3 times greater than the "old" CVS method counts in the ODU samples over the 14 - 21 ppt salinity range. On the other hand, the monitoring data suggest that Versar and ODU field counts of adult *Eurytemora affinis* might be comparable (Figure 9).

Does the CVS method undercount mesozooplankton?

The "old" CVS method very clearly undercounted mesozooplankton. Comparisons of "new" and "old" CVS method counts show that total counts and most taxa counts increased significantly when a smaller sieve was added. Hence, most counts obtained with the "old" CVS method (i.e. the 1985-1998 Virginia monitoring data) are undercounted. The lower split sample counts obtained with the "old" CVS method appear to be due primarily to sieving losses through the bottom 200 micron sieve. While the CVS method as originally described in Alden et al. (1982) employed four large-mesh sieves in combination with a 150 micron and a 75 micron mesh sieve, the "old" CVS method used by the Virginia mesozooplankton monitoring program since its inception employed five large-mesh sieves (2000, 850,600, 300, 200 microns) and no small-mesh sieves (i.e. <200 microns).

The significantly lower counts produced by the "new" CVS method in Round 2 of the Split Sample Study indicate one or both methods are not producing counts representative of actual mesozooplankton abundances in the field. Is the Stempel pipette method biasing counts above actual sample levels, or the "new" CVS method biasing counts below actual sample levels, or both? The possibility of bias in the Stempel pipette counts caused by clumping was tested for several years by Versar, and did not appear to be occurring (W.Burton, personal communication). Also, replicate sample counts perform regularly by Versar indicate good repeatability (W. Burton, personal communication). These QA/QC data could be further analyzed if needed to check the accuracy of the existing Versar counts. Information from several zooplankton methodology manuals suggest that aspects of the "new" CVS method could be causing it to undercount the ODU samples. First, several distinct taxa with significantly lower ODU split sample counts are large but also narrow, e.g. *Eurytemora affinis* and *Acartia tonsa* copepodites. A review of the lengths and widths of commonly found mesozooplankton taxa in Chesapeake Bay (Table 11) suggests many immature copepods could be extruded head-first through the bottom 64 micron mesh sieve of the "new" CVS method as the sieves are shaken during the sieving process. Second, several methodology manuals suggest animals stick to the walls of sample splitters and a percentage could be lost during the CVS method splitting steps with the Folsom splitter (e.g. Edmondson and Winberg,1971, pg 130; APHA, 1995). Finally, use of an unleveled Folsom splitter will produce biases in the subsamples which increase with repeated splitting (APHA, 1995). One or more of these causes of bias could be responsible for the lower ODU counts, but further tests would be need to done to determine if they are in fact occurring.

Versar-ODU Differences in Taxa Richness and Diversity

The modified mesozooplankton counting methods do not produce comparable taxa richness measures (Figure 10). While taxa richness increased when a sieving step (850 microns) was added to the Versar laboratory protocol and counts for larger, rarer taxa were reinstated, the Split Sample Study shows that other issues still need to be resolved before taxa richness or taxa diversity indices can be used bay-wide. First, level of taxonomic identification is not consistent between the states. Side-by-side count comparisons by program staff at the March 1999 meeting served to move the two laboratories closer to a common level of taxonomy, but the species lists are not identical yet. The problem was overcome in the split sample study by "lumping" species counts into higher taxonomic categories, but this is not desirable long-term solution. Second, fewer species were observed in the ODU counts (Figure 10). While the CVS sieving steps and the addition of an 850 micron sieving step to the Versar method both help to bring forward large-sized, rare species for counting, other aspects of the CVS method are making the number of observed taxa in the ODU splits lower than those in the Versar splits. The second issue, in combination with the lower ODU total counts, brings into question the usefulness of Margalef's Diversity Index as a bay-wide indicator of zooplankton community health at the present time. Taxa richness (number of observed taxa) is a variable in the index numerator and total abundance (number of organisms per sample) is a variable in the denominator. When richness is divided by abundance, as in Margalef's Diversity Index, the resulting proportion does not reflect the lower taxa richness and lower total abundance of the CVS method counts. Thus, the Virginia and Maryland diversity indexes were approximately the same (Figure 10). The Shannon-Wiener, Pielou, and Simpson indices of diversity would be similarly affected because they also rely on measures of species proportional abundance.

Sample Variances

A higher level of sample variance was observed in the ODU counts (Figures 2, 4). This reflects Versar's choice of a $\pm$20% error level and ODU's choice of a 35% error level (see Introduction, Appendix A). Versar achieves its lower error level by producing relatively large raw counts (Table 12). The 20% and 35% error levels are for total mesozooplankton counts, and error levels for individual taxa are usually much higher. This was evident in the split sample results for rarer taxa which typically had very high % differences. The higher sample variance and subsequently lower estimates of precision for the ODU sample counts make it more difficult to identify significant trends in the Virginia data as compared to the Maryland data. These difficulties are overcome by time in long-term data sets. However, the CBP in its search for ecosystem responses to nutrient reductions is very interested in year to year trend changes in the monitoring parameters. Both programs should probably take this management need into consideration when future approaches and levels of effort are discussed.

Next Steps

Monitoring program representatives did not reach a consensus on which method should be adopted by both laboratories after they reviewed the Split Sample Study results. To help them decide, they agreed to perform additional split sample comparisons to determine if laboratory differences were due to procedural bias or identification bias, or both. If the results confirm this report's conclusions and bias is shown to be method dependent, the representatives agreed that one method should be selected for both laboratories and used in the future to provide directly comparable mesozooplankton monitoring data to the CBP.

Conclusions

1. Inter-laboratory split sample comparisons between ODU and Versar indicate that the laboratories do not produce comparable abundance data for most species. There were:

- Persistent differences in level-of-taxonomy for some taxa groups
- Persistent differences in the taxonomic identifications for at least chydorid cladocerans, "other" cladocerans, ostracods, and several copepod taxa
- Significantly higher Versar counts for "total mesozooplankton"
- Significantly higher Versar counts for "total copepod"

  Within the copepod group, Versar counted significantly higher "total calanoid copepods," and calanoid copepodite life stages (i.e. *Acartia*, *Eurytemora*) while ODU counted significantly higher "total harpacticoid copepods" and the laboratories produced roughly comparable counts for "total cyclopoid copepod."
- Significantly higher Versar counts for "total cladocerans"
- Slightly higher Versar counts for "total miscellaneous" (includes ostracods, polychaetes larvae, immature barnacles, and other meroplankton larvae)
- Greater taxa richness in the Versar samples
- Lower coefficients of variance (CV) in the Versar split samples than in ODU samples

In general, mesozooplankton with the smallest body sizes and/or the narrowest body shapes appear to be most affected by the CVS counting method, i.e. ODU count differences with Versar are frequently greatest in these taxa. Calanoid copepodites may be especially undercounted by the ODU method.

2. Split sample comparisons between counts produced with the "new" Versar method and the "new" ODU method identified areas of uncertainty and areas of agreement/improvement:

- Possible taxonomic differences between counters within at least one of the laboratories during 1998
- Counts of four taxa are in general agreement a) between laboratories, and b) between "old" and "new" ODU methods.

  Counts for these four taxa could possible be used for long-term trends, thereby maintaining some backward comparability in Virginia. The four taxa showed no significant differences *on average* between the "old" and "new" ODU counts, although their sample variances were at times large. ODU counts of these taxa were also generally comparable to Versar counts. These taxa are: adult *Eurytemora affinis* (frequent common calanoid copepod species in tidal freshwaters), adult *Acartia* spp. (dominant calanoid copepod genus in mesohaline/polyhaline salinities), Podonidae (mesohaline/polyhaline cladoceran family), and possibly *Bosmina longirostris* (seasonally dominant cladoceran in freshwater).
- Improvement in the quality of Versar and ODU taxa counts as a result of site visits, side-by-side taxonomic comparisons, and the split sample study

3. The split sample data indicate that there is a consistent bias between the Virginia and Maryland data due to differences in identification and laboratory procedures. To separate identification biases from procedural biases, the following actions are recommended:

a) *ODU should perform the modified Stempel pipette method on all samples collected in CY 2000.*

b) *ODU should perform both the modified Stempel pipette method and the "New" CVS method on a subset of CY 2000 samples. This subset should encompass the complete range of mesozooplankton community structure.*

The purposes of these are:

- Split samples between ODU and Versar can be analyzed with the modified Stempel pipette, permitting a clear comparison of the laboratories' taxonomic identifications.
- Stempel pipette vs. CVS differences will be attributed to methodology alone, assuming that identification bias would not occur within ODU.
- This data should be used to assess the effect of changing methodology on Virginia's data analysis and interpretation. This also may provide data conversion factors for use in combining CVS data and Stempel pipette data in Virginia waters.

c) *ODU should identify possible sources of bias in the CVS method.* It is possible that some bias is inherent in the method and cannot be eliminated. For example, copepodites under 64μ in length or width may pass through the bottom CVS screen, but are captured and counted in the Stempel pipette method.

Bias from the Folsom splitter should be estimated as described in Standard Methods for the Examination of Water and Wastes, 19th edition (APHA, 1995). Sieving loss should be assessed by sieving and counting a single sample successively, e.g., 3-5 times, to see if recoveries diminish.

d) *Versar and ODU should check the Stempel pipette subsampling and sorting bias as described in section 2.1.8 of the IPB Handbook (Edmondson and Winberg, 1971).*

4. Quality assurance counts within each laboratory and between laboratories should be rigorously maintained, documented, and periodically reviewed to ensure comparable, high quality mesozooplankton counts. Quality assurance procedures should be maintained in each laboratory to ensure adequate taxonomic training of new technical staff. Quality assurance (repeated) counts for each laboratory should be regularly submitted to the states, the Chesapeake Bay Program or their designees for independent analysis. Regular site visits between the two states' technical staffs should be carried out to ensure comparable interstate taxonomy. A split sample study should be done annually for at least the next few years to ensure interstate count comparability.

5. Both laboratories should work from an identical taxon list, to the same level of taxonomy, and they should enumerate the same life stages. A record of the mesozooplankton taxa identified in the CBP zooplankton monitoring program should be maintained in both laboratories (e.g. a type specimen collection, a photographic record). Laboratory differences in taxonomic identifications can be reconciled during side-by-side

comparisons and through the assembly of a photographic or type specimen collection for Chesapeake Bay mesozooplankton. The goal would be to standardize the level of taxonomy and avoid discrepancies in taxonomic identification between laboratories.

Literature Cited

APHA. 1995. Standard Methods for the Examination of Water and Wastes. 19th Edition.

Alden, R. W., III, R. C. Dahiya and R. J. Young Jr. 1982. A method for the enumeration of zooplankton subsamples. *Exp. Mar. Biol. Ecol.* 59:185-206.

Edmondson, W. T. and G. G. Winberg (editors). 1971. IBP Handbook No. 17. A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications, Oxford, 358pp.

Harris, R., P. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley. 2000. ICES Zooplankton Methodology Manual. *Academic Press.* 684pp.

Day, J.W., C.A.S. Hall, W.M. Kemp and A. Yanez-Arancibia. 1989. Estuarine Ecology. John Wiley and Sons, New York.

Sieburt, J. McN., V. Smetacek, and J. Lenz. 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanog.* 23(6):1256-1263.

Figure 1. Comparison of Total Mesozooplankton Counts in Round 2 First Ten Split Samples. The average % difference between the Versar and ODU total mesozooplankton counts for these samples was +13%. The % difference of the pooled sample data is +69.0%. In most cases, the individual sample % differences were greater than +20%, suggesting a high degree of variability in the counts from one or both laboratories. Note: the RET3.1 May sample was counted twice by ODU. Details: Percent (%) difference is the difference of the Versar and ODU counts for a split sample, divided by their mean. Positive values indicate the Versar count was highest. Negative values indicate the ODU count was highest. Values higher than +20% or lower than -20% can be considered significantly different ($p<0.05$). The average % difference is the average of all the individual sample % differences. The % difference of the pooled sample data is obtained by calculating % difference on the sum of all Versar counts and the sum of all ODU counts. By summing the Versar and ODU counts, arbitrary biases introduced by small raw counts in a few of the split samples is minimized.

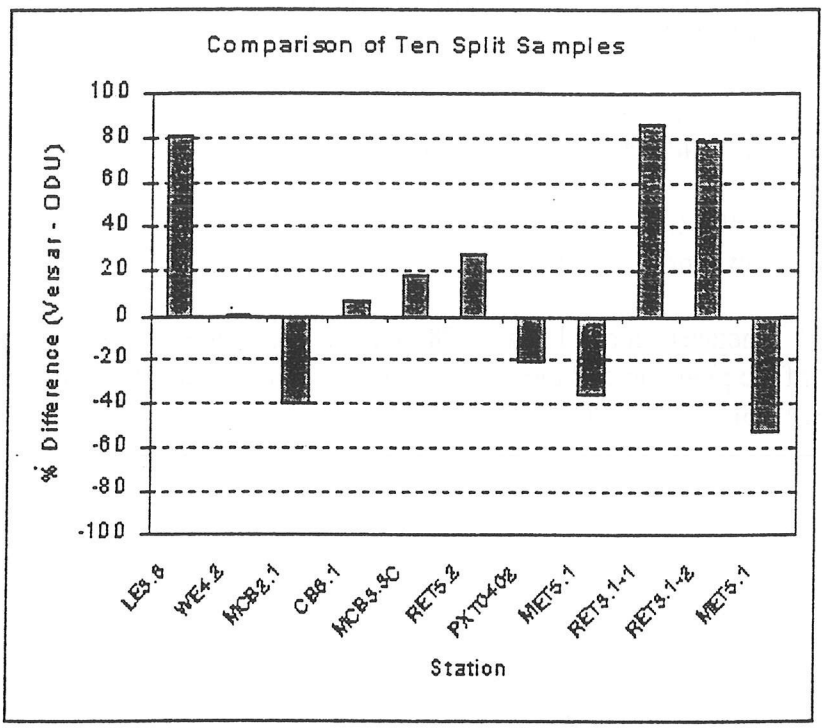

Figure 2. Plot of Versar Coefficient of Variation vs ODU Coefficient of Variation in Round 2 First Ten Split Samples (Elgin Perry 15:11 Thursday, January 28, 1999). VCV = Versar Coefficient of Variation, OCV = ODU Coefficient of Variation. Legend: A = 1 obs, B = 2 obs, etc. N = 71 (i.e. splits samples where taxon counts are available from both laboratories.) The results indicate the ODU split samples have a higher coefficient of variation than the Versar split samples (i.e. they fall below the VCV=OCV diagonal line).

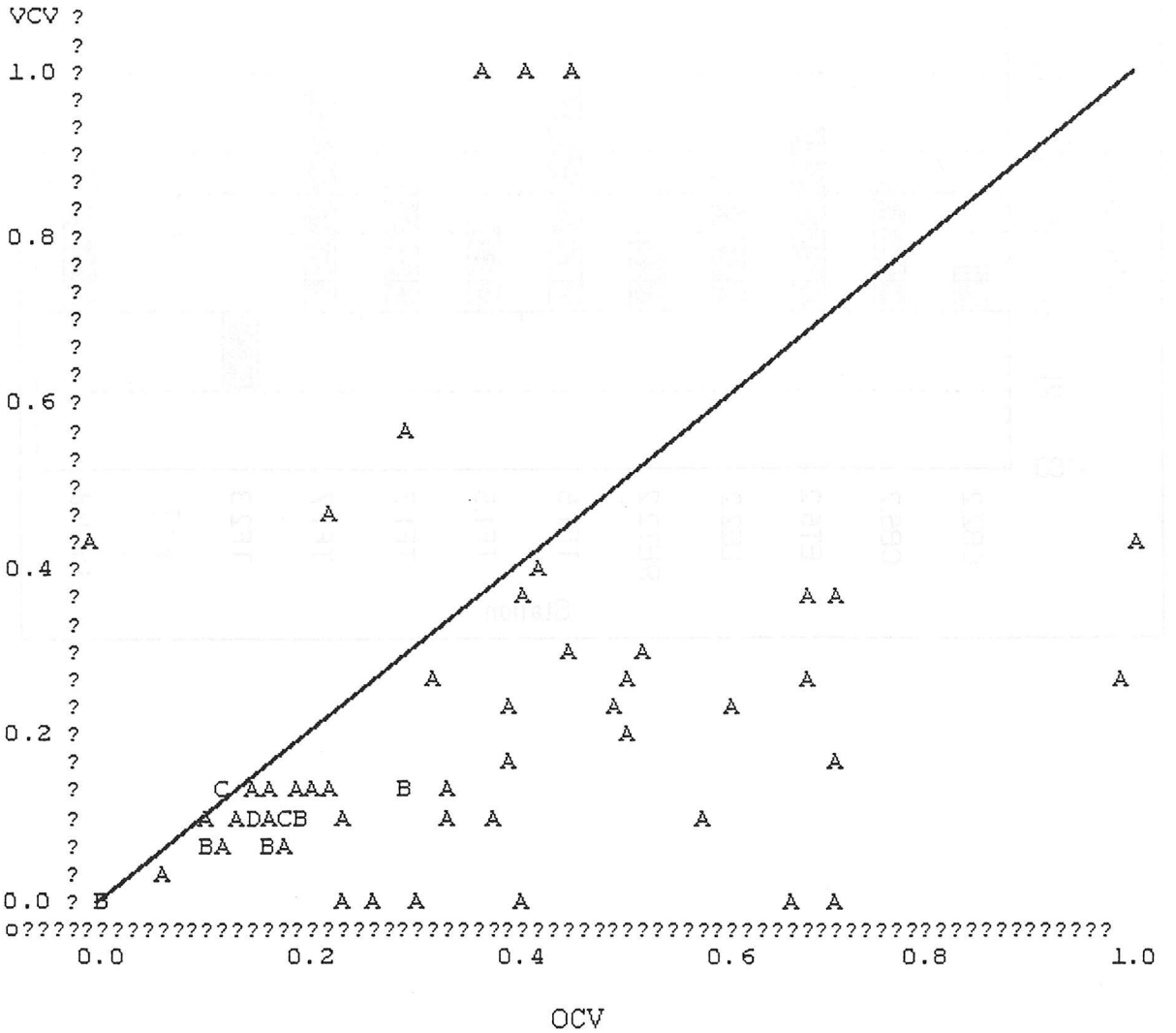

Figure 3. Percent (%) difference between ODU and Versar total mesozooplankton counts, by sample, for the Round 2 Last Ten split samples. (See Figure 1 caption for details.) The average % difference of the 10 samples is 74.9%. The % difference between the pooled ODU and pooled Versar counts is 67.2%.

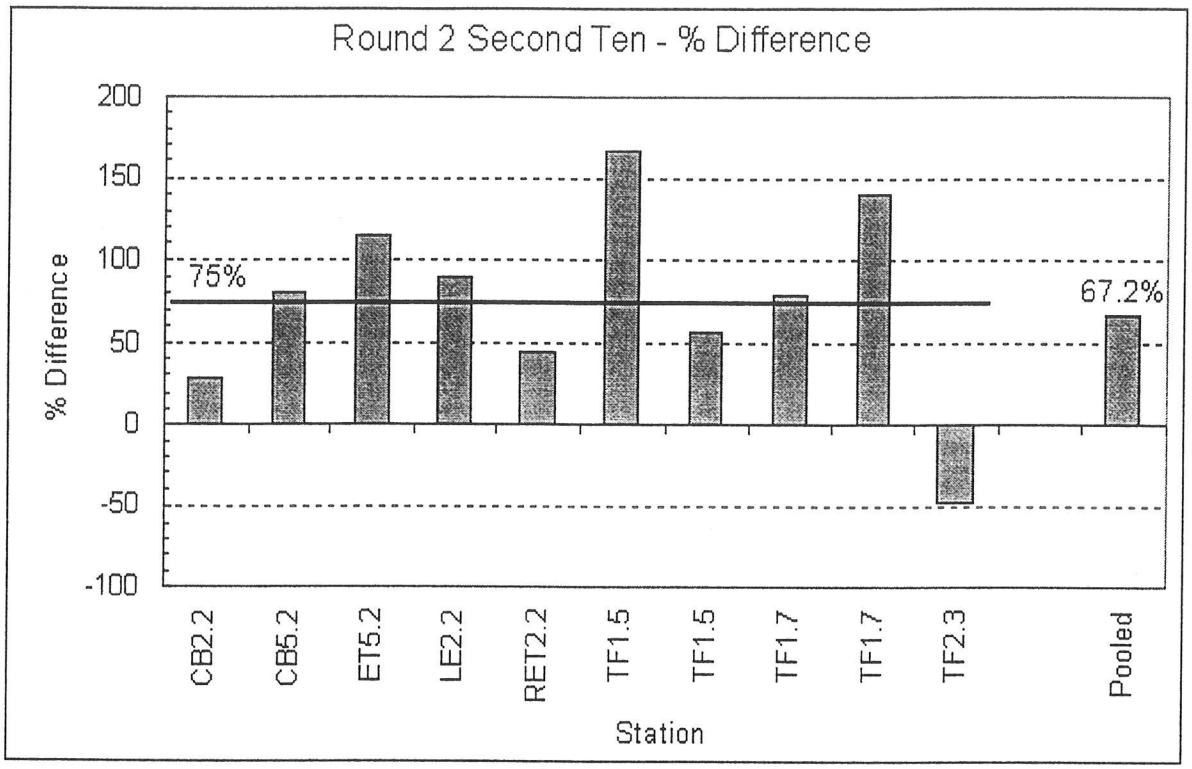

Figure 4. Plot of Versar Coefficient of Variation vs ODU Coefficient of Variation in Round 2 Second Ten Split Samples. N = 62 (i.e. splits samples where taxon counts are available from both laboratories.) The results indicate the ODU split samples have a higher coefficient of variation than the Versar split samples (i.e. they fall below the VCV=OCV diagonal line).

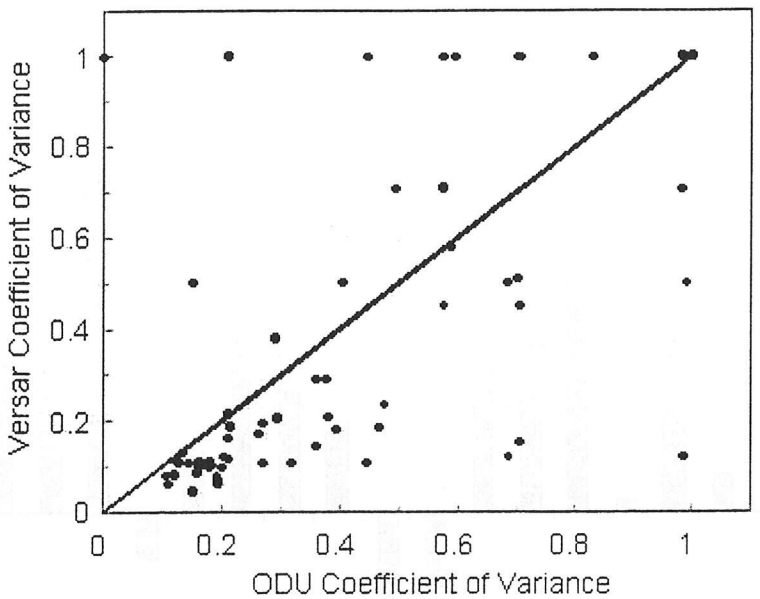

Figure 5. Percent (%) difference between ODU and Versar total mesozooplankton counts, by sample, for all Round 2 split samples (n = 21). (See Figure 1 caption for details.) There is a preponderance of positive % differences in the 21 counts of total mesozooplankton, indicating Versar counts are generally higher. The average % difference is +42.9%. The % difference of the pooled sample data is +69.0%. Note: the RET3.1 May sample was counted twice by ODU, so there are 21 counts for 20 split samples.

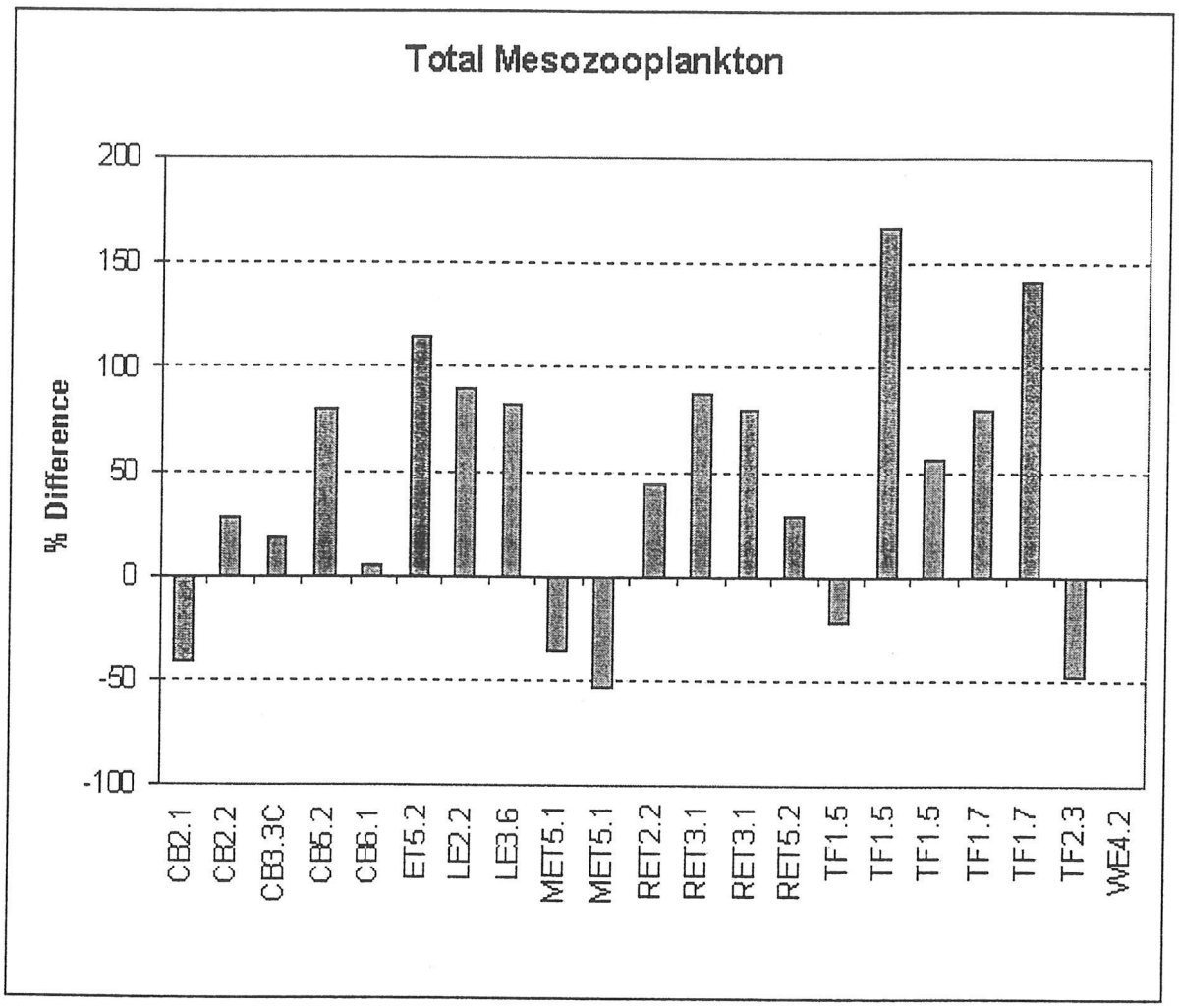

Figure 6. Percent (%) differences of pooled taxa data for Round 2 "First Ten" and "Second Ten" of the Split Sample Study. (See Figure 1 caption for details.) "Mesozooplankton" consists of all the mesozooplankton taxa (dark colored bars). "Miscellaneous" (primarily meroplankton larvae and ostracods), "cladocera" and "copepod" are the three major taxonomic groupings of mesozooplankton in estuaries (light colored bars). "Calanoid," "cyclopoid," and "harpacticoid" are three orders of copepod (white bars). The % difference for the "Miscellaneous" and "Cladocera" taxonomic groupings are only shown for the second ten split samples because taxonomic identification changes made by ODU and Versar after the first ten split samples affect the earlier results. Only the second ten split sample results for "Harpacticoid" are shown because counts of harpacticoids in the first ten were relatively small. Data for groups that are not know at this time to have taxonomic identification problems can be pooled for all Round 2 split samples, and their overall % differences are: total mesozooplankton, +69.0%; calanoid copepods, +65.1%; cyclopoid copepods, -2.8%; harpacticoid copepods, -45.0%. The most abundant copepod group in Chesapeake Bay is the calanoid copepod, and counts for this group tend to dominate the "copepod" results (see graph).

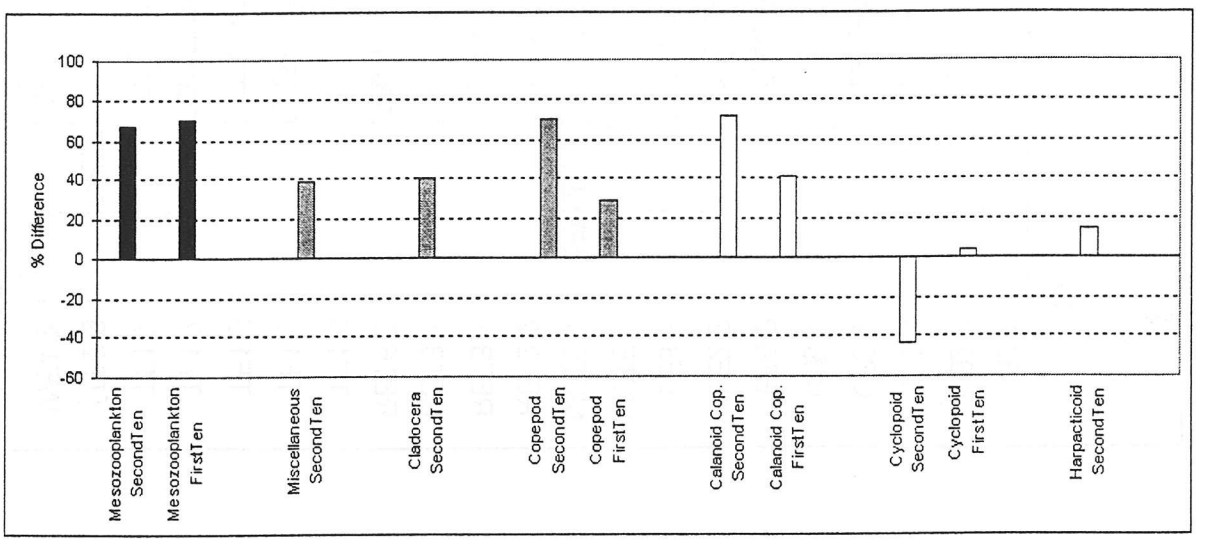

Figure 7. Percent (%) difference for ODU and Versar total copepod counts for all Round 2 split samples (see Figure 1 caption for details). The average % difference of the total copepod counts is +48.1%. The % difference of the pooled sample data is +59.0%.

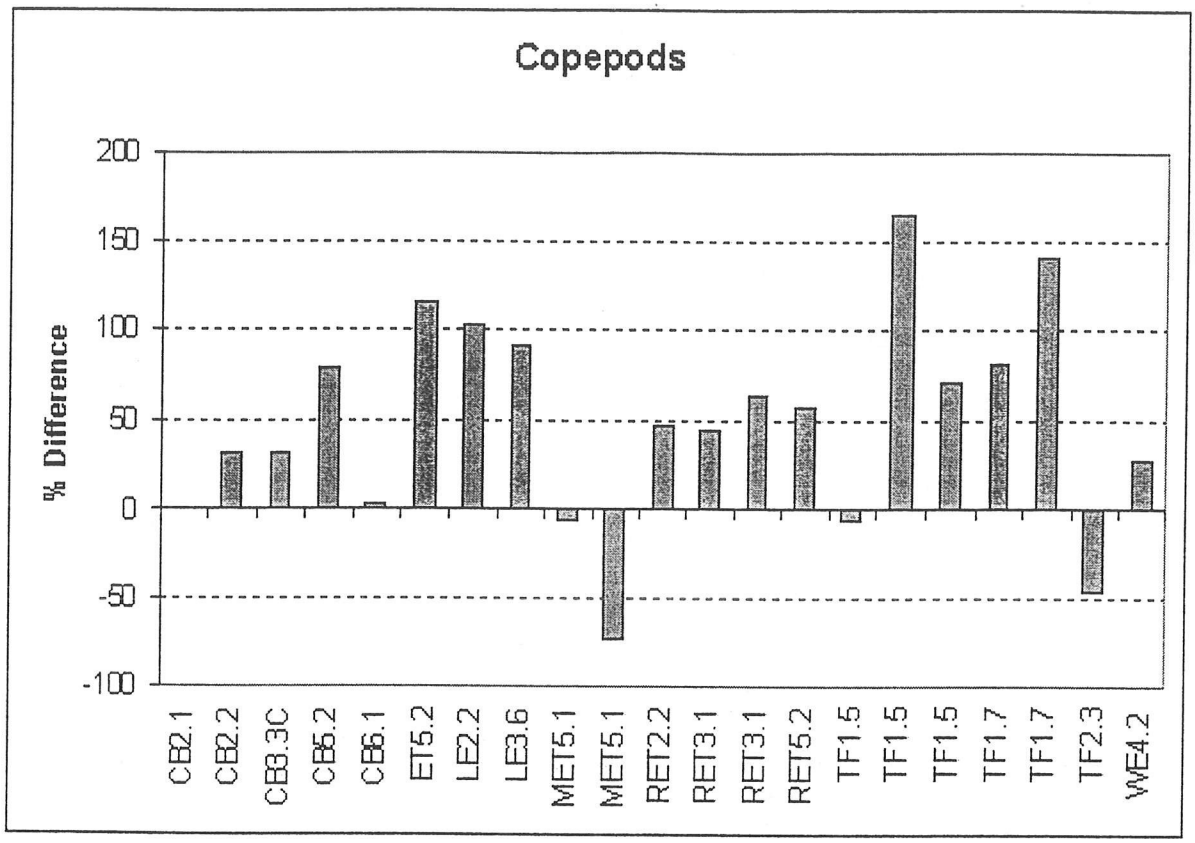

Figure 8. Comparison of Versar, Inc. and Old Dominion Univeristy (ODU) estimates of Acartia tonsa adult abundances in oligohaline, mesohaline and polyhaline salinities of the Chesapeake Bay mainstem versus salinity (all sample dates between August 1984 and December 1998). Absences, or zero values, are excluded. The graphs demonstrate the euryhaline nature of Acartia tonsa, i.e. salinity between approximately 5 and 32 ppt do not affect abundances. Secchi depths experienced in this salinity range overlapped strongly and are not a reason for the laboratory differences (i.e. secchi depths of 0.4 - 4.5 m were experienced in ODU data and secchi depths of 0.5 - 5.5 m were experienced in Versar data). Versar and ODU counts for adult Acartia tonsa were significantly different ($p<0.01$) at the adjacent CB5.2 (Maryland) and CB6.1 (Virginia) mainstem stations, the most comparable of the Maryland and Virginia mainstem stations. Versar's counts were approximately 4.33 times greater than ODU's (C. Buchanan, unpublished).

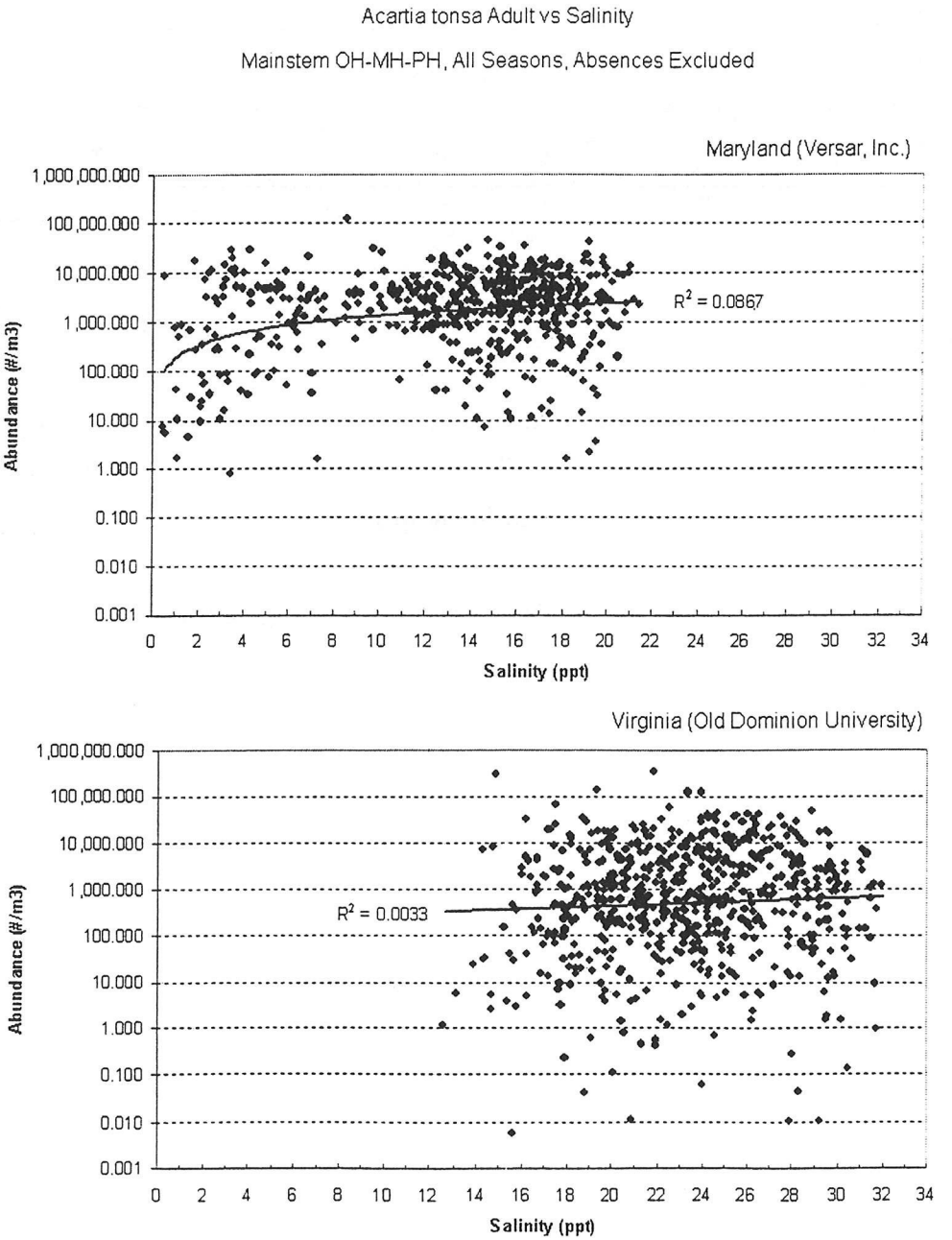

Figure 9. Adult Eurytemora affinis monitoring data from the Maryland and Virginia mainstem (1985 - 1998), for salinities that occur in both states (14 - 21 ppt). Light points and line: Virginia data; dark points and line: Maryland data. Although primarily an oligohaline/low mesohaline species, Eurytemora affinis is found in the Chesapeake Bay middle mainstem. The effect of salinity can be seen in the negative slopes of the regression lines. The Maryland and Virginia regressions are nearly identical, suggesting that Versar Stempel pipette counts and ODU "old" CVS method counts could be comparable.

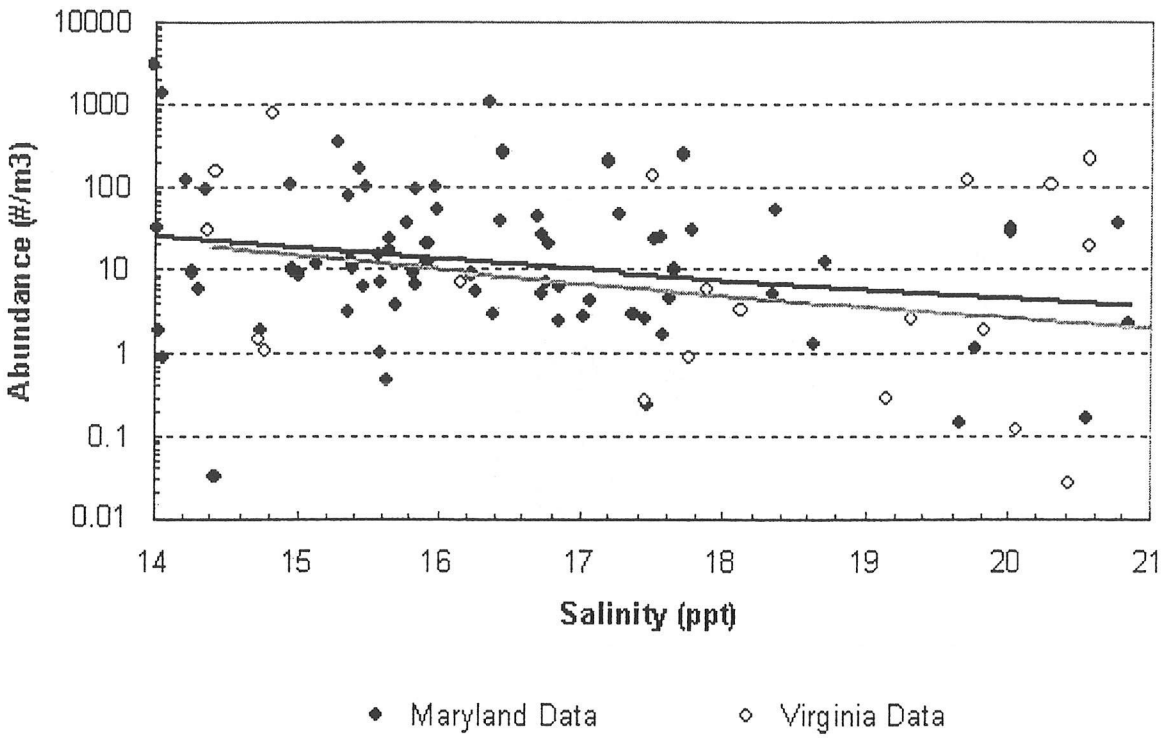

Figure 10. Total mesozooplankton abundance, species richness and Margalef Diversity Index for "Last Ten" split samples of Round 2. The % differences of the pooled data (see Figure 1 caption for details) are shown in the graphs.

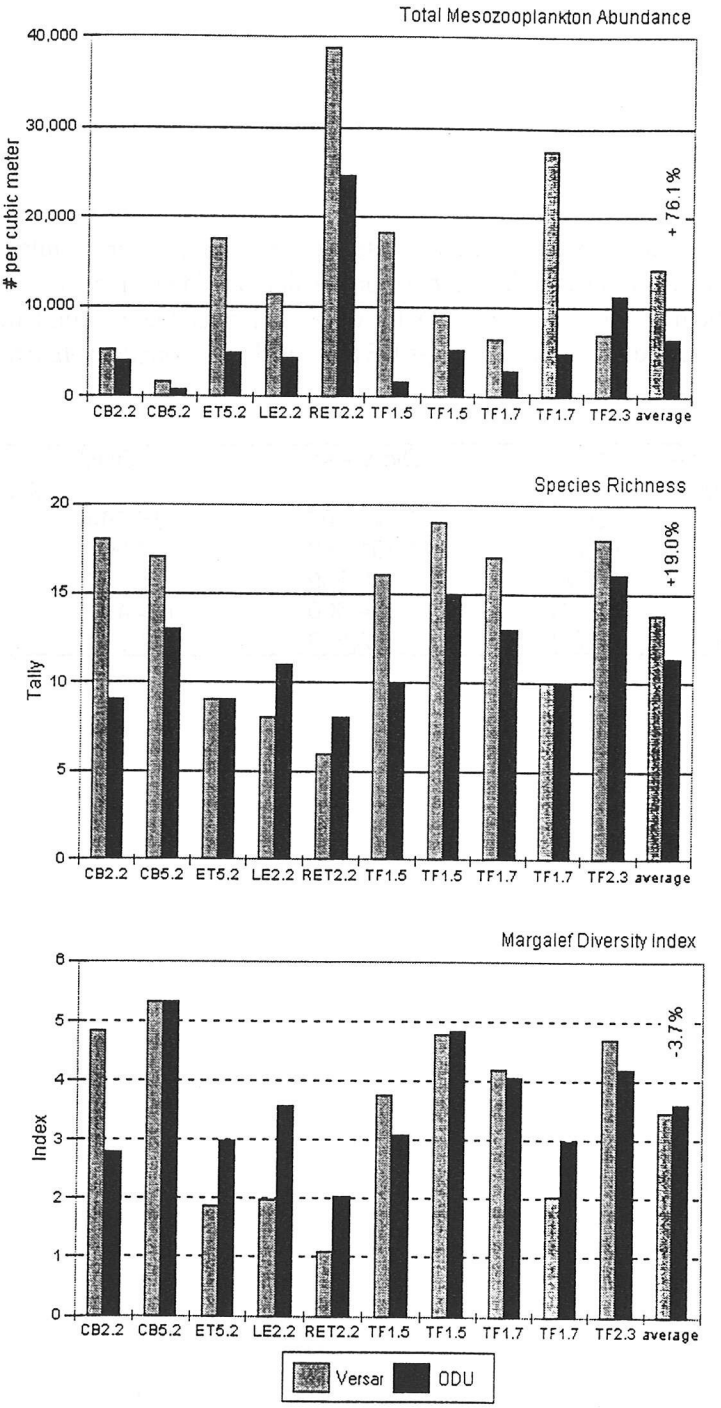

Table 1. Total, non-normalized mesozooplankton counts (i.e. total number per sample jar, estimated from raw subsample counts and total sample volume) for the side-by-side split sample comparisons done at ODU in early 1998. The high Versar count at PXT0402 (= TF1.5) is due to abundant *Bosmina longirostris*. The high Versar count at XDA1177 (= RET2.2) is due to abundant *Acartia tonsa* adults and copepodites.

Salinity	Station	Sample Date	Taxa	Old Versar	Old ODU	ODU count is this % of the Versar count
TF	PXT0402	n/a	TOTAL	7,346,400	65,664	0.89
OH	XDA1177	n/a	TOTAL	1,301,082	154,967	11.91
OH	MCB2.2	n/a	TOTAL	18,639	6,236	33.46
MH	MCB4.3C	n/a	TOTAL	264,000	164,460	62.30
MH	MLE2.2	n/a	TOTAL	32,550	1,632	5.01

Table 2. Mesozooplankton Summary Statistics Comparison, September 11-12, 1998, Using New Methods. Units are number per m^3 (abundance) or ug Carbon per m^3 (biomass), except for the diversity index.*

Station	Date	Counting Agency	TotMes Abundance	Calanoid Abundance	Cladoceran Abundance	Cyclopoid Abundance	Cal:Cla&Cyc Ratio	Ostracod Abundance	Polychaete Abundance
CB6.1	5/18/98	Versar	1,881.62	1,521.83	173.81	2.18	8.65	23.29	4.16
CB6.1	5/18/98	ODU	715.18	560.17	109.11	0.00	5.13	0.00	0.00
CB7.3E	3/6/98	Versar	140.87	134.46	0.21	3.17	39.77	1.48	0.42
CB7.3E	3/6/98	ODU	7.90	2.66	2.33	0.09	1.11	0.00	0.00
CB7.4	4/8/98	Versar	5,031.44	2,301.99	62.16	1,026.67	2.11	6.02	50.13
CB7.4	4/8/98	ODU	10,186.69	6,182.64	800.80	1,334.67	2.90	0.00	369.60
LE3.6	6/1/98	Versar	756.33	724.41	7.19	0.00	100.71	16.44	0.00
LE3.6	6/1/98	ODU	604.40	427.54	119.03	0.00	3.59	0.00	0.00
MCB2.1	5/6/98	Versar	706.10	104.88	496.82	73.82	0.18	1.16	0.00
MCB2.1	5/6/98	ODU	686.48	33.49	589.00	51.17	0.05	0.00	0.00
MCB3.3C	5/5/98	Versar	6,271.03	4,850.43	0.01	8.46	572.27	3.05	384.62
MCB3.3C	5/5/98	ODU	3,457.09	3,227.08	6.30	1.56	410.40	0.00	0.00
MCB4.3C	4/7/98	Versar	39,776.83	39,425.37	0.00	25.86	1,524.45	12.93	90.52
MCB4.3C	4/7/98	ODU	14,643.10	12,424.83	971.03	11.03	12.65	0.00	0.00
MCB4.3C	6/2/98	Versar	2,316.99	2,272.88	1.63	0.00	1,391.00	16.34	3.27
MCB4.3C	6/2/98	ODU	1,354.94	1,326.08	1.08	0.03	1,193.49	0.00	0.00
MET5.1	4/7/98	Versar	10,753.29	3,295.92	7,294.01	86.74	0.45	25.51	0.00
MET5.1	4/7/98	ODU	3,466.94	230.82	3,157.76	23.67	0.07	32.65	0.00
MET5.1	5/5/98	Versar	38,800.20	1,396.10	24,775.72	12,548.70	0.04	32.47	0.00
MET5.1	5/5/98	ODU	60,614.81	531.95	45,958.18	13,651.43	0.01	33.25	0.00
MLE2.2	5/4/98	Versar	17,561.10	14,890.93	291.67	20.83	47.65	520.83	62.50
MLE2.2	5/4/98	ODU	23,327.83	18,476.17	551.17	0.00	33.52	171.00	0.00
MWT5.1	6/3/98	Versar	16,926.86	15,235.29	51.47	12.26	239.08	39.22	19.61
MWT5.1	6/3/98	ODU	4,573.33	3,917.65	49.41	28.33	50.39	3.14	0.00
PXT0402	4/13/98	Versar	31,186.12	29,364.16	1,345.12	175.45	19.31	184.97	23.12
PXT0402	4/13/98	ODU	19,167.40	16,369.48	2,146.24	177.57	7.04	252.02	0.00
PXT0402	6/8/98	Versar	10,905.23	4,809.06	5,971.45	91.76	0.79	0.00	0.00
PXT0402	6/8/98	ODU	10,782.85	5,378.58	4,933.01	339.68	1.02	75.60	0.00
RET3.1	5/6/98	Versar	127,047.47	6,875.27	116,811.08	3,175.43	0.06	43.46	0.36
RET3.1	5/6/98	ODU	8,286.80	4,195.89	3,993.02	29.76	1.04	0.00	0.00
RET4.3	6/10/98	Versar	390.99	289.79	10.42	3.48	20.85	7.64	0.00
RET4.3	6/10/98	ODU	193.05	104.93	13.34	1.25	7.19	1.11	0.00
RET5.2	4/22/98	Versar	11,853.31	957.51	10,346.98	520.73	0.09	12.44	4.15
RET5.2	4/22/98	ODU	8,091.15	854.38	6,774.47	445.85	0.12	0.00	0.00
SBE5	5/14/98	Versar	1,692.85	1,630.41	1.40	2.37	432.59	2.37	0.21
SBE5	5/14/98	ODU	1,183.39	1,101.63	0.00	0.00	110,163.16	0.00	0.00
TF3.3	6/10/98	Versar	24,345.22	13,766.09	6,922.53	3,510.89	1.32	0.30	0.00
TF3.3	6/10/98	ODU	26,136.32	1,824.79	20,035.11	3,842.14	0.08	0.00	0.00
TF4.2	4/10/98	Versar	994.55	943.89	26.19	11.08	25.32	0.59	1.01
TF4.2	4/10/98	ODU	921.35	828.85	49.56	0.00	16.72	0.00	10.32
TF5.5	5/20/98	Versar	2,116.40	1,747.52	225.41	140.12	4.78	0.00	0.00
TF5.5	5/20/98	ODU	4,921.85	2,321.76	305.92	2.96	7.52	18.35	0.00
WE4.2	4/6/98	Versar	862.10	567.66	49.27	0.51	11.40	8.64	16.25
WE4.2	4/6/98	ODU	1,224.18	601.06	66.97	0.00	8.98	0.00	22.11
XDE5339	4/13/98	Versar	5,406.42	3,494.74	5.26	15.79	166.00	5.26	31.58
XDE5339	4/13/98	ODU	1,532.63	957.90	16.00	0.21	59.09	0.00	0.00
XEA6596	6/1/98	Versar	5,678.40	2,493.87	1,086.31	2,098.21	0.78	0.00	0.00
XEA6596	6/1/98	ODU	311.79	120.00	75.36	115.36	0.63	0.00	0.00

Table 2 (Cont.)

Mesozp Biomass	Copepod Biomass	Adult Copepod Biomass	Copepodite Biomass	Total Copepod Abundance	Adult Copepod Abundance	Copepodite Abundance	Margalef Diversity	Station	Date
3,801.37	3,620.65	2,874.71	745.95	1,524.01	900.31	623.70	2.75	CB6.1	5/18/98
1,819.95	1,756.97	1,736.10	20.88	560.17	542.87	17.30	1.75	CB6.1	5/18/98
294.03	290.31	204.18	86.13	137.63	65.78	71.85	8.38	CB7.3E	3/6/98
9.43	7.48	6.72	0.76	2.75	2.11	0.63	11.14	CB7.3E	3/6/98
5,964.36	4,632.30	2,163.16	2,469.14	3,328.66	1,463.81	1,864.85	4.86	CB7.4	4/8/98
17,651.32	15,428.83	11,800.40	3,628.43	7,517.31	4,827.23	2,690.07	3.74	CB7.4	4/8/98
1,703.25	1,689.25	1,314.43	374.83	724.41	411.02	313.40	2.08	LE3.6	6/1/98
1,463.17	1,354.41	1,335.53	18.88	432.80	417.02	15.78	4.31	LE3.6	6/1/98
645.44	306.02	99.86	206.16	190.28	42.09	148.19	8.78	MCB2.1	5/6/98
609.31	234.95	215.12	19.83	93.03	78.18	14.85	7.05	MCB2.1	5/6/98
10,676.90	8,979.29	4,969.69	4,009.60	4,860.69	1,649.45	3,211.23	3.16	MCB3.3C	5/5/98
9,438.87	9,265.73	8,980.14	285.59	3,228.65	3,002.76	225.89	2.54	MCB3.3C	5/5/98
70,310.73	69,771.96	29,064.36	40,707.59	39,451.23	10,917.28	28,533.94	4.13	MCB4.3C	4/7/98
24,780.44	24,066.38	12,888.82	11,177.56	12,435.86	4,889.66	7,546.21	2.16	MCB4.3C	4/7/98
5,171.66	5,141.37	3,871.11	1,270.26	2,272.88	1,210.78	1,062.09	1.78	MCB4.3C	6/2/98
4,051.83	4,031.57	3,908.03	123.55	1,326.11	1,222.81	103.30	2.55	MCB4.3C	6/2/98
9,507.47	5,128.50	596.01	4,532.49	3,408.16	234.69	3,173.47	3.97	MET5.1	4/7/98
2,496.54	605.61	501.57	104.04	263.47	193.27	70.20	3.39	MET5.1	4/7/98
38,732.72	23,329.41	9,824.29	13,505.12	13,944.81	3,754.06	10,190.75	4.79	MET5.1	5/5/98
60,720.45	32,169.41	27,055.53	5,113.88	14,183.64	10,327.01	3,856.62	4.39	MET5.1	5/5/98
33,847.95	31,875.71	22,414.96	9,460.75	14,911.76	7,015.93	7,895.83	2.12	MLE2.2	5/4/98
61,181.34	57,707.33	56,890.86	816.47	18,476.17	17,793.50	682.67	2.06	MLE2.2	5/4/98
25,560.10	24,296.68	9,656.71	14,639.98	15,247.55	3,019.61	12,227.94	2.60	MWT5.1	6/3/98
12,445.68	11,977.52	11,594.57	382.96	3,946.08	3,625.88	320.20	3.83	MWT5.1	6/3/98
54,221.89	53,305.78	24,453.30	28,852.49	29,562.74	9,447.13	20,115.61	3.78	PXT0402	4/13/98
39,044.58	37,623.28	30,430.18	7,193.10	16,621.04	11,767.40	4,853.64	3.27	PXT0402	4/13/98
12,737.80	9,210.77	4,901.24	4,309.53	4,913.76	1,900.25	3,013.52	3.96	PXT0402	6/8/98
18,147.48	15,099.66	15,099.66	0.00	5,718.25	5,718.25	0.00	3.22	PXT0402	6/8/98
89,740.31	19,388.48	11,814.69	7,573.79	10,073.88	4,542.95	5,530.93	5.68	RET3.1	5/6/98
16,428.18	14,009.31	14,009.31	0.00	4,255.34	4,255.34	0.00	2.30	RET3.1	5/6/98
2,165.51	885.96	855.72	30.25	293.67	268.65	25.02	9.26	RET4.3	6/10/98
1,457.11	131.44	5.91	125.53	106.74	2.09	104.66	6.56	RET4.3	6/10/98
8,926.84	2,476.01	891.76	1,584.25	1,482.38	342.49	1,139.90	6.63	RET5.2	4/22/98
6,840.14	2,692.20	1,662.14	1,030.06	1,300.23	605.18	695.05	3.33	RET5.2	4/22/98
4,829.33	4,061.25	3,358.52	702.73	1,651.77	1,070.34	581.43	7.43	SBE5	5/14/98
5,600.96	3,314.11	3,129.05	185.06	1,130.11	975.38	154.73	3.58	SBE5	5/14/98
33,628.84	29,233.98	13,154.52	16,079.46	17,357.38	5,614.74	11,742.64	6.38	TF3.3	6/10/98
32,826.01	13,400.32	10,695.98	2,704.34	5,941.37	4,116.58	1,824.79	2.49	TF3.3	6/10/98
1,837.38	1,819.58	1,005.59	814.00	957.99	388.84	569.16	7.67	TF4.2	4/10/98
3,599.31	2,231.95	1,840.75	391.19	834.01	563.07	270.94	6.75	TF4.2	4/10/98
3,670.14	3,535.62	1,843.28	1,692.35	1,890.80	707.86	1,182.94	6.92	TF5.5	5/20/98
6,207.63	3,923.72	836.51	3,087.21	2,336.96	253.82	2,083.14	3.79	TF5.5	5/20/98
1,837.18	1,618.57	1,489.52	129.05	568.17	464.04	104.12	5.11	WE4.2	4/6/98
2,398.01	1,940.98	1,940.98	0.00	601.06	601.06	0.00	3.24	WE4.2	4/6/98
7,315.57	5,755.17	2,384.06	3,371.11	3,521.05	763.16	2,757.90	3.22	XDE5339	4/13/98
3,224.48	2,730.48	2,519.89	210.59	961.47	789.47	172.00	2.83	XDE5339	4/13/98
9,657.26	6,655.26	2,234.76	4,420.50	4,592.09	922.62	3,669.47	2.66	XEA6596	6/1/98
783.66	628.79	627.52	1.26	236.31	235.36	0.95	4.01	XEA6596	6/1/98

Table 3. Round 1 split sample results as percent differences for specific taxon groups (%). Percent difference was calculated as follows: (Versar # - ODU #)/((Versar # + ODU #)/2) * 100. Positive numbers indicate Versar's counts are higher, negative numbers indicate ODU's counts are higher.

STATION	DATE	TOTAL MESOZOOP ABUNDANCE	CALANOID COPEPOD ABUNDANCE	CLADOCERAN ABUNDANCE	CYCLOPOID COPEPOD ABUNDANCE	OSTRACOD ABUNDANCE	POLYCHEATE ABUNDANCE	COPEPOD ABUNDANCE	COPEPOD ABUNDANCE - ADULTS	COPEPOD ABUNDANCE - COPEPODITES	MARGAELF DIVERSITY
CB6.1	5/18/98	89.84	92.38	45.74	200.00	200.00	200.00	92.49	49.54	189.21	44.27
CB7.3E	3/6/98	178.75	192.23	-166.72	189.55	200.00	200.00	192.17	187.55	196.50	-28.31
CB7.4	4/8/98	-67.75	-91.47	-171.19	-26.09	200.00	-152.23	-77.24	-106.93	-36.23	26.05
LE3.6	6/1/98	22.33	51.54	-177.21		200.00		50.40	-1.45	180.82	-69.71
MCB2.1	5/6/98	2.82	103.19	-16.98	36.24	200.00		68.66	-60.02	163.57	21.79
MCB3.3C	5/5/98	57.85	40.19	-199.24	137.65	200.00	200.00	40.35	-58.18	173.71	21.64
MCB4.3C	4/7/98	92.37	104.15	-200.00	80.38	200.00	200.00	104.13	76.27	116.34	62.62
MCB4.3C	6/2/98	52.40	52.62	41.00	-200.00	200.00	200.00	52.61	-0.99	164.54	-35.55
MET5.1	4/7/98	102.48	173.82	79.15	114.23	-24.56		171.30	19.36	191.34	15.74
MET5.1	5/5/98	-43.89	89.64	-59.89	-8.42	-2.37		-1.70	-93.36	90.18	8.78
MLE2.2	5/4/98	-28.21	-21.49	-61.58	200.00	101.13	200.00	-21.35	-86.88	168.17	2.87
MWT5.1	6/3/98	114.92	118.18	4.08	-79.23	170.37	200.00	117.76	-18.25	189.79	-38.10
PXT0402	4/13/98	47.74	56.83	-45.89	-1.20	-30.69	200.00	56.04	-21.87	122.25	14.58
PXT0402	6/8/98	1.13	-11.18	19.05	-114.93	-200.00		-15.13	-100.23	200.00	20.56
RET3.1	5/6/98	175.51	48.40	186.78	196.29	200.00	200.00	81.21	6.54	200.00	84.85
RET4.3	6/10/98	67.78	93.66	-24.56	94.12	149.20		93.37	196.92	-122.83	34.08
RET5.2	4/22/98	37.73	11.38	41.73	15.49	200.00	200.00	13.09	-55.44	48.49	66.32
SBE5	5/14/98	35.43	38.71	200.00	200.00	200.00	200.00	37.50	9.28	115.93	70.01
TF3.3	6/10/98	-7.10	153.18	-97.28	-9.01	200.00		98.00	30.79	146.20	87.75
TF4.2	4/10/98	7.64	12.98	-61.70	200.00	200.00	-164.42	13.84	-36.61	71.00	12.84
TF5.5	5/20/98	-79.72	-28.22	-30.30	191.72	-200.00		-21.11	94.43	-55.12	58.35
WE4.2	4/6/98	-34.71	-5.71	-30.45	200.00	200.00	-30.51	-5.63	-25.73	200.00	44.82
XDE5339	4/13/98	111.65	113.95	-100.99	194.73	200.00	200.00	114.20	-3.39	176.52	12.91
XEA6596	6/1/98	179.18	181.64	174.05	179.15			180.42	118.70	199.90	-40.34
Average % Difference		**46.51**	**65.44**	**-27.18**	**86.55**	**128.83**	**136.86**	**59.81**	**5.00**	**128.76**	**20.78**

Table 4. Round 2 "First Ten" Split Samples (Elgin Perry 15:11 Thursday, January 28, 1999). Univariate Procedure, Variable = CVDIFF = Versar Coefficient of Variation minus ODU Coefficient of Variation.

Moments

N	65	Sum Wgts	65	
Mean	-0.11482	Sum	-7.46348	
Std Dev	0.261908	Variance	0.068596	
Skewness	0.436674	Kurtosis	1.944496	
USS	5.247104	CSS	4.390127	
CV	-228.098	Std Mean	0.032486	
T:Mean=0	-3.53456	Pr>|T|	0.0008	
Num ^= 0	63	Num > 0	10	
M(Sign)	-21.5	Pr>=|M|	0.0001	= Significant difference between the paired CV's w/ higher CV at ODU
Sgn Rank	-680	Pr>=|S|	0.0001	= Significant difference between the paired CV's w/ higher CV at ODU

Quantiles (Def=5)

100% Max	0.643628	99%	0.643628		
75% Q3	-0.02629	95%	0.442719		
50% Med	-0.07317	90%	0.00891		
25% Q1	-0.23403	10%	-0.413		
0% Min	-0.72278	5%	-0.5563	1%	-0.72278

Range	1.366407
Q3-Q1	0.207746
Mode	0

Extremes

Five lowest and five highest observations (check for outliers)

5 Lowest (Obs #)	5 Highest (Obs #)
-0.72278 (152)	0.288705 (26)
-0.70642 (44)	0.442719 (121)
-0.66144 (196)	0.551221 (52)
-0.5563 (51)	0.601356 (142)
-0.5266 (6)	0.643628 (28)

Table 5. Results of Wilcoxon Signed Rank Analysis for Each Taxa in Round 2 "First Ten" Split Samples. The sign of the Signed Rank statistic indicates the direction of the difference. Positive values indicate that Versar estimates a greater abundance of the taxa while negative values indicate that ODU estimates a greater abundance of the taxa. For exploratory purposes, one might use a p-value of 0.05 bearing in mind that about 1 in every 20 tests will be a false positive by this criterion. (From Elgin Perry).

Wilcoxon Signed rank statistics by taxa with p-values.

TSN	NEWNODC	LIFESTG	TAX_NAME	SGNRNK	p
064358	5001	97	POLYCHAETA	9.50	0.06250
069296	501401	BL	PISCICOLIDAE	0.50	1.00000
069459	51	97	GASTROPODA	5.00	0.12500
081388	551546	0	PISIDIIDAE	-3.00	0.25000
081388	551546	97	PISIDIIDAE	-1.50	0.50000
083833	6109	98	EUCLADOCERA	-0.50	1.00000
083833	6109	BL	EUCLADOCERA	10.00	0.10940
083873	61090201	BL	DAPHNIA	4.00	0.57810
083936	61090301	BL	BOSMINA	-8.50	0.43160
083964	61090502	BL	PODON	-2.50	0.62500
084195	6110	BL	OSTRACODA	8.50	0.43160
085761	61181701	12	CENTROPAGES	0.50	1.00000
085761	61181701	98	CENTROPAGES	0.50	1.00000
085780	61181801	12	DIAPTOMUS	3.00	0.25000
085780	61181801	98	DIAPTOMUS	3.00	0.25000
085848	61181902	12	PSEUDODIAPTOMUS	0.00	1.00000
085848	61181902	98	PSEUDODIAPTOMUS	-2.00	0.50000
085862	61182002	12	EURYTEMORA	13.00	0.03130
085862	61182002	98	EURYTEMORA	2.50	0.82030
085874	61182003	12	TEMORA	-4.00	0.25000
085874	61182003	98	TEMORA	-12.00	0.04690
086084	61182901	12	ACARTIA	10.50	0.03130
086084	61182901	98	ACARTIA	7.00	0.38280
086099	61183001	98	TORTANUS	0.50	1.00000
086110	6119	98	HARPACTICOIDA	-10.50	0.03130
086110	6119	BL	HARPACTICOIDA	5.50	0.31250
088599	61200501	BL	ERGASILUS	7.50	0.06250
088628	61200602	98	SAPHIRELLA	1.50	0.50000
088634	612008	12	CYCLOPIDAE	2.00	0.62500
088634	612008	98	CYCLOPIDAE	-7.50	0.06250
088634	612008	BL	CYCLOPIDAE	-2.00	0.62500
088802	61200901	98	OITHONA	-1.50	0.81250
089599	613402	11	BALANIDAE	9.50	0.35940
089599	613402	17	BALANIDAE	-14.00	0.01560
090054	61530115	BL	NEOMYSIS	3.00	0.25000
092120	6158	BL	ISOPODA	-5.50	0.31250
093294	6168	BL	AMPHIPODA	9.50	0.06250
096383	61791103	31	PALAEMONETES	1.50	0.50000
097107	61792201	31	CRANGON	-0.50	1.00000
098763	61890206	31	HEXAPANOPEUS	-0.50	1.00000
098974	61890602	31	PINNOTHERES	-0.50	1.00000
	61HYDRAC	BL	HYDRACTINIA	1.50	0.50000
102467	6251	BL	PLECOPTERA	0.50	1.00000
118831	6481	21	DIPTERA	3.00	0.25000
118831	6481	97	DIPTERA	0.00	1.00000
155457	770001	98	PHORONIDAE	-1.50	0.50000
159664	8412	98	APPENDICULARIA	-0.50	1.00000
167676	88357502	97	MORONE	-3.00	0.25000
171788	88470106	97	GOBIOSOMA	-0.50	1.00000

Table 6. Comparison of number of taxa identified in each split sample in "First Ten." Life stages of individual taxon are not counted as separate taxa. However, if two or more closely related taxa are identified, they are kept separate (e.g. *Acartia* sp. and *Acartia tonsa* are counted as separate taxa). In the ODU count, "*Acartia* sp. j" and *Acartia* (ODU code 297) are assumed to be *Acartia tonsa* copepodite; "*Eurytemora* sp. j" and *Eurytemora* (ODU code 437) are assumed to be *Eurytemora affinis* copepodite; "*Mesocyclops* sp. j" is assumed to be *Mesocyclops edax* copepodite. Salinity: TF = tidal freshwater; OH = oligohaline; MH = mesohaline; PH = polyhaline.

Station (Salinity/Location)	Date	Rep	ODU Round 1	ODU Round 2	Versar Round 1
CB2.1 (OH/mainstem)	5/6/1998	1	17	12	23
CB3.3C (MH/mainstem)	5/5/1998	1	6	12	11
CB6.1 (MH-PH/mainstem)	5/18/1998	1	4	6	9
ET5.1 (TF/Choptank R.)	4/7/1998	1	13	9	15
ET5.1 (TF/Choptank R.)	5/5/1998	1	19	15	19
LE3.6 (MH-PH/Rappahannock R.)	6/1/1998	1	10	10	6
RET3.1 (TF/Rappahannock R.)	5/6/1998	1,2	10	13	21
RET5.2 (PH/James R.)	4/21/1998	1	13	12	20
TF1.5 (TF/Patuxent R.)	6/8/1998	1	13	12	15
WE4.2 (PH/York R.)	4/16/1998	1	10	14	12
Mean Number of Taxa			11.5	11.5	15.1

Table 7. Results of Wilcoxon Signed Rank Analysis for Each Taxa, Round 2 "Last Ten" Split Samples. The sign of the Signed Rank statistic indicates the direction of the difference. Positive values indicate that Versar estimates a greater abundance of the taxa while negative values indicate that ODU estimates a greater abundance of the taxa. Rankings with p-values of 1 or -1 indicate only one lab counted the identified species. For exploratory purposes, one might use a p-value ("Pr>=|S|") of 0.05 bearing in mind that about 1 in every 20 tests will be a false positive by this criterion. (From Elgin Perry, 9/1/99)

NEWNODC	LIFE_STG	NODCNAME	Sign Rank	Pr>=|S|
3702	98	HYDROIDA	0.5	1.0000
50	98	TROCHOPHORE	-3.0	0.2500
5001	97	POLYCHAETA	22.5	0.0039
5001	98	POLYCHAETA	-1.5	0.5000
51	98	GASTROPODA	-3.0	0.2500
55	97	BIVALVIA	5.0	0.1250
55	98	PELECYPODA	-1.5	0.5000
61090102	98	DIAPHANOSOMA BRACHYURUM	-3.0	0.3750
61090103	98	SIDA CRYSTALLINA	0.5	1.0000
61090201	98	DAPHNIA	0.5	1.0000
61090201	98	DAPHNIA LONGISPINA	2.0	0.6250
61090201	98	DAPHNIA PULEX	1.5	0.5000
61090301	98	BOSMINA LONGIROSTRIS	-7.0	0.3828
61090502	98	PODON POLYPHEMOIDES	-3.0	0.3750
61090601	98	LEPTODORA KINDTII	0.5	1.0000
61090701	98	ALONA	-0.5	1.0000
61090702	98	CHYDORUS	5.0	0.1250
61090705	98	LEYDIGIA QUADRANGULARIS	0.5	1.0000
61090805	98	ILYOCRYPTUS SPINIFER	5.0	0.1250
6110	98	OSTRACODA	-9.0	0.2500
61180505	12	PSEUDOCALANUS	-14.0	0.0156
61180505	98	PSEUDOCALANUS	-18.0	0.0078
61181701	12	CENTROPAGES	3.0	0.2500
61181701	98	CENTROPAGES HAMATUS	-0.5	1.0000
61181701	98	CENTROPAGES TYPICUS	0.5	1.0000
61181801	12	DIAPTOMUS	2.5	0.5625
61181801	98	DIAPTOMUS	-4.5	0.4375
61181902	12	PSEUDODIAPTOMUS CORONATUS	0.5	1.0000
61181902	98	PSEUDODIAPTOMUS CORONATUS	0.5	1.0000
61182002	12	EURYTEMORA	25.5	0.0059
61182002	98	EURYTEMORA AFFINIS	17.5	0.0840
61182003	12	TEMORA TURBINATA	0.5	1.0000
61182003	98	TEMORA LONGICORNIS	-0.5	1.0000
61182901	12	ACARTIA	4.5	0.3125
61182901	12	ACARTIA TONSA	1.5	0.5000
61182901	98	ACARTIA TONSA	6.0	0.3750
6119	98	HARPACTICOIDA	10.5	0.0313
61190502	98	CANUELLA ELONGATA	-7.5	0.0625
61191401	98	EUTERPINA ACUTIFRONS	-0.5	1.0000
61200801	12	HALICYCLOPS	-3.0	0.2500
61200801	98	HALICYCLOPS	-0.5	1.0000
61200802	12	CYCLOPS	10.5	0.0313
61200802	98	CYCLOPS BICUSPIDATUS	3.0	0.2500
61200802	98	CYCLOPS VERNALIS	0.5	1.0000
61200803	12	MESOCYCLOPS	-1.5	0.5000
61200803	12	MESOCYCLOPS EDAX	0.5	1.0000
61200803	98	MESOCYCLOPS EDAX	-1.5	0.5000
61200804	98	EUCYCLOPS AGILIS	0.5	1.0000
61200804	98	EUCYCLOPS SPERATUS	1.5	0.5000
61200807	98	TROPOCYCLOPS PRASINUS	0.5	1.0000
61200901	12	OITHONA	-0.5	1.0000
61200901	98	OITHONA	-1.5	0.5000
61200901	98	OITHONA COLCARVA	0.5	1.0000
613402	11	BALANIDAE	10.5	0.0313

613402	17	BALANIDAE	3.0	0.2500
61340201	11	BALANUS	-10.5	0.0313
61340201	17	BALANUS	-14.0	0.0156
615301	98	MYSIDAE	1.5	0.5000
61530115	93	NEOMYSIS AMERICANA	-3.0	0.2500
61530115	98	NEOMYSIS AMERICANA	7.5	0.0625
61530121	98	MYSIDOPSIS BIGELOWI	0.5	1.0000
6154	98	CUMACEA	0.5	1.0000
61540508	98	OXYUROSTYLIS SMITHI	0.5	1.0000
61691502	98	COROPHIUM LACUSTRE	3.0	0.2500
61692107	98	GAMMARUS	3.5	0.5625
61693708	98	MONOCULODES	7.5	0.0625
61792201	31	CRANGON SEPTEMSPINOSA	-3.0	0.2500
61792201	98	CRANGON SEPTEMSPINOSA	1.5	0.5000
64890502	98	CHAOBORUS PUNCTIPENNIS	0.0	1.0000
648933	21	CHIRONOMIDAE	1.5	0.5000
648933	97	CHIRONOMIDAE	7.5	0.0625

Table 8. Comparisons of Taxa Totals for Round 2, Last Ten Samples, show count increases due to the addition of a 64 micron mesh sieve to the CVS method used by ODU. However, ODU counts are still lower than Versar counts. Features: S = small, M = medium, L = large, VL = very large. Versar Total: calculated total number for this taxa in all ten split samples. ODUwout - Total: calculated total for this taxa in all ten split samples, method is CVS without 64 micron mesh seive ("old" method used prior to 1999). ODUw - Total: calculated total for this taxa in all ten split samples, method is CVS with 64 micron mesh seive. % Difference: (VersarTotal) minus (ODUw -Total) divided by the mean of [(VersarTotal) and (ODUw-Total)] and then multiplied by 100, where a positive % means Versar counts more individuals and a negative percent means ODU counts more individuals. Z: general results of z-score statistical analysis on paired split samples (from Appendix D or Table 9), where NS = no/few significant differences observed and/or no preponderance of higher counts by one laboratory; S(V) = many significant differences observed with a preponderance of higher Versar counts; rare = split counts were mostly ≤ 2000/sample, so % error is relatively high; "-" = z-score not calculated. Conversion factor: multiplier that could be used to convert pre-1999 ODU counts to values comparable to Versar counts. A factor is only calculated for individual taxa with grand totals (total in 10 split samples) of >30,000 in both of the splits.

Features	Taxa	VersarTotal	ODUwout - Total	ODUw - Total	% Difference	Z	Conversion Factor
	Total Cladocera	188,308	84,322	125,666	**39.9**	-	
S-M round	*Bosmina*	77,850	65,966	87,278	-11.42	NS	1.1802
S-M round	Chydorus/chydorids	82,800	1,024	17,408	130.51	rare	
S-L round	*Daphnia* spp.	14,600	3,090	5,906	84.79	rare	
M round	Podonidae (*Podon & Evadne*)	12,800	13,952	14,272	-10.87	rare	
	all other Cladocera	258	290	802		rare	
	Total Miscellaneous	328,513	204,773	222,309	**38.56**	-	
soft	Mollusc & annelid larvae (polychaetes, gastropod, pelecypod)	7,621	20,362	21,130	-93.97	rare	
S-M round	Ostracods	27,450	50,679	64,247	-80.26	rare	
S-M round	Barnacle cypris	6,200	10,184	10,504	-51.53	rare	
S	Barnacle nauplii	287,242	123,548	126,428	77.75	S(V)	2.3249
	Total Copepods	7,842,884	2,872,598	3,797,622	**69.5**	-	
VL	Eurytemora adults	1,756,400	1,549,058	1,557,250	12.02	S(V)	1.1339
M	Eurytemora copepodites	5,794,800	963,947	1,533,803	116.28	S(V)	6.0115
L	Acartia adults	98,400	98,650	100,698	-2.31	S(V)	0.9731
S	Acartia copepodites	69,435	17,552	30,896	76.82	S(V)	3.9560
	Other Calanoids ad&cop	22,272	189,621	437,781	-180.64	-	
	All Calanoids adults and copepodites	7,738,907	2,818,828	3,660,428	71.56	-	
	Mesocyclops	402	4,228	7,044	-178.4	rare	
S	Oithona	800	1,281	36,129	-191.33	rare	
	All Cyclopoids adults and copepodites	67,799	22,944	105,856	-43.83	-	0.6405
	All Harpactacoids adults and copepodites	36,178	30,826	31,338	14.34	-	1.1736
	GRAND TOTALS OF TEN SAMPLES	8,359,705	3,161,693	4,145,597	**67.40**	-	

Table 9. Z-Scores for Barnacle Nauplii, Selected Copepods, Small-Round Taxa, and Polychaete Larvae. The estimated total and the variance estimate for four size-based or shape-based groupings* were computed. The methods for these computations are described in Appendix B. The analysis were run on the NEWNODC code field which contains higher level (more general) taxonomic codes. The estimated total and it's sampling variance were computed for each sample and grouping (and sieve in the case of ODU). For ODU, estimated counts and their variances were summed across sieves. A z-score was calculated from the variances and used to compare the labs on a sample by sample/taxa by taxa basis. Variables names are: STATION, DATE, VETOT = Versar Estimated Total, OETOT = ODU Estimated Total, VEVTOT = Versar Estimated Variance of Total, OEVTOT = ODU Estimated Variance of Total, VCV = Vesar Coefficient of Variation, OCV = ODU Coefficient of Variation, DIFF and VDIFF are calculation steps, and Z = Z-score for difference of counts. A z-score of 2 (**bold** numbers below) has about a 1/20 chance of occurring by accident and a z-score of 3 has about a 1/100 chance of occurring by accident. (From Elgin Perry 10/28/99)

NODCGRP=barnacle nauplii

STATION	MONTH	VETOT	OETOT	VEVTOT	OEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	1600	0	638400	0	0.49937	.	1600	640000	**2.00000**
CB5.2	3	82000	32321	81918000	26717632	0.11038	0.15992	49679	108749953	**4.76385**
ET5.2	4	4400	1032	1755600	261176	0.30113	0.49521	3368	2022208	**2.36842**
LE2.2	3	192000	77842	383808000	132042768	0.10204	0.14762	114158	516120610	**5.02494**
RET2.2	4	0	8192	0	67100672	.	0.99994	-8192	67108864	-1.00000
TF1.5	4	400	0	159600	0	0.99875	.	400	160000	1.00000
TF1.7	3	0	1	0	0	.	0.00000	-1	1	-1.00000
TF1.7	4	7200	7040	2872800	2860160	0.23541	0.24023	160	5747200	0.06674

NODCGRP=Polychaetes AND Trochophores

STATION	MONTH	VETOT	OETOT	VEVTOT	OEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	2000.00	6200	798000.00	12576824	0.44665	0.57200	-4200.00	13383024.00	-1.14808
CB5.2	3	1000.00	0	199000.00	0	0.44609	.	1000.00	200000.00	**2.23607**
ET5.2	4	235.29	768	55128.03	195840	0.99787	0.57622	-532.71	251971.32	-1.06124
LE2.2	3	2000.00	2560	798000.00	4322816	0.44665	0.81216	-560.00	5125376.00	-0.24736
TF1.5	3	1600.00	0	1278400.00	0	0.70666	.	1600.00	1280000.00	1.41421
TF1.5	4	400.00	8	159600.00	24	0.99875	0.61237	392.00	160032.00	0.97990
TF1.7	3	400.00	0	159600.00	0	0.99875	.	400.00	160000.00	1.00000
TF1.7	4	400.00	0	159600.00	0	0.99875	.	400.00	160000.00	1.00000
TF2.3	4	250.00	0	62250.00	0	0.99800	.	250.00	62500.00	1.00000

NODCGRP="Round" organisms

STATION	MONTH	VETOT	OETOT	VEVTOT	OEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	1600	5130	638400	6286346	0.49937	0.48874	-3530	6931476	-1.3408
CB5.2	3	800	2112	159200	2097088	0.49875	0.68567	-1312	2259200	-0.8729
ET5.2	4	2000	3634	798000	4584272	0.44665	0.58918	-1634	5387906	-0.7040
LE2.2	3	11600	12032	4628400	6738176	0.18546	0.21574	-432	11390208	-0.1280
RET2.2	4	250	16576	62250	268431168	0.99800	0.98841	-16326	268510244	-0.9963
TF1.5	3	99200	7444	79260800	2833396	0.08975	0.22612	91756	82200840	**10.1204**
TF1.5	4	49450	103196	19693050	291597640	0.08974	0.16547	-53746	311443336	**-3.0455**
TF1.7	3	7600	4224	3032400	1412992	0.22913	0.28141	3376	4457216	1.5991
TF1.7	4	750	773	186750	294144	0.57619	0.70162	-23	482417	-0.0331
TF2.3	4	41708	75444	16563300	373692850	0.09758	0.25623	-33736	390373302	-1.7075

NODCGRP=Selected Copepods

STATION	MONTH	VETOT	OETOT	VEVTOT	OEVTOT	VCV	OCV	DIFF	VDIFF	Z
CB2.2	3	186705.88	301702	365579384	1064396614	0.1024	0.1081	-114996.12	1430464405	**-3.0405**
CB5.2	3	50612.00	23200	10069400	7881056	0.0627	0.1210	27412.00	18024268	**6.4567**
ET5.2	4	220400.00	143701	423939600	282326648	0.0934	0.1169	76699.00	706630349	**2.8853**
LE2.2	3	81200.00	39459	32398800	52258334	0.0701	0.1832	41741.00	84777793	**4.5334**
RET2.2	4	452800.00	1054772	1707067200	31294744624	0.0912	0.1677	-601972.00	33003319396	**-3.3136**
TF1.5	3	359400.00	74340	542860600	109403948	0.0648	0.1407	285060.00	652698288	**11.1578**
TF1.5	4	225850.00	370573	352476650	1128880756	0.0831	0.0906	-144723.00	1481953829	**-3.7594**
TF1.7	3	112235.29	166927	88903128	292385792	0.0840	0.1024	-54691.71	381568082	**-2.7999**
TF1.7	4	284051.00	268863	977148450	424666624	0.1100	0.0766	15188.00	1402367988	0.4056
TF2.3	4	81802.00	583932	63848200	7262394360	0.0976	0.1459	-502130.00	7326908294	**-5.8662**

* Grouping Assignments were made to the taxa identified by Versar and by ODU as follows:

Versar

NEWNODC	NODC Code	Life_Stg	Name
Balanidae	613402	11	BALANIDAE
PolyTroc	5001	97	POLYCHAETA
Round	61090102	98	DIAPHANOSOMA
Round	61090103	98	SIDA CRYSTALLINA
Round	61090201	98	DAPHNIA
Round	61090301	98	BOSMINA LONGIROSTRIS
Round	61090502	98	PODON POLYPHEMOIDES
Round	61090601	98	LEPTODORA KINDTII
Round	61090702	98	CHYDORUS
Round	61090705	98	LEYDIGIA QUADRANGULARIS
Round	61090805	98	ILYOCRYPTUS SPINIFER
Round	6110	98	OSTRACODA
S_Cope	61180505	12	PSEUDOCALANUS MINUTUS
S_Cope	61180505	98	PSEUDOCALANUS MINUTUS
S_Cope	61181701	12	CENTROPAGES
S_Cope	61181701	98	CENTROPAGES HAMATUS
S_Cope	61181801	12	DIAPTOMUS
S_Cope	61181801	98	DIAPTOMUS
S_Cope	61181902	12	PSEUDODIAPTOMUS CORONATUS
S_Cope	61181902	98	PSEUDODIAPTOMUS CORONATUS
S_Cope	61182002	98	EURYTEMORA AFFINIS
S_Cope	61182003	12	TEMORA TURBINATA
S_Cope	61182901	12	ACARTIA TONSA
S_Cope	61182901	98	ACARTIA TONSA
S_Cope	6119	98	HARPACTICOIDA
S_Cope	61190502	98	CANUELLA ELONGATA
S_Cope	61200801	98	HALICYCLOPS
S_Cope	61200802	12	CYCLOPS
S_Cope	61200802	98	CYCLOPS BICUSPIDATUS
S_Cope	61200803	12	MESOCYCLOPS EDAX
S_Cope	61200803	98	MESOCYCLOPS EDAX
S_Cope	61200804	98	EUCYCLOPS AGILIS
S_Cope	61200807	98	TROPOCYCLOPS PRASINUS
S_Cope	61200901	98	OITHONA COLCARVA

Old Dominion University

NEWNODC	NODC Code	Life_Stg	Name
Balanidae	61340201	11	BALANUS
PolyTroc	50	98	TROCHOPHORE
PolyTroc	5001	97	POLYCHAETA
PolyTroc	5001	98	POLYCHAETA
Round	61090102	98	DIAPHANOSOMA BRACHYURUM
Round	61090201	98	DAPHNIA LONGISPINA
Round	61090301	98	BOSMINA LONGIROSTRIS
Round	61090502	98	PODON POLYPHEMOIDES
Round	61090701	98	ALONA
Round	6110	98	OSTRACODA
S_Cope	61180505	12	PSEUDOCALANUS
S_Cope	61180505	98	PSEUDOCALANUS
S_Cope	61181701	12	CENTROPAGES
S_Cope	61181701	98	CENTROPAGES HAMATUS
S_Cope	61181801	12	DIAPTOMUS
S_Cope	61181801	98	DIAPTOMUS
S_Cope	61182002	98	EURYTEMORA AFFINIS
S_Cope	61182003	98	TEMORA LONGICORNIS
S_Cope	61182901	12	ACARTIA
S_Cope	61182901	98	ACARTIA TONSA
S_Cope	61190502	98	CANUELLA ELONGATA
S_Cope	61191401	98	EUTERPINA ACUTIFRONS
S_Cope	61200801	12	HALICYCLOPS
S_Cope	61200801	98	HALICYCLOPS
S_Cope	61200803	12	MESOCYCLOPS
S_Cope	61200803	98	MESOCYCLOPS EDAX
S_Cope	61200901	12	OITHONA
S_Cope	61200901	98	OITHONA

Table 10. Comparisons of total mesozooplankton counts made with the "old" and "new" CVS methods in Round 2. Numbers are total, non-normalized numbers per sample jar.

Batch	Station	Rep	Month	"Old" CVS Method w/out 64u sieve	"New" CVS Method w/ 64u sieve	New/Old
First Ten	CB2.1	1	5	65,315	73,379	1.1235
First Ten	CB3.3C	1	5	361,277	426,301	1.1800
First Ten	CB6.1	1	5	151,632	166,096	1.0954
First Ten	ET5.1	1	4	92,486	150,854	1.6311
First Ten	ET5.1	1	5	208,535	511,639	2.4535
First Ten	LE3.6	1	6	9,860	15,420	1.5639
First Ten	RET3.1	1	5	2,726,006	2,982,006	1.0939
First Ten	RET3.1	2	5	3,168,902	3,379,590	1.0665
First Ten	RET5.2	1	4	267,207	424,647	1.5892
First Ten	TF1.5	1	6	311,685	414,213	1.3289
First Ten	WE4.2	1	4	63,030	82,230	1.3046
SecondTen	CB2.2	1	3	365,527	398,852	1.0912
SecondTen	CB5.2	1	3	58,468	60,356	1.0323
SecondTen	ET5.2	1	4	169,439	213,471	1.2599
SecondTen	LE2.2	1	3	160,856	174,296	1.0836
SecondTen	RET2.2	1	4	1,605,108	1,620,084	1.0093
SecondTen	TF1.5	1	3	89,060	98,660	1.1078
SecondTen	TF1.5	1	4	288,976	344,784	1.1931
SecondTen	TF1.7	1	3	120,767	137,535	1.1388
SecondTen	TF1.7	1	4	160,454	241,458	1.5048
SecondTen	TF2.3	1	4	151,950	864,654	5.6904
					AVERAGE	1.5019

Table 11. Approximate range of body lengths [a] and widths [b] for some taxa found in Chesapeake Bay. A typical length to width (L:W) ratio for each taxa was determined from drawings and photographs in the available literature.[a] Length and width estimates do not include the dimensions of antennae, spines, caudal rami, etc.

	Length (μ)	Width (μ)	L:W ratio
Adult Copepods			
Eurytemora affinis adult	1,400-1,800	350-450	4:1
Acartia tonsa female adult	1,250-1,500	270-325	4.6:1
Acartia tonsa male adult	1,000-1,150	215-250	4.6:1
Pseudocalanus adult	700-1,500	175-375	4:1
Copepodites			
Acartia copepodite stages I-III	350-570	95-150	3.75:1
Eurytemora affinis copepodite I-V	475-1,275	135-365	3.5:1
Cladocera (immatures & adults)			
Podon polyphemoides	200-800	120-470	1.7:1
Evadne nordmanni	200-1000	120-590	1.7:1
Bosmina longirostris	180-2000	140-1,540	1.3:1
Daphnia pulex	50-2,200	30-1,220	1.8:1

[a] derived from several sources including 1) Todd and Laverack. 1991. Coastal marine zooplankton: a practical manual for students. Cambridge University Press. 2) Conover, R.J. 1956. Comparative development of *A. clausi* and *A. tonsa*. Bull. Bingham Oceanogr. Coll. 15:156-233. 3) Wilson, C. B. 1932. The copepods of the Woods Hole Region, Massachusetts. 4) Edmondson, W. T. (ed.) 1959. Freshwater Biology, 2nd edition. John Wiley & Sons, Inc. 5) Pennak, R. W. 1978. Freshwater Invertebrates of the United States. John Wiley & Sons, Inc. 6) Dodson, S. I. 1981. Morphological variation of *Daphnia pulex* Leydig (Crustacea:Cladocera) and related species from North America. Hydrobiologia 83:101-114. 7) Huff (Appendix 15)

[b] derived from length and L:W ratio

Table 12. Mean and range of raw counts tallied for taxa identified in the Round 2 - Last Ten split samples. Versar raw counts tended to be higher than ODU raw counts for the relatively common taxa. This observation reflects Versar's laboratory objective of counting at least 60 individuals of the dominant and subdominant taxa which gives these counts an error level of about ±25% or better ($p<0.05$). It reflects ODU's laboratory objective of counting 20-42 individuals of the dominant and subdominant taxa to obtain an error level of ±35% ($p<0.05$). See Appendix A for more detail. Versar and ODU raw counts were roughly equivalent for the moderately abundant taxa. Size categories of adults are given for comparisons purposes. The categories are based on the mean adult lengths obtained from literature values, and are determined by the following size (length) fractions: small (S) is < 500µ, medium (M) is 500µ - 800µ, large (L) is 800µ - 1,200µ, and very large (VL) is > 1,200µ.

	Adult Size Category	ODU Mean Cnt	Range	Versar Mean Cnt	Range	
"Common" taxa						
Acartia tonsa adults	L	26	2-48	47	6-74	
Acartia copepodites		32	19-52	31	1-82	
balanus nauplii		28	1-61	35	1-96	
Eurytemora affinis adults	VL	73	4-121	95	2-200	
Eurytemora copepodites		72	1-140	185	4-522	
"Rarer" taxa						
Bosmina longirostris	S	18	1-62	28	1-91	
Podon polyphemoides	M	15	1-40	11	1-29	
Polychaeta		11	2-28	2	1-5	
Daphnia	L-VL	11	1-32	3	1-12	
Ostracoda	mixed	9	2-17	10	1-23	
Neomysis americana	VL	8	1-18	46	11-75	Whole Cnt

Appendix A: Pre-1998 Mesozooplankton Methodologies

	MARYLAND	VIRGINIA
NUMBER OF STATIONS	16 (3 MAINSTEM, 9 TRIB, 4 SEASONAL)	14 (4 MAINSTEM, 10 TRIB)
SAMPLE COLLECTION TYPE	COMPOSITE	COMPOSITE
FIELD COLLECTION PROCEDURES	Two stepped oblique, replicate tows are taken at each station through the entire water column. Steps are taken in 1-4 meter increments depending on total station depth. There are always 5 step levels per station. Tows last between 5 to 10 minutes depending on zooplankton abundance. One of the paired nets is used for taxonomic purposes (counting), the other for biomass measurements. The count sample is preserved.	Monthly net collections of 5 minutes oblique tows, over the water column, are made at 15 stations (located in the Bay and Tributaries) using a paired ½ meter bongo frame containing a set of 202 micron mesh nets, each equipped with flow meter. After the tow, each net will be washed down to collect the sample into bottles which are preserved with 7% buffered formalin. These two paired samples represent replicate samples for that station. Flowmeter readings were taken before and after each tow to determine the volume of water filtered
MESOZOOPLANKTON ENUMERATION TECHNIQUE	A hierarchical counting technique is employed to obtain density estimates. This procedure consists of first counting at least 60 individuals of the most dominant forms (e.g. Acartia tonsa) in a small subsample (usually 1 - 2 milliliters), followed by 5- and 10- milliliter subsamples from which all species that had counts less than 60 in the previous subsample are counted.	Processing and analysis of samples is conducted by the coefficient of variation stabilizing method (Alden et al. 1982). Size fractionation of each sample produces 5 size classes (200, 300,600, 850, 2000 microns). Size classes in which the organisms are too numerous to count in their entirety are split with a folsom plankton splitter until an appropriate sample size is reached for statistically valid counts of the dominant species. The chosen error level of 35% requires that each species of interest be counted to achieve a range of between 20 and 42 individuals in any given split. Species observed to be subdominant in the final split are counted until they have achieved the range for the 35% error level. Taxon abundance is recorded as numbers per unit volume.

	MARYLAND	VIRGINIA
MESOZOOPLANKTON BIOMASS DETERMINATION TECHNIQUE	Dry weights and ash weights are measured by gravimetric methods for detritus-free samples. Samples containing detritus are not processed and are disposed of after the final report is completed. A regression-based computer program is used to estimate mesozooplankton biomass in samples containing detritus. In detritus contaminated samples values for dry weight are based on the known weight (from literature or by weighing of organisms) multiplied by the number present and summed across all taxa in the sample.	ONLY DRYWEIGHT DETERMINATION IS PERFORMED. NO CURRENT METHODOLOGY ON FILE. Data are NOT CONSISTENTLY COLLECTED.
BIOVOLUME DETERMINATION TECHNIQUE	Cnidarians (true jellyfish, hydromedusae) and ctenophores (comb jellies) are separated from the samples in the field after sample preservation. The separate settled volumes of the jellyfish are then measured and recorded. Settled volumes are measured from the correlative count sample for each biomass sample. Samples are poured into Imhoff cones and left undisturbed for 2 - 4 days as plankton settles to the bottom of the cone. After settling time, the reading(top of settled material) is recorded in the lab notebook.	NO METHODOLOGY ON FILE . STARTED REPORTING SETTLED VOLUMES AS OF JAN 96.
ARCHIVE SAMPLES	?	?
NUMBER OF OBSERVED TAXA	157	451
PRESERVATIVES	FORMALIN	Lugol's Solution

	MARYLAND	VIRGINIA
METHOD OF CALCULATION FOR TAXON DENSITY	DENSITY = A * (B/(C * FVOL_M3)), where DENSITY = density in numbers per cubic meter A = number of individuals counted in the subsample, B = volume in milliliters of sample from which subsamples are taken, C = subsample volume in milliliters, FVOL_M3 = volume of water filtered by the bongo nets in cubic meters = (3.14*(r**2)) * (Y*(26,873/999,999)) , where r = radius of the bongo net, Y = flowmeter count (i.e. difference between beginning count and end count, 26,873 = a rotor constant equal to the standard distance traveled in meters for 999,999 revolutions of the flow meter, 999,999 = the maximum revolutions that can be read by flowmeter	The following formula is used to calculate distance traveled by the bongo net during a tow. DISTANCE=(STOP-START)*BLADE CONSTANT Where STOP is the number of revolutions recorded on the bongo net flow meter at the end of the tow, START is the number of revolutions on the meter at the beginning of the tow, DISTANCE is the distance traveled by the bongo net during the tow. The blade constant is equal to 26873/999999.The sample volume is calculated using the following equation: SAMPLE VOLUME= DISTANCE*AREA where AREA + 0.18776 square meters. Densities are first calculated for each size class and then a total density is calculated. The size classes range from 200 to 2000 microns and represent the sieve sizes used to separate organisms into categories prior to identification and enumeration. SC2000= ((2**SC2000S)*SC2000C)/VOLSC850 = ((2**SC850S)*SC850C)/VOLSC600 = ((2**SC600S)*SC600C)/VOLSC300 = ((2**SC300S)*SC300C)/VOLSC200 = ((2**SC2000S)*SC200C)/VOLT_DENS= SC2000+SC850+SC600+SC300+SC200SC= Density of taxa

Appendix B: Methods for Comparing Results from Two Laboratories Participating in Plankton Split Sample Studies

Elgin Perry

The objective of the comparison is to determine is the differences in the estimated sample counts computed by two laboratories consistently differ by more than the chance variation that results from the original split that divides the sample for the two laboratories coupled with the variance that results from subsampling within the laboratories. This evaluation will proceed on two levels. The first level will compare the counts obtained by the laboratories on a sample by sample basis for each taxonomic group. The second level will consider the cumulative evidence across all samples.

For each sample, it is possible to compute the sampling variance of the estimated count. This computation requires that each organism in the sample have equal probability of being selected by the lab's subsampling procedure and that probability must be equal to the proportion of the sample that is fully enumerated by the lab. In addition, the original split that divides the sample for the two labs introduces some variation between the counts that are obtained by the two labs and this variance will also contribute to differences between the labs. The details of computing this variance estimate are given below in "Formulation of Sampling Variance Estimate and Splitting Variance Estimate." By combining the subsampling variance and the splitting variance, it is possible to estimate the variance of the difference between the two labs. The difference between the two labs divided by its standard deviation forms a z-score the absolute value of which should exceed 1.96 only 5% of the time if the null hypothesis of no relative bias between the labs is true. This z-score will be the basis of comparing the labs on a sample by sample basis.

In the second level of analysis, the combined evidence of all the samples will be assessed using the Wilcoxon signed rank test. This test requires a minimum of distributional assumptions. The data should be from a continuous distribution to minimize ties and the data should be from a symmetric distribution. Some data transformation may be required to achieve symmetry. From this test we will learn if there is a consistent bias of one lab relative to the other based on all the samples.

General guidance for interpreting the results from these analyses is as follows. If the z-score test shows occasional differences and these differences are not always in the same direction and the Wilcoxon test does not confirm a consistent bias, we will conclude that the methods and taxonomy employed by the labs are comparable. If the majority of z-score tests indicate differences and these differences are not in the same direction and the Wilcoxon test does not confirm a consistent bias, it is likely that some source of extra-binomial variance is affecting the sample processing and this source of variance should be identified and removed. If the z-score tests indicate a high frequency of differences between labs and these differences are consistently in one direction and the Wilcoxon test confirms that there is a consistent bias, we will conclude that data from the labs are not comparable. There are other outcomes that may warrant attention. If for example a low frequency of differences are identified by the z-score test which are all it the same direction and all from a single salinity zone, this may indicate a taxonomy problem in that salinity zone.

To lend perspective to the differences between labs, this difference may be expressed as a percentage of the average of the two labs.

$$\text{percent difference} = 200\left[\frac{n_1 - n_2}{n_1 + n_2}\right]$$

Where: n_1 = the count for lab 1, and
n_2= the count for lab 2

If these percentages are combined over taxonomic groups or across samples, a weighted averaging formulation will be used to insure that large percentages that result from small numbers of organisms do not distort the results.

Formulation of Sampling Variance Estimate and Splitting Variance Estimate

Let p be the proportion fo the sample that is enumerated.
N is the number of organisms in the sample.
n is the number in the subsample that are enumerated.

Rules for evaluating moments applied to the binomial distribution tell us that

Var(n) = Npq

Where q = (1-p).

N is unknown, but can be estimated by

$\hat{N} = n/p$

Substituting this into the equation above yields

$s_n^2 = \hat{N}pq = (n/p)pq = nq$

When comparing counts between laboratories, it is the sampling variance of $\hat{N}$ that is needed. When a random variable, for example n is multiplied by a constant, for example $1/p$ then the variance of this product is obtained by multiplying the variance of the random variable ($s_n 2$) by the square of the constant. Applying this rule we obtain

$$var(\hat{N}) = s_{n(\frac{1}{p})}^2 = \frac{nq}{p^2} = s_N^2$$

This quantifies the variance due to subsampling within labs. It remains to quantify the variance due to the original split that divided the sample between the labs. At this point we add a subscript I to distinguish between labs. That is: $\hat{N}_i$ is the estimated count from lab i and s_i^2 is the estimated variance for lab i.

Assume that there are N total organisms in a sample to be split. As a result of the split, x organisms go to one lab and N-x go to the other. The difference between the two labs is $2x$-N. If the p of the original split was 0.5 then the $E(2x\text{-}N) = 0$ and the variance of $2x$-N is

$$\text{Var}(2x\text{-}N) = 4\,\text{Var}(x) - \text{Var}(N) = 4N\left(\frac{1}{2}\right)\left(\frac{1}{2}\right) = N.$$

Our best estimate of the total number of organisms before the split is $\hat{N} = \hat{N}_1 + \hat{N}_2$.

Combining the results from above, the estimate of the variance of the difference between labs is given by

$$s^2_{diff} = (\hat{N}_1 + \hat{N}_2) + s^2_{N_1} + s^2_{N_2} = \frac{n_1}{p_1} + \frac{n_2}{p_2} + \frac{n_1 q_1}{p_1^2} + \frac{n_2 q_2}{p_2^2}$$

Appendix C: Letter from Versar to Maryland Department of Natural Resources following March 10-12, 1999 meeting at Old Dominion University.

April 6, 1999

Bruce Michael
Tidewater Ecosystem Assessments
Maryland Department of Natural Resources
Tawes State Office Building, D-2
580 Taylor Avenue
Annapolis, Maryland 21401

Dear Bruce:

On March 10, 1999 Craig M. Bruce from Versar, Inc visited the zooplankton laboratory at Old Dominion University (ODU) for three days discussing techniques, taxonomy, nomenclature, and ways to make laboratory processing more compatible between the Maryland and Virginia programs. The purpose of this letter is to summarize the results of this meeting and to identify solutions.

Historically, Versar and ODU identified rotifers such as Brachionus sp. to the genus level. While they are seen in samples collected with a 202-µm mesh net, the microzooplankton program generates more accurate numbers for this taxa. Therefore, both organizations have agreed stop counting rotifers in the mesozooplankton samples. Several in-house taxanomic differences were identified at ODU for cyclopoids, isopods, and amhipods. The differences were related to the level of identification and it was resolved that when possible animals should be identified to genera (e.g., gammarus vs. unidentified amphipod).

We determined that Versar had been misidentifying barnacle cypris (eggs) as ostracods in high salinity stations. Although the taxonomic issue has been resolved beginning with March 1999 samples, Versar will query historic data to determine when and where barnacle nauplii and the misidentified ostracods were present together. In this situation it is most likely that organisms identified as ostracods were barnacle cypris. If this is the case we will recode the species as barnacle cypris. Versar will contact Jackie Johnson to correct the data.

One of the ODU taxonomist had been misidentifying Eurytemora sp. as Temora sp. at some stations. This error was most likely due to inexperience. The taxonomist presently can identify the difference between the genera. Versar has not reported Eurytemora americana whereas ODU has. The differences between E. affinis vs. E. americana were discussed based on descriptions in C. *Wilson's Copepods of Woods Hole Region Massachusetts.* In lieu of the descriptions, it appears that ODU has been misidentifying E. affinis as E. americana; however, both groups will be alert for E. americana. The taxonomic issue has been resolved (E. affinis had four segments on the urosome while E. americana has five segments).

The lumping of E. affinis and E. hirundoides into E. affinis was questioned. However, according to Frank Ferrari at the Smithsonian Institution, the two species names are now considered to be synonymous. George Mateja is going to follow up on this reclassification by asking Paul Fofonoff of the Smithsonian Environmental Research Center to evaluate several specimens.

We discussed the nomenclature changes such as Cyclops vernalis to Acanthocyclops vernalis and Cyclops bicuspidatus to Diacyclops thomasi. Versar has changed the names in its database to the new designations.

The consistency of identification levels between Versar and ODU was discussed. It was agreed that:

- The most common species of Daphnia will be identified to species level.
- The most common species of Harpacticoid will be identified to genus and/or species level.
- The most common Diptera will be identified to family or genus.
- The most common Amphipod will be identified to family or genus.
- Crab zoea and megalops will be identified to species level.
- Specific larval stages (e.g. trochophore and spionidae) will not be deffientiated. Instead they will be reported as polychaete larvae.

Versar does not currently count fish eggs, fish larvae, or protochordates; ODU does count these organisms but the information is not reported. Versar will continue not to count these organisms based on an earlier decision by the Chesapeake Bay Program that the current field gear does not effectively sample these organisms.

We noticed that ODU did not count Bosmina longirostris in sample WE4.2 during Round 1 of the split sample but reported a density of 326 m^3 in Round two of the comparison. ODU felt that the occurrence of B. longirostris was possibly due to sample contamination during sample sieving/splitting. We decided that for Round 3 of the comparison, 10 new samples would be examined. The 10 new samples will be used to rule out any previous sample contamination and should better represent the consistency of taxonomic techniques. All 10 samples will be from the regular Maryland collections to avoid additional costs to the program split. The first half of the new samples will be taken from March and the second half of the samples will be taken from April.

The list of specimens in Table 1 were either examined or discussed during our meeting.

Table 1. List of species discussed at ODU and Versar meeting in March 1999

Acartia tonsa	Centropages hamatus
Ameroculodes species complex	Centropages typicus
Argulus	Chaoborus punctipennis
Barnacle cypris	Chironomidae larvae
Barnacle nauplii	Crab megalops unid.
Bivalvia	Crab zoea unid.
Brachionus	Crangon septemspinosa
Cumacea	Neomysis americana
Cyclops bicuspidatus (Diacyclops thomasi)	Oithona
Cyclops vernalis (Acanthocyclops vernalis)	Ostracoda
Daphnia	Paguridae
Diaphansoma sp.	Pagurus longicarpus
Diaptomus	Pagurus pollicaris
Ergasilus	Palaemonetes sp. zoea
Eurytemora affinis	Phronidae
Hexarthra sp.	Podon polyphemoides
Hydroid	Polychaete larvae
Ilyocryptus spinifer	Pseudodiaptomus coronatus
Isopoda	Rhithropanopeus
Labidocera aestiva	Saphirella
Leptodora kindtii	Gammarus
Mesocyclops edax	Temora turbinata
Moina	Tortanus discaudatus
Mysidopsis	Gastropod unid.
Mysidopsis almyra	Halicyclops
Mysidopsis bigelowi	Tropocyclops prasinus
	Harpacticoid

The meeting between Versar and ODU was very productive. However, given laboratory personnel turn over rates (especially ODU graduate students) annual workshops to discuss taxonomy and laboratory

counting techniques should be continued to ensure the continued development of a bay-wide zooplankton indicators.

Sincerely,

William Burton

cc: C. Bruce
C. Buchanan
F. Jacobs
File: 4337-101
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Appendix D: Z-Score Statistical Analysis of Round 2 Last Ten Split Samples

The estimated total and the variance estimate for each taxonomic group and sample were computed. The methods for these computations are described in Appendix B. The estimated total and it's sampling variance were computed for each sample, taxa, and lifestage (and sieve in the case of ODU). For ODU, estimated counts and their variances were summed across sieves. A z-score was calculated from the variances and used to compare the labs on a sample by sample/taxa by taxa basis. Variables names are: STATION, DATE, VETOT = Versar Estimated Total, OETOT = ODU Estimated Total, VEVTOT = Versar Estimated Variance of Total, OEVTOT = ODU Estimated Variance of Total, VCV = Vesar Coefficient of Variation, OCV = ODU Coefficient of Variation, Z = Z-score for difference of counts. A z-score of 2 has about a 1/20 chance of occurring by accident and a z-score of 3 has about a 1/100 chance of occurring by accident. Z-score $\geq$ 2.0 are **bolded**. (From Elgin Perry 9/1/1999)

NEWNODC	LIFE_STG	TAXON	STATION	MONTH	VETOT	OETOT	VEVTOT	OEVTOT	VCV	OCV	Z
3702	98	HYDROIDA	TF2.3	4	2.0	0.0	.	0.0	.	.	.
50	98	TROCHOPHORE	LE2.2	3	0.0	2048.0	0.0	4192256.0	.	0.99976	-1.00000
50	98	TROCHOPHORE	ET5.2	4	0.0	768.0	0.0	195840.0	.	0.57622	-1.73205
50	98	TROCHOPHORE	CB2.2	3	0.0	6144.0	0.0	12576768.0	.	0.57721	-1.73205
51	98	GASTROPODA	TF1.5	4	400.0	4096.0	159600.0	16773120.0	0.99875	0.99988	-0.89807
51	98	GASTROPODA	TF1.7	3	235.3	320.0	55128.0	36544.0	0.99787	0.59739	-0.27892
51	98	GASTROPODA	CB2.2	3	0.0	2.0	0.0	2.0	.	0.70711	-1.00000
55	97	BIVALVIA	ET5.2	4	4.0	0.0	.	0.0	.	.	.
55	97	BIVALVIA	CB2.2	3	3.0	0.0	.	0.0	.	.	.
55	97	BIVALVIA	LE2.2	3	1.0	0.0	.	0.0	.	.	.
55	97	BIVALVIA	CB5.2	3	200.0	0.0	39800.0	0.0	0.99750	.	1.00000
55	98	PELECYPODA	ET5.2	4	0.0	8.0	0.0	56.0	.	0.93541	-1.00000
55	98	PELECYPODA	TF2.3	4	0.0	7168.0	0.0	7332864.0	.	0.37778	**-2.64575**
87	90	GNATHOSTOMATA	TF1.7	4	0.0	3.0	0.0	0.0	.	0.00000	-1.73205
87	90	GNATHOSTOMATA	TF1.5	4	0.0	92.0	0.0	276.0	.	0.18058	**-4.79583**
5001	97	POLYCHAETA	TF2.3	4	250.0	0.0	62250.0	0.0	0.99800	.	1.00000
5001	97	POLYCHAETA	TF1.7	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
5001	97	POLYCHAETA	ET5.2	4	235.3	0.0	55128.0	0.0	0.99787	.	1.00000
5001	97	POLYCHAETA	CB5.2	3	1000.0	0.0	199000.0	0.0	0.44609	.	**2.23607**
5001	97	POLYCHAETA	CB2.2	3	2000.0	0.0	798000.0	0.0	0.44665	.	**2.23607**
5001	97	POLYCHAETA	TF1.5	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
5001	97	POLYCHAETA	LE2.2	3	2000.0	512.0	798000.0	130560.0	0.44665	0.70572	1.54210
5001	97	POLYCHAETA	TF1.5	3	1600.0	0.0	1278400.0	0.0	0.70666	.	1.41421
5001	97	POLYCHAETA	TF1.7	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000

5001	98	POLYCHAETA		TF1.5	4	0.0	8.0	0.0	24.0	.	0.61237	-1.41421
5001	98	POLYCHAETA		CB2.2	3	0.0	56.0	0.0	56.0	.	0.13363	**-5.29150**
6110	98	OSTRACODA		RET2.2	4	0.0	16512.0	0.0	268427136.0	.	0.99223	-1.00780
6110	98	OSTRACODA		CB2.2	3	0.0	4618.0	0.0	6024714.0	.	0.53151	-1.88070
6110	98	OSTRACODA		TF1.5	4	9200.0	24647.0	3670800.0	89106372.0	0.20825	0.38299	-1.60341
6110	98	OSTRACODA		TF1.5	3	14400.0	1424.0	11505600.0	460656.0	0.23555	0.47663	**3.74865**
6110	98	OSTRACODA		TF1.7	3	0.0	128.0	0.0	8064.0	.	0.70156	-1.41421
6110	98	OSTRACODA		TF1.7	4	250.0	2.0	62250.0	0.0	0.99800	0.00000	0.99198
6110	98	OSTRACODA		TF2.3	4	1200.0	14988.0	478800.0	77661580.0	0.57663	0.58798	-1.55962
6110	98	OSTRACODA		ET5.2	4	1600.0	1544.0	638400.0	391704.0	0.49937	0.40535	0.05509
6117	11	COPEPODA		TF1.7	4	800.0	0.0	319200.0	0.0	0.70622	.	1.41421
6117	11	COPEPODA		TF1.5	3	20800.0	0.0	16619200.0	0.0	0.19599	.	**5.09902**
6117	11	COPEPODA		TF1.5	4	800.0	0.0	319200.0	0.0	0.70622	.	1.41421
6117	11	COPEPODA		TF1.7	3	470.6	0.0	110256.1	0.0	0.70560	.	1.41421
6117	11	COPEPODA		LE2.2	3	800.0	384.0	319200.0	48768.0	0.70622	0.57509	0.68468
6117	11	COPEPODA		ET5.2	4	235.3	0.0	55128.0	0.0	0.99787	.	1.00000
6117	11	COPEPODA		CB2.2	3	1600.0	0.0	638400.0	0.0	0.49937	.	**2.00000**
6119	98	HARPACTICOIDA		TF1.7	3	800.0	0.0	319200.0	0.0	0.70622	.	1.41421
6119	98	HARPACTICOIDA		CB5.2	3	200.0	0.0	39800.0	0.0	0.99750	.	1.00000
6119	98	HARPACTICOIDA		TF1.5	3	12800.0	0.0	10227200.0	0.0	0.24984	.	**4.00000**
6119	98	HARPACTICOIDA		CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
6119	98	HARPACTICOIDA		TF1.5	4	2800.0	0.0	1117200.0	0.0	0.37749	.	**2.64575**
6119	98	HARPACTICOIDA		LE2.2	3	1600.0	0.0	638400.0	0.0	0.49937	.	**2.00000**
6154	98	CUMACEA		TF1.5	3	1.0	0.0	.	0.0	.	.	.
615301	98	MYSIDAE		CB2.2	3	2.0	0.0	.	0.0	.	.	.
615301	98	MYSIDAE		TF1.7	3	1.0	0.0	.	0.0	.	.	.
648933	21	CHIRONOMIDAE		TF1.5	4	21.0	0.0	.	0.0	.	.	.
648933	21	CHIRONOMIDAE		RET2.2	4	1.0	0.0	.	0.0	.	.	.
648933	97	CHIRONOMIDAE		TF1.5	4	5.0	0.0	.	0.0	.	.	.
648933	97	CHIRONOMIDAE		RET2.2	4	1.0	0.0	.	0.0	.	.	.
648933	97	CHIRONOMIDAE		TF1.7	3	1.0	0.0	.	0.0	.	.	.
648933	97	CHIRONOMIDAE		ET5.2	4	1.0	0.0	.	0.0	.	.	.
648933	97	CHIRONOMIDAE		TF2.3	4	6.0	0.0	.	0.0	.	.	.
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF1.7	3	0.0	512.0	0.0	261632.0	.	0.99902	-1.00000
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF2.3	4	250.0	2.0	62250.0	2.0	0.99800	0.70711	0.99197
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF1.5	4	0.0	256.0	0.0	65280.0	.	0.99804	-1.00000
61090102	98	DIAPHANOSOMA	BRACHYURUM	TF1.5	3	0.0	32.0	0.0	992.0	.	0.98425	-1.00000

61090103	98	SIDA	CRYSTALLINA	TF2.3	4	1.0	0.0	.	0.0	.	.	.
61090201	98	DAPHNIA	LONGISPINA	TF1.7	3	2000.0	1536.0	798000.0	784896.0	0.44665	0.57679	0.36839
61090201	98	DAPHNIA	PULEX	RET2.2	4	250.0	0.0	62250.0	0.0	0.99800	.	1.00000
61090201	98	DAPHNIA		CB2.2	3	800.0	0.0	319200.0	0.0	0.70622	.	1.41421
61090201	98	DAPHNIA	LONGISPINA	TF1.5	4	1450.0	2056.0	541050.0	2095128.0	0.50728	0.70402	-0.37299
61090201	98	DAPHNIA	LONGISPINA	TF2.3	4	250.0	38.0	62250.0	998.0	0.99800	0.83135	0.84106
61090201	98	DAPHNIA	LONGISPINA	TF1.5	3	9600.0	2276.0	7670400.0	675108.0	0.28849	0.36101	**2.53345**
61090201	98	DAPHNIA	PULEX	TF1.7	4	250.0	0.0	62250.0	0.0	0.99800	.	1.00000
61090301	98	BOSMINA	LONGIROSTRIS	ET5.2	4	400.0	2082.0	159600.0	4192480.0	0.99875	0.98346	-0.80603
61090301	98	BOSMINA	LONGIROSTRIS	TF1.5	3	24000.0	2208.0	19176000.0	1063776.0	0.18246	0.46712	**4.84076**
61090301	98	BOSMINA	LONGIROSTRIS	CB2.2	3	0.0	512.0	0.0	261632.0	.	0.99902	-1.00000
61090301	98	BOSMINA	LONGIROSTRIS	TF2.3	4	36400.0	39648.0	14523600.0	159366494.0	0.10470	0.31840	-0.24625
61090301	98	BOSMINA	LONGIROSTRIS	TF1.7	3	2800.0	2048.0	1117200.0	358400.0	0.37749	0.29232	0.61805
61090301	98	BOSMINA	LONGIROSTRIS	TF1.7	4	250.0	771.0	62250.0	294144.0	0.99800	0.70344	-0.87147
61090301	98	BOSMINA	LONGIROSTRIS	TF1.5	4	14000.0	39945.0	5586000.0	110584856.0	0.16882	0.26326	**-2.40660**
61090301	98	BOSMINA	LONGIROSTRIS	RET2.2	4	0.0	64.0	0.0	4032.0	.	0.99216	-1.00000
61090502	98	PODON	POLYPHEMOIDES	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61090502	98	PODON	POLYPHEMOIDES	CB5.2	3	800.0	2112.0	159200.0	2097088.0	0.49875	0.68567	-0.87288
61090502	98	PODON	POLYPHEMOIDES	LE2.2	3	11600.0	12032.0	4628400.0	6738176.0	0.18546	0.21574	-0.12800
61090502	98	PODON	POLYPHEMOIDES	TF2.3	4	0.0	128.0	0.0	16256.0	.	0.99609	-1.00000
61090601	98	LEPTODORA	KINDTII	TF2.3	4	7.0	0.0	.	0.0	.	.	.
61090701	98	ALONA		TF2.3	4	0.0	17408.0	0.0	134724608.0	.	0.66677	-1.49968
61090702	98	CHYDORUS		TF1.7	3	800.0	0.0	319200.0	0.0	0.70622	.	1.41421
61090702	98	CHYDORUS		CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61090702	98	CHYDORUS		TF1.5	3	4800.0	0.0	3835200.0	0.0	0.40799	.	**2.44949**
61090702	98	CHYDORUS		TF2.3	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61090705	98	LEYDIGIA	QUADRANGULARIS	TF2.3	4	1200.0	0.0	478800.0	0.0	0.57663	.	1.73205
61090805	98	ILYOCRYPTUS	SPINIFER	TF2.3	4	2000.0	0.0	798000.0	0.0	0.44665	.	**2.23607**
61090805	98	ILYOCRYPTUS	SPINIFER	TF1.7	3	2000.0	0.0	798000.0	0.0	0.44665	.	**2.23607**
61090805	98	ILYOCRYPTUS	SPINIFER	TF1.5	4	24800.0	0.0	9895200.0	0.0	0.12684	.	**7.87401**
61090805	98	ILYOCRYPTUS	SPINIFER	TF1.5	3	46400.0	0.0	37073600.0	0.0	0.13122	.	**7.61577**
61180505	12	PSEUDOCALANUS		TF1.5	4	0.0	39424.0	0.0	45835776.0	.	0.17173	**-5.82065**
61180505	12	PSEUDOCALANUS		RET2.2	4	0.0	512.0	0.0	261632.0	.	0.99902	-1.00000
61180505	12	PSEUDOCALANUS		TF1.7	4	0.0	33792.0	0.0	34569216.0	.	0.17399	**-5.74456**
61180505	12	PSEUDOCALANUS		CB5.2	3	200.0	384.0	39800.0	48768.0	0.99750	0.57509	-0.61624
61180505	12	PSEUDOCALANUS		CB2.2	3	0.0	46592.0	0.0	74271232.0	.	0.18497	**-5.40462**
61180505	12	PSEUDOCALANUS		ET5.2	4	0.0	2048.0	0.0	4192256.0	.	0.99976	-1.00000
61180505	12	PSEUDOCALANUS		TF2.3	4	0.0	183296.0	0.0	1414870016.0	.	0.20521	**-4.87267**

61180505	98	PSEUDOCALANUS		TF1.5	3	0.0	4096.0	0.0	8384512.0	.	0.70693	-1.41421
61180505	98	PSEUDOCALANUS		TF1.7	3	0.0	15489.0	0.0	7406464.0	.	0.17570	**-5.68544**
61180505	98	PSEUDOCALANUS		TF1.5	4	0.0	63.0	0.0	94.0	.	0.15389	**-5.02795**
61180505	98	PSEUDOCALANUS		TF2.3	4	0.0	2586.0	0.0	206490.0	.	0.17572	**-5.65557**
61180505	98	PSEUDOCALANUS		RET2.2	4	0.0	32768.0	0.0	1073709056.0	.	0.99998	-1.00000
61180505	98	PSEUDOCALANUS		TF1.7	4	0.0	57871.0	0.0	107224576.0	.	0.17893	**-5.58723**
61180505	98	PSEUDOCALANUS		LE2.2	3	0.0	2048.0	0.0	2095104.0	.	0.70676	-1.41421
61180505	98	PSEUDOCALANUS		CB5.2	3	2400.0	2752.0	477600.0	1090880.0	0.28795	0.37952	-0.28060
61181701	12	CENTROPAGES		TF2.3	4	500.0	0.0	124500.0	0.0	0.70569	.	1.41421
61181701	12	CENTROPAGES		CB5.2	3	6200.0	736.0	1233800.0	84256.0	0.17916	0.39439	**4.74683**
61181701	12	CENTROPAGES		LE2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61181701	98	CENTROPAGES	TYPICUS	CB5.2	3	5408.0	2240.0	1074600.0	370496.0	0.19168	0.27173	**2.62839**
61181701	98	CENTROPAGES	HAMATUS	LE2.2	3	0.0	2.0	0.0	2.0	.	0.70711	-1.00000
61181801	12	DIAPTOMUS		TF1.5	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61181801	12	DIAPTOMUS		TF1.5	3	1600.0	384.0	1278400.0	48768.0	0.70666	0.57509	1.05474
61181801	12	DIAPTOMUS		TF1.7	4	250.0	0.0	62250.0	0.0	0.99800	.	1.00000
61181801	12	DIAPTOMUS		TF1.7	3	0.0	3008.0	0.0	877632.0	.	0.31144	**-3.20537**
61181801	12	DIAPTOMUS		TF2.3	4	250.0	0.0	62250.0	0.0	0.99800	.	1.00000
61181801	98	DIAPTOMUS		TF2.3	4	250.0	0.0	62250.0	0.0	0.99800	.	1.00000
61181801	98	DIAPTOMUS		CB5.2	3	0.0	1024.0	0.0	523264.0	.	0.70642	-1.41421
61181801	98	DIAPTOMUS		LE2.2	3	0.0	5512.0	0.0	1682056.0	.	0.23529	**-4.24305**
61181801	98	DIAPTOMUS		TF1.5	3	1000.0	32.0	499000.0	992.0	0.70640	0.98425	1.36756
61181801	98	DIAPTOMUS		TF1.7	4	1.0	0.0	.	0.0	.	.	.
61181801	98	DIAPTOMUS		TF1.7	3	0.0	1026.0	0.0	1047552.0	.	0.99756	-1.00195
61181902	12	PSEUDODIAPTOMUS	CORONATUS	CB5.2	3	1800.0	0.0	358200.0	0.0	0.33250	.	**3.00000**
61181902	98	PSEUDODIAPTOMUS	CORONATUS	CB5.2	3	2000.0	0.0	398000.0	0.0	0.31544	.	**3.16228**
61182002	12	EURYTEMORA		TF1.7	3	198000.0	34305.0	395802000.0	31685120.0	0.10048	0.16409	**7.91510**
61182002	12	EURYTEMORA		TF1.7	4	1096000.0	31746.0	4382904000.0	38765568.0	0.06040	0.19613	**16.00280**
61182002	12	EURYTEMORA		TF2.3	4	408000.0	462864.0	1631592000.0	7270166542.0	0.09900	0.18421	-0.58150
61182002	12	EURYTEMORA		ET5.2	4	550000.0	139640.0	1099450000.0	247327248.0	0.06029	0.11262	**11.17910**
61182002	12	EURYTEMORA		CB2.2	3	334000.0	155474.0	667666000.0	289370322.0	0.07736	0.10941	**5.76930**
61182002	12	EURYTEMORA		CB5.2	3	800.0	64.0	159200.0	4032.0	0.49875	0.99216	1.81690
61182002	12	EURYTEMORA		RET2.2	4	2088000.0	563149.0	8349912000.0	7455168588.0	0.04376	0.15332	**12.12810**
61182002	12	EURYTEMORA		LE2.2	3	170000.0	56353.0	339830000.0	53683230.0	0.10844	0.13002	**5.72730**
61182002	12	EURYTEMORA		TF1.5	3	608000.0	30560.0	2431392000.0	13759648.0	0.08110	0.12138	**11.67610**
61182002	12	EURYTEMORA		TF1.5	4	342000.0	59648.0	683658000.0	132126464.0	0.07645	0.19271	**9.88320**
61182002	98	EURYTEMORA	AFFINIS	TF2.3	4	78400.0	68376.0	62641600.0	134509846.0	0.10095	0.16962	0.71360

61182002	98	EURYTEMORA	AFFINIS	ET5.2	4	210000.0	49749.0	419790000.0	100621176.0	0.09757	0.20163	**7.02290**
61182002	98	EURYTEMORA	AFFINIS	CB5.2	3	400.0	256.0	79600.0	16128.0	0.70534	0.49608	0.46380
61182002	98	EURYTEMORA	AFFINIS	TF1.7	4	240000.0	88623.0	959760000.0	145925632.0	0.12908	0.13631	**4.55180**
61182002	98	EURYTEMORA	AFFINIS	TF1.7	3	110400.0	78860.0	88209600.0	160353280.0	0.08507	0.16058	1.99980
61182002	98	EURYTEMORA	AFFINIS	CB2.2	3	182000.0	177286.0	363818000.0	680873286.0	0.10480	0.14718	0.14580
61182002	98	EURYTEMORA	AFFINIS	LE2.2	3	27200.0	2072.0	10852800.0	4192276.0	0.12112	0.98818	**6.47200**
61182002	98	EURYTEMORA	AFFINIS	TF1.5	4	164000.0	115002.0	327836000.0	439828270.0	0.11040	0.18236	1.76810
61182002	98	EURYTEMORA	AFFINIS	RET2.2	4	424000.0	929842.0	1695576000.0	28060012590.0	0.09712	0.18015	**-2.93240**
61182002	98	EURYTEMORA	AFFINIS	TF1.5	3	**320000.0***	47184.0	511680000.0	84625648.0	0.07069	0.19496	**11.16870**
61182003	12	TEMORA	TURBINATA	CB5.2	3	4.0	0.0	.	0.0	.	.	.
61182003	98	TEMORA	LONGICORNIS	CB5.2	3	0.0	96.0	0.0	2976.0	.	0.56826	-1.73205
61182901	12	ACARTIA		RET2.2	4	400.0	1408.0	159600.0	88704.0	0.99875	0.21153	**-2.01555**
61182901	12	ACARTIA		TF1.7	4	15600.0	2816.0	6224400.0	357632.0	0.15993	0.21237	**4.97600**
61182901	12	ACARTIA	TONSA	TF1.7	3	235.3	0.0	55128.0	0.0	0.99787	.	1.00000
61182901	12	ACARTIA		LE2.2	3	34800.0	11776.0	13885200.0	10080768.0	0.10708	0.26962	**4.69853**
61182901	12	ACARTIA	TONSA	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61182901	12	ACARTIA		ET5.2	4	1600.0	11024.0	638400.0	2807280.0	0.49937	0.15199	**-5.06762**
61182901	12	ACARTIA		CB5.2	3	16400.0	3872.0	3263600.0	402656.0	0.11016	0.16388	**6.52489**
61182901	98	ACARTIA	TONSA	CB5.2	3	14800.0	10880.0	2945200.0	5252480.0	0.11596	0.21065	1.36698
61182901	98	ACARTIA	TONSA	ET5.2	4	8800.0	5520.0	3511200.0	1371888.0	0.21293	0.21219	1.48215
61182901	98	ACARTIA	TONSA	CB2.2	3	2400.0	0.0	957600.0	0.0	0.40774	.	**2.44949**
61182901	98	ACARTIA	TONSA	LE2.2	3	17200.0	2048.0	6862800.0	2095104.0	0.15231	0.70676	**5.05709**
61182901	98	ACARTIA	TONSA	RET2.2	4	28000.0	67394.0	11172000.0	2147535042.0	0.11937	0.68762	-0.84786
61182901	98	ACARTIA	TONSA	TF1.7	4	27200.0	13825.0	10852800.0	8112640.0	0.12112	0.20602	**3.06791**
61182901	98	ACARTIA	TONSA	TF2.3	4	0.0	1031.0	0.0	1047558.0	.	0.99273	-1.00683
61190502	98	CANUELLA	ELONGATA	TF2.3	4	0.0	32.0	0.0	992.0	.	0.98425	-1.00000
61190502	98	CANUELLA	ELONGATA	TF1.7	3	0.0	192.0	0.0	12096.0	.	0.57282	-1.73205
61190502	98	CANUELLA	ELONGATA	TF1.7	4	250.0	2560.0	62250.0	1308160.0	0.99800	0.44678	-1.97125
61190502	98	CANUELLA	ELONGATA	TF1.5	4	20000.0	25289.0	7980000.0	83883334.0	0.14124	0.36216	-0.55169
61190502	98	CANUELLA	ELONGATA	TF1.5	3	0.0	3008.0	0.0	4537408.0	.	0.70815	-1.41166
61191401	98	EUTERPINA	ACUTIFRONS	LE2.2	3	0.0	257.0	0.0	32512.0	.	0.70160	-1.41972
61200801	12	HALICYCLOPS		TF1.5	4	0.0	16896.0	0.0	8633856.0	.	0.17391	**-5.74456**
61200801	12	HALICYCLOPS		LE2.2	3	0.0	768.0	0.0	195840.0	.	0.57622	-1.73205
61200801	12	HALICYCLOPS		CB5.2	3	0.0	64.0	0.0	1984.0	.	0.69597	-1.41421
61200801	98	HALICYCLOPS		CB5.2	3	0.0	128.0	0.0	16256.0	.	0.99609	-1.00000
61200801	98	HALICYCLOPS		TF1.5	4	36000.0	10241.0	14364000.0	20961280.0	0.10528	0.44706	**4.33114**
61200801	98	HALICYCLOPS		TF1.7	4	0.0	384.0	0.0	48768.0	.	0.57509	-1.73205

61200801	98	HALICYCLOPS		TF2.3	4	0.0	26752.0	0.0	202364800.0	.	0.53175	-1.88044
61200801	98	HALICYCLOPS		TF1.5	3	19200.0	7450.0	15340800.0	4875258.0	0.20400	0.29638	**2.61158**
61200802	12	CYCLOPS		TF2.3	4	1200.0	0.0	478800.0	0.0	0.57663	.	1.73205
61200802	12	CYCLOPS		TF1.7	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61200802	12	CYCLOPS		CB2.2	3	705.9	0.0	165384.1	0.0	0.57612	.	1.73205
61200802	12	CYCLOPS		TF1.5	3	3200.0	0.0	2556800.0	0.0	0.49969	.	**2.00000**
61200802	12	CYCLOPS		TF1.5	4	2000.0	0.0	798000.0	0.0	0.44665	.	**2.23607**
61200802	12	CYCLOPS		TF1.7	4	750.0	0.0	186750.0	0.0	0.57619	.	1.73205
61200802	98	CYCLOPS	BICUSPIDATUS	RET2.2	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61200802	98	CYCLOPS	BICUSPIDATUS	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61200802	98	CYCLOPS	VERNALIS	TF1.5	3	1600.0	0.0	1278400.0	0.0	0.70666	.	1.41421
61200802	98	CYCLOPS	BICUSPIDATUS	TF1.5	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61200803	12	MESOCYCLOPS		CB2.2	3	0.0	1792.0	0.0	456960.0	.	0.37723	**-2.64575**
61200803	12	MESOCYCLOPS	EDAX	TF2.3	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61200803	12	MESOCYCLOPS		TF1.5	4	0.0	1024.0	0.0	523264.0	.	0.70642	-1.41421
61200803	98	MESOCYCLOPS	EDAX	TF2.3	4	2.0	3202.0	.	4731778.0	.	0.67935	.
61200803	98	MESOCYCLOPS	EDAX	TF1.5	4	0.0	1026.0	0.0	523266.0	.	0.70504	-1.41697
61200804	98	EUCYCLOPS	SPERATUS	CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61200804	98	EUCYCLOPS	SPERATUS	TF1.5	4	250.0	0.0	62250.0	0.0	0.99800	.	1.00000
61200804	98	EUCYCLOPS	AGILIS	TF2.3	4	800.0	0.0	319200.0	0.0	0.70622	.	1.41421
61200807	98	TROPOCYCLOPS	PRASINUS	TF1.7	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61200901	12	OITHONA		CB5.2	3	0.0	32.0	0.0	992.0	.	0.98425	-1.00000
61200901	98	OITHONA		TF1.7	4	0.0	2048.0	0.0	2095104.0	.	0.70676	-1.41421
61200901	98	OITHONA	COLCARVA	CB5.2	3	800.0	0.0	159200.0	0.0	0.49875	.	**2.00000**
61200901	98	OITHONA		TF2.3	4	0.0	34049.0	0.0	537918208.0	.	0.68117	-1.46802
613402	11	BALANIDAE		CB2.2	3	1600.0	0.0	638400.0	0.0	0.49937	.	**2.00000**
613402	11	BALANIDAE		CB5.2	3	82000.0	0.0	81918000.0	0.0	0.11038	.	**9.05539**
613402	11	BALANIDAE		LE2.2	3	192000.0	0.0	383808000.0	0.0	0.10204	.	**9.79796**
613402	11	BALANIDAE		TF1.5	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
613402	11	BALANIDAE		TF1.7	4	7200.0	0.0	2872800.0	0.0	0.23541	.	**4.24264**
613402	11	BALANIDAE		ET5.2	4	4400.0	0.0	1755600.0	0.0	0.30113	.	**3.31662**
613402	17	BALANIDAE		ET5.2	4	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
613402	17	BALANIDAE		CB5.2	3	5400.0	0.0	1074600.0	0.0	0.19197	.	**5.19615**
613402	17	BALANIDAE		CB2.2	3	400.0	0.0	159600.0	0.0	0.99875	.	1.00000
61340201	11	BALANUS		ET5.2	4	0.0	1032.0	0.0	261176.0	.	0.49521	**-2.01538**
61340201	11	BALANUS		LE2.2	3	0.0	77842.0	0.0	132042768.0	.	0.14762	**-6.77218**
61340201	11	BALANUS		RET2.2	4	0.0	8192.0	0.0	67100672.0	.	0.99994	-1.00000

61340201	11	BALANUS		CB5.2	3	0.0	32321.0	0.0	26717632.0	.	0.15992	**-6.24918**
61340201	11	BALANUS		TF1.7	3	0.0	1.0	0.0	0.0	.	0.00000	-1.00000
61340201	11	BALANUS		TF1.7	4	0.0	7040.0	0.0	2860160.0	.	0.24023	**-4.15761**
61340201	17	BALANUS		TF2.3	4	0.0	3078.0	0.0	3142662.0	.	0.57594	-1.73543
61340201	17	BALANUS		TF1.7	3	0.0	1.0	0.0	0.0	.	0.00000	-1.00000
61340201	17	BALANUS		CB5.2	3	0.0	3392.0	0.0	1581760.0	.	0.37078	**-2.69414**
61340201	17	BALANUS		CB2.2	3	0.0	2048.0	0.0	4192256.0	.	0.99976	-1.00000
61340201	17	BALANUS		TF1.5	4	0.0	768.0	0.0	326912.0	.	0.74448	-1.34164
61340201	17	BALANUS		RET2.2	4	0.0	193.0	0.0	12096.0	.	0.56985	-1.74100
61340201	17	BALANUS		LE2.2	3	0.0	1024.0	0.0	1047552.0	.	0.99951	-1.00000
61530115	93	NEOMYSIS	AMERICANA	TF1.7	3	0.0	2.0	0.0	0.0	.	0.00000	-1.41421
61530115	93	NEOMYSIS	AMERICANA	RET2.2	4	0.0	17.0	0.0	12.0	.	0.20377	**-3.15682**
61530115	93	NEOMYSIS	AMERICANA	CB2.2	3	0.0	4302.0	0.0	16785230.0	.	0.95234	-1.04991
61530115	98	NEOMYSIS	AMERICANA	CB2.2	3	75.0	26.0	.	26.0	.	0.19612	.
61530115	98	NEOMYSIS	AMERICANA	CB5.2	3	11.0	1.0	.	0.0	.	0.00000	.
61530115	98	NEOMYSIS	AMERICANA	RET2.2	4	56.0	31.0	.	26.0	.	0.16448	.
61530115	98	NEOMYSIS	AMERICANA	TF1.7	3	17.0	7.0	.	0.0	.	0.00000	.
61530115	98	NEOMYSIS	AMERICANA	ET5.2	4	70.0	48.0	.	408.0	.	0.42081	.
61530121	98	MYSIDOPSIS	BIGELOWI	CB2.2	3	2.0	0.0	.	0.0	.	.	.
61540508	98	OXYUROSTYLIS	SMITHI	CB5.2	3	2.0	0.0	.	0.0	.	.	.
61691502	98	COROPHIUM	LACUSTRE	TF1.5	3	2.0	0.0	.	0.0	.	.	.
61691502	98	COROPHIUM	LACUSTRE	TF1.5	4	12.0	0.0	.	0.0	.	.	.
61691502	98	COROPHIUM	LACUSTRE	TF1.7	3	6.0	0.0	.	0.0	.	.	.
61692107	98	GAMMARUS		CB2.2	3	11.0	0.0	.	0.0	.	.	.
61692107	98	GAMMARUS		RET2.2	4	0.0	2.0	0.0	2.0	.	0.70711	-1.00000
61692107	98	GAMMARUS		TF1.5	3	80.0	0.0	.	0.0	.	.	.
61692107	98	GAMMARUS		TF1.5	4	117.0	4363.0	.	16781080.0	.	0.93891	.
61692107	98	GAMMARUS		TF1.7	3	52.0	2.0	.	0.0	.	0.00000	.
61692107	98	GAMMARUS		TF2.3	4	13.0	8.0	.	8.0	.	0.35355	.
61693708	98	MONOCULODES		CB2.2	3	19.0	0.0	.	0.0	.	.	.
61693708	98	MONOCULODES		CB5.2	3	1.0	0.0	.	0.0	.	.	.
61693708	98	MONOCULODES		TF1.5	3	10.0	0.0	.	0.0	.	.	.
61693708	98	MONOCULODES		TF1.5	4	23.0	0.0	.	0.0	.	.	.
61693708	98	MONOCULODES		TF1.7	3	1.0	0.0	.	0.0	.	.	.
61792201	31	CRANGON	SEPTEMSPINOSA	CB5.2	3	0.0	2.0	0.0	0.0	.	0.00000	-1.41421
61792201	31	CRANGON	SEPTEMSPINOSA	LE2.2	3	0.0	2.0	0.0	2.0	.	0.70711	-1.00000
61792201	31	CRANGON	SEPTEMSPINOSA	TF1.7	3	0.0	1.0	0.0	0.0	.	0.00000	-1.00000

61792201	98	CRANGON	SEPTEMSPINOSA	CB5.2	3	24.0	0.0	.	0.0	.	.	.
61792201	98	CRANGON	SEPTEMSPINOSA	LE2.2	3	3.0	0.0	.	0.0	.	.	.
64890502	98	CHAOBORUS	PUNCTIPENNIS	TF1.5	3	3.0	0.0	.	0.0	.	.	.
64890502	98	CHAOBORUS	PUNCTIPENNIS	CB2.2	3	2.0	0.0	.	0.0	.	.	.
64890502	98	CHAOBORUS	PUNCTIPENNIS	TF1.5	4	0.0	32.0	0.0	992.0	.	0.98425	-1.00000

* The corrected number is 1,216,000.

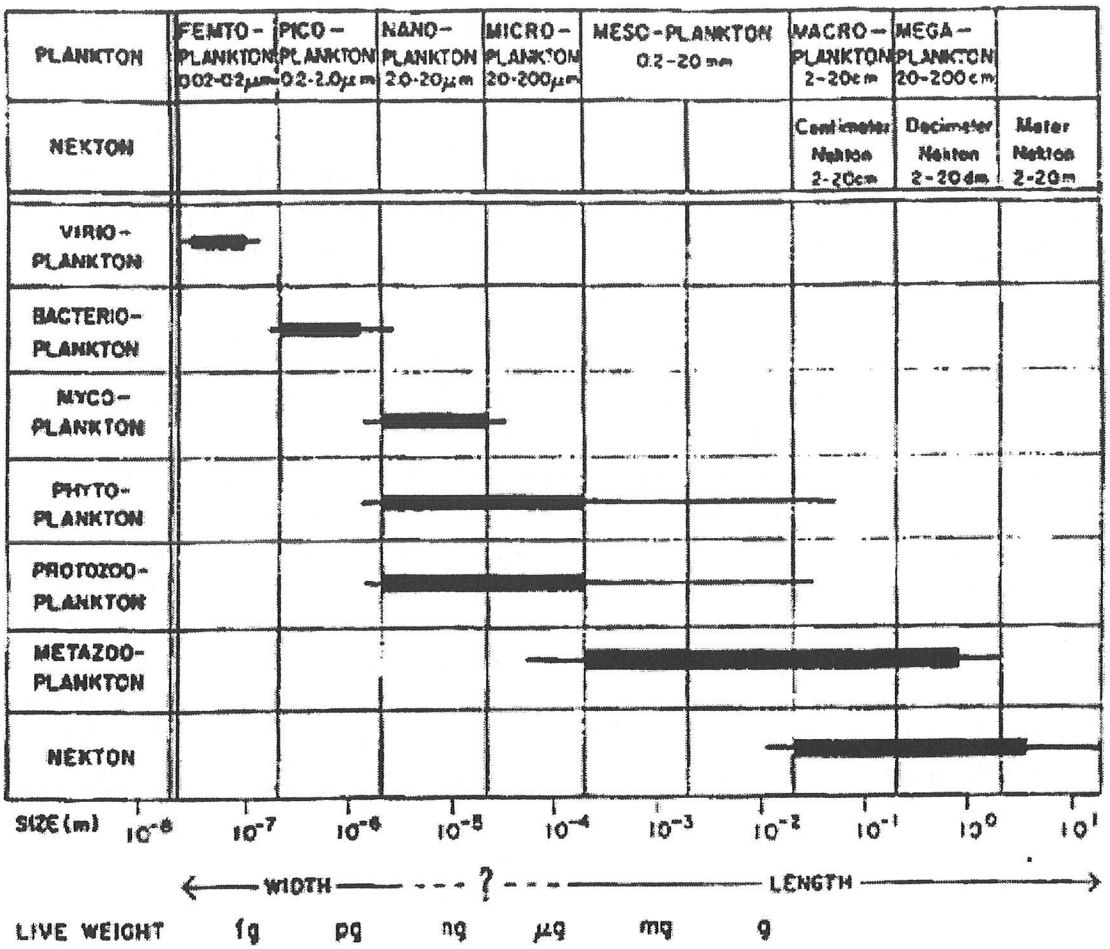
Appendix E: Zooplankton Classifications

Classifying the plankton on the basis of characteristics such as structural organization, reproduction and growth rate, and mode of nutrition "provides the ecologist with a rational system for putting the components of the pelagic ecosystem into compartments and then equating these with plankton size fractions and methods for estimating their populations" (Sieburt et al. 1978). The size fractions are at times an artificial division of the compartments and functional groups that is forced on the ecologist by the mesh sizes of the nets used to collect plankton (Sieburt et al. 1978). Size, however, can prove to be a useful means of dividing the plankton because it "is a decisive factor in governing growth rate and doubling time of plankton organisms. Since within the pelagic food web most predators swallow their prey organisms undivided, body size also determines food-chain relationships" (Harris et al. 2000).

The "metazooplankton" were identified by Sieburt et al. (1978) as a compartment of the pelagic ecosystem consisting of "multicellular ingesting forms." They typically span a length range of approximately 200 microns to 100 centimeters, but immature forms can be as small as 50 microns and some individuals reach 200 cm in length (Figure E-1). Sieburth et al. (1978) divided the metazooplankton into three functional groups and equated them with three size fractions: the "mesoplankton" (200 micron - 20 mm), the "macroplankton" (20 mm - 20 cm), and the "megaplankton" (20 - 200 cm). Mesozooplankton in ocean settings consist mainly of copepods whose copepodite and adult sizes almost exactly match the length limits of the size fraction. This functional group also includes cladocera, meroplanktic larvae, small hydromedusae and ctenophores, chaetognaths, appendicularians, doliolids, ostracods, and fish eggs and small fish larvae (Sieburth et al. 1987, Harris et al. 2000, Day et al. 1989). The macrozooplankton are generally active swimmers and carnivorous. They include large crustaceans such as hyperiid amphipods, mysids, and euphausiids, the larger ctenophores, hydromedusae, and scyphomedusae, and the larger fish larvae. Megazooplankton are the still larger, drifting forms such as cnidarians and pelagic tunicates. This classification scheme is widely used and was recently affirmed by International Council for the Exploration of the Sea, or ICES (Harris et al. 2000). In order to catch representative samples of the three functional groups, plankton collection methodology is usually tailored to the population characteristics of each size fraction in the body of water being surveyed.

The protozooplankton are another plankton compartment in the pelagic ecosystem and consist of the nanozooplankton and the microzooplankton functional groups (Sieburth et al. 1978). Nanozooplankton are the apochlorotic (heterotrophic) flagellates and amoeboid forms, and are equated to the smaller, 2 - 20 micron size fraction. Microzooplankton are the rotifers, ciliates, and the eggs and early life stages of crustacean plankton and meroplanktic larvae, and they are equated to the 20 - 200 micron "microplankton" size fraction although some ciliates are smaller than 20 microns (Harris et al. 2000).

Figure E-1. Size spectrum of different taxonomic-trophic compartments of plankton including the size range of nekton (from Sieburth et al. 1978).



Appendix F: Tracking Sheet for Reviews of the April 2000 Draft Report on the Mesozooplankton Split Sample Study

June 6, 2000

Recommendations and comments received from reviewers by May 17, 2000 were incorporated into this tracking sheet. Text referenced in the recommendations and comments was copied and highlighted in the tracking sheet. Minor changes, and suggested spelling and punctuation corrections, were simply fixed and are not listed here. Specific change(s) to the report were proposed in response to the recommendations and comments. These proposed changes were reviewed, somewhat modified, and approved by the Chesapeake Bay Program Monitoring Subcommittee coordinator and the program managers in the Virginia Department of Environmental Quality and the Maryland Department of Natural Resources, and then implemented. They are listed as "action(s)" in this tracking sheet.

Report structure

Recommendation: The report as it is now should be reorganized to emphasize the results of the last 20 split samples. The results of the first round are of some historical interest but should not be considered when making judgements about the CVS versus the stempel pipette method. Some of the discussion in the first round should be saved. However, the results of the entire 20 samples from the second round are the results that really count and these should be considered in toto as the main part of the report. (Kent Carpenter)

Comments:

- I find the report hard to follow since it is basically a compendium of the various minutes of the zooplankton conference and various conference calls. (Kent Carpenter)
- Most of the background information in the first paragraph [page 6] is irrelevant to the results and conclusion. Please state the total number of samples in Round 2. It's a little confusing - you have to read this section carefully to know if 20 or 25 samples were done. (Mary Ellen Ley)
- If you haven't done so already, combine the discussion, results and tables from the 1st five with the 1st ten. It would make more sense to do data analyses on all 20 samples instead of the 1st ten and 2nd ten. (Mary Ellen Ley)

Action: Text of the Round 1, "Plankton Summit," and Round 2 sections was condensed. The various subsections in Round 2 were left separate because changes (e.g. counting methods, species identifications) occurred between the first five, the second five and the last ten split sample counts. The Round 2 text, however, was reworked to try and make it clearer. A Discussion section was added to further discuss issues raised by all reviewers and summarize key points. This tracking sheet was created.

Definition of "mesozooplankton" and extrusion/clogging problems with towed plankton nets

Recommendation: The short comings of sampling and counting zooplankton smaller than 200 microns was discussed at length during the split sampling program and yet nowhere in this report is it mentioned. I recommend that the argument be included in order to show that all aspects of the mesozooplankton monitoring was thoroughly discussed. (Kent Carpenter)

Comments:

- The definition of mesozooplankton are those heterotrophic organisms in the size range of 200 microns to 2000 microns. This classification is based on taxon and trophic considerations (ICES, 2000). Nowhere in the ICES manual (or anywhere else in the published scientific literature) as far as I can tell does it state that the definition of mesozooplankton is defined as the plankton that is retained on a 200 micron sampling net. The plankton that is retained on a 200 micron sampling net is closely approximated as mesozooplankton for OCEANOGRAPHIC purposes because the density of zooplankton is relatively small and detritus is not a clogging factor in the open ocean. However, under ESTUARINE conditions, productivity and hence densities of zooplankton are much higher and there is often considerable detritus. Both these factors effectively reduce the mesh size of the plankton net due to clogging. "It is fairly obvious that as clogging increases the mesh size will decrease, with a corresponding effect on mesh selection. Clogging is greater with fine meshes and in highly productive waters." (UNESCO, 1968). Therefore, a large proportion of individuals smaller than 200 microns can be collected on a 200 micron mesh net and subsequently counted as mesozooplankton under estuarine conditions when they are in fact, microzooplankton. Furthermore, the density of zooplankton and detritus in an estuarine condition are highly variable and very patchy and therefore the effective mesh size of a 200 micron plankton net will vary depending on conditions. This means that this zooplankton smaller than 200 microns (microzooplankton) cannot be reliably or accurately measured, especially under estuarine conditions. Furthermore, even in waters that are relatively oligotrophic, extrusion of zooplankton at normal towing speeds reduces reliability of sampling organisms larger than the mesh size of the plankton net (UNESCO, 1968). Because of this, "it is advisable to use a net with a mesh size of about 75% of the width of the smallest organisms to be sampled." (Omori and Ikeda, 1992). In other words, to reliably sample zooplankton even at the 200 micron size, a plankton mesh size of 150 microns would be required. These limitations to reliable sampling of zooplankton less than 200 microns, given the mesh size of the sampling net are the reasons the Virginia subsampling methodology used a lower limit of 200 microns in their CVS method. (Kent Carpenter)
- I disagree with Kent's comment that *"nowhere in the ICES Manual (or anywhere else in the published scientific literature) as far as I can tell does it state that the definition of mesozooplankton is defined as the plankton that is retained on a 200 micron sampling net."* On page 320 of the ICES manual (Harris et al. 2000) it is stated that, "Meso- and macrozooplankton are defined as being retained on meshes of 200um and 2000um, respectively..... " The net size is 200u and should remain that size. Everything retained in that net needs to be retained prior to any kind of enumeration. This is what we mean by the term mesozooplankton. Therefore, any field or laboratory handling (sieving) after collection of the sample should be filtered through mesh sizes smaller than 200u. Zooplankton programs at VIMS, City University of New York, and Lamont-Doughery at Columbia .....CBI, ANS, VIMS and Versar.....all followed this procedure. We should include this as a recommended procedure for handling of samples. Rinsing the sampling thoroughly is also extremely important to ensure that all organisms caught in the sample find their way into the cod end. Otherwise, lots of organisms, especially the smaller ones, are likely to remain stuck on the meshes, won't ever be enumerated, and an underestimate

of density will result. The goal of the shipboard and laboratory handling is to assure that everything captured in the net somehow is represented in a density estimate. I can't stress this enough. (Fred Jacobs)

- Reply to some of Kent's points (Claire Buchanan):
  - In its Introduction (pages 1-13) and elsewhere (e.g. pg 320 mentioned by Fred Jacobs), the ICES Manual offers general definitions of the various zooplankton types, including mesozooplankton, and discusses the need for and the uses of zooplankton size classifications. This information is largely derived from Sieburth et al. (1978) and earlier authors. Sieburth et al. (1978) point out that zooplankton as a whole span a wide size spectrum (six orders of magnitude) which necessitates grouping them into size fractions that can be effectively collected. The upper and lower limits chosen for each size fraction were selected so that they encompass the bulk of an individual zooplankton category (for example, "mesozooplankton"). Since nets were - and still are - the primary means of collected zooplankton greater than 20 micron, this meant that plankton nets with mesh openings equal to the lower size limit should collect the bulk of an individual zooplankton category when towed correctly in the water. A size range of 200 micron - 20 mm (body length) was selected for the mesozooplankton even though immature individuals of some species are smaller than 200 microns and hence not adequately sampled by the 200 micron mesh plankton net.
  - Microzooplankton taxa (e.g. copepod nauplii, rotifers) are indeed caught in the 200 micron plankton nets and counted by the mesozooplankton monitoring programs, however these taxa are not included in calculations of bay-wide mesozooplankton indicators.
  - Clogging and extrusion are important issues to consider when using plankton nets to collect mesozooplankton as well as while handling samples on ship-board and in the laboratory. However, clogging/extrusion problems experienced with *towed plankton nets* are not identical to clogging/extrusion problems experienced with *sieves*. The "limitations to reliable sampling of zooplankton less than 200 microns" with a 200 micron towed plankton net in the field are not valid reasons for using a 200 micron sieve on the bottom of the CVS method stack of sieves in the laboratory.

Action: Appendix E (definitions of mesozooplankton and microzooplankton) inserted. Paragraphs on "what are mesozooplankton?" and counting mesozooplankton smaller than 200 micron inserted in new Discussion section of report.

General comments on Draft Executive Summary

- I believe the Executive Summary is well written and gets across the major points. (Fred Jacobs)
- Add sentence that states that the [CVS method] patch didn't work and the methods do not produce comparable data. Hence, need to use a single method for baywide determinations. (Mary Ellen Ley)

Action: Added the sentence "A single method needs to be selected and implemented because the modified laboratory methods of the two programs still do not produce comparable results."

Text about taxa lost by CVS sieving protocol and resulting undercount

"The Maryland and Virginia mesozooplankton monitoring programs implemented modifications to their respective laboratory counting protocols in 1998 in order to better estimate species richness in Maryland and to eliminate large sieving losses of smaller taxa in Virginia." (Draft Executive Summary, pg 1, first paragraph.)

"However, the "new" ODU method still produces split sample results with significantly lower total counts than those of Versar. It appears to selectively undercount key taxa, particularly the immature (copepodite) life stage of calanoid copepods, a common and frequently dominant taxonomic group." (Draft Executive Summary, page 1, first paragraph.)

Recommendation: Replace "eliminate...." with "add coverage of zooplankton smaller than 200 microns." (Kent Carpenter)

Recommendations: Please replace "It appears...." with: "It consistently counts less of certain" and "dominant taxonomic group largely occurring in the below 200 micron size range." (Kent Carpenter)

Comments:

- The so called sieving losses of smaller taxa was built into the design of the Virginia CVS subsampling method because the mesh size of the sampling net in the field is 200 microns. This 200 micron mesh size was chosen as the lowest mesh size of the Virginia subsampling method because the intent was to monitor MESOZOOPLANKTON. (Kent Carpenter)
- While small taxa can be caught length-wise on the sieve, body width is the critical dimension that determines retention. The ICES Manual (Harris et al. 2000), the earlier IBP Handbook No 17 (Edmondson and Winberg 1971), and other methodology papers recommend using sieves with mesh openings that are less than the length or width of the smallest individuals the investigator wants to retain. The "old" CVS method has employed five sieves since the start of the Virginia mesozooplankton monitoring program: 2000, 850, 600, 300, and 200 microns. None are smaller than 200 microns. Comparisons of counts obtained with the "new" CVS method and the "old" CVS method show that total counts increased significantly when smaller sieves were included in the "new" CVS protocol. This result demonstrates that the "old" CVS method *undercounted* Virginia mesozooplankton samples, and supports the statement in question. (Claire Buchanan)
- Reply to Kent's recommendation to change wording to "dominant taxonomic group largely occurring in the below 200 micron size range...." Length-width information presented in the report (new Table 11) shows that the lengths of adults and copepodites of *Acartia tonsa* and *Eurytemora affinis*, two of the dominant calanoid copepod taxa, do not largely occur "in the below 200 micron size range." Likewise, the lengths of immature and adult *Bosmina longirostris*, a seasonally dominant cladoceran species, does not largely occur in the <200 micron range. (Claire Buchanan)
- The IBP Manual No. 17 (Edmondson and Winberg, 1971) makes the following recommendation (pg. 136-137): "If subsampling with a pipette is necessary, one should show that a sorting bias is not introduced. This can be done by fractionating a whole sample and counting subsamples from the beginning and end of the series; there should be no significant tendency for one kine of animal (the largest or smallest) to be in the subsamples taken first or last. Subsampling should be practiced and subsamples counted

until the operator is able to show that the aliquotes counts are randomly distributed." (Mary Ellen Ley)

- See also Sample handling in CVS method below

Actions: Changed text to "...eliminate laboratory sieving losses of small mesozooplankton taxa and life stages in Virginia." Changed text to "However, the "new" ODU method still produces significantly lower total counts than the Versar method. The method consistently counts less of certain taxa, particularly the immature (copepodite) life stage of calanoid copepods which are a common and frequently dominant taxonomic group."

Clumping in sample jars

Recommendation: Include the following text in the executive summary: "It is not clear if the Versar method overcounts these taxa because of potential clumping in their subsampling method or if the ODU method somehow undercounts these taxa." (Kent Carpenter)

Comments:

- It is well know that clumping can occur in plankton samples (e.g. Longhurst and Seibert, 1967) and that zooplankton have different densities and will suspend in fluids differently depending on animal density and shape. For example, the ICES manual (p 151) when discussing enumerating techniques states "cladocerans, tend to float in the surface film." These differences could affect distribution of zooplankton even in a sample that is being mixed prior to subsampling with a stempel pipette. Since the possibility of clumping while subsampling exists for the stempel pipette and there is no evidence in the data that suggests the CVS method somehow eliminates taxa selectively, it should not be assumed that the difference in the observed abundances is due to an inadequacy of the CVS method. It could just as well be due to bias in the stempel pipette method employed by VERSAR. (Kent Carpenter)
- There does not appear to be a problem with clumping in the Stempel pipette using the Versar method as far as we can tell. Versar applies a methodology to ensure homogeneity in the sample prior to subsampling with the pipette. Early in the program Versar conducted lab counts using 3 sample replicate subsamples of the same volume. The results indicated that sample counts were usually with 5% of each other and almost always within 10%. Willie can probably dig up these old data sheets we if need to. While anything is possible, I do not think there is any basis to change the text as Kent suggests on Page 2, where he wants to introduce clumping as a potential source of error for the Versar method. (Fred Jacobs)
- Section 2.1.8 of the IBP Manual (pag 137) describes in general terms a procedure to check sampling bias. (Mary Ellen Ley)

Action: None

Sample handling in CVS method

Comments:

- *The motorized siever used at ODU for the CVS method is an accurate and reliable machine with minimal losses of mesozooplankton.* There is an impression that has been circulating that the motorized siever is overly destructive to zooplankton during its operation and that zooplankton are typically lost in the process of sieving. True, the motorized siever does make noise, and does shake in order to facilitate the sieving

process. *However, the allegations that it is overly destructive to zooplankton and unreliable are unfounded.* There is no data or casual observation that supports this idea. We have placed a passive 45 micron sieve below the 63 micron passive sieve that collects all discharge from the motorized siever and find only the smallest organisms that would be expected to pass through these sieves. There is no evidence for the destructive nature of the motorized siever. The methodology I propose for the next round of split samples should test this. (Kent Carpenter)

- Standard Methods (APHA, 1995) says about the Folsom Splitter: "Exercise care to provide unbiased splits. Even when using the Folsom splitter unbiased subsamples cannot be unquestioningly assumed (McEwen et al. 1954); therefore, count animals in several subsamples from the same sample to verify that the splitter is unbiased and to determine the sampling error introduced by using it." (Mary Ellen Ley)
- Question to Fred and William: Sieburth et al (1978) make an interesting observation about how long (90u), thin (1.5u) bacteria get through a 3u millipore filter which they apparently do well (pg 1261): "...as water flows through small screens and perforated membranes, streamlines are apparently formed on a microscale, inwhich the smaller particles line up longitudinally so that only their widths affects retention." I've seen something like this while watching preserved copepods get sucked up into a pipette. Considering the water flow inside the stacked sieves of the CVS method, this seem like a likely hypothesis to explain why the long, thin mesozooplankton taxa (e.g. copepodites) get through small meshes and why George isn't seeing many broken zooplankton parts in the 64u mesh sieve collecting passively at the bottom of the stack....Do you think it's a viable hypothesis? (Claire Buchanan)
- Reply to Claire's question (above): I think your thoughts on the Sieburth paper may certainly be a possibility for the difference and could certainly be mentioned, but I believe there may be a more general principal that applies as well. My gut feel has always been that the more you handle these samples, the greater the loss that will occur, even if the methodology for handling the sample may appear to be more sophisticated than a simpler method. So even if, all things being equal, the statistics on a splitter that has 4 splitting chambers are acceptable, the precision will be sequentially less if a sample is really dense and you need to go to say a 1/64th or 1/128th split, etc. The more sieves, splits, rinses, etc. the greater potential for error. (Fred Jacobs)

Action: points summarized in new Discussion section

Text about ODU count precision

"The study determined that counts produced with the "new" ODU protocol have variances that are much higher than counts produced with the Versar protocol, hence the ODU counts are less precise." (Draft Executive Summary, page 1, first paragraph.)

"The coefficients of variation in the ODU taxa counts were again larger than those for the Versar counts, indicating that count precision was poorer in the ODU counts (Figure 6)." (Draft Report page 14, third bullet)

Recommendation:

- Replace text in first sentence with "higher than counts produced with the Versar protocol, although this is expected in the results since Versar is counting more individuals of the smaller taxa." Delete text in second sentence. (Kent Carpenter)

- Replace text in first sentence with "... higher than counts produced with the Versar protocol, hence the ODU estimates of precision are lower." (Elgin Perry)
- Include 1-2 paragraphs in the body of the report summarizing Ray Alden's paper and describing of how the CVS method is intended to change the coefficient of variation for certain kinds of species. (Mary Ellen Ley)
- On the last conference call Kent argued with the statement "ODU counts are less precise". Table 7 indicates that the first part of the sentence is true. If Elgin agrees, I would say something like: *"Based on 20(or 10?) pairs of ODU/Versar CVs, there is a significant difference in the paired CVs, with higher CVs at ODU."* In Conclusion 1, (p.14), last bullet, keep statement that says that ODU's coefficients of variance are higher than Versar's. (Mary Ellen Ley)

Comments:

- The concepts of bias, accuracy and precision, the relationship of precision to sample variance, and the relationship of sample variance to raw count numbers were discussed during the April conference call. (Claire Buchanan)
- Kent states in comment 2.B. that the CVS method is an accurate method. This has not been demonstrated. (Mary Ellen Ley)

Action: Sentence changed to "The study determined that counts produced with the "new" ODU protocol have variances that are higher than counts produced with the Versar protocol, hence the ODU estimates of precision are lower." Paragraphs added to the new Discussion section of the report.

Text about species richness

"Furthermore, the number of taxa identified per sample was on average lower in the ODU counts." (Draft Executive Summary, page 1, first paragraph)

On *average*, ODU identified fewer unique taxa per sample than Versar (Table 9). This observation suggests that the CVS method as it is currently implemented does not produce more accurate estimates of species richness." (Draft Report, page 9, second paragraph, last bullet)

Recommendation:

- Replace phrase in Executive Summary with "Furthermore, the diversity measures between the two modified methods are not significantly different although the modified Versar method identified on average more taxa than the ODU counts. However, these additional taxa are mostly the smallest taxa that cannot be reliably counted as mesozooplankton." (Kent Carpenter)
- As already stated above, the additional species appear to be smaller taxa that may be expected to be undercounted in the ODU method. Therefore this statement should not be one of accuracy but simply of consistency between the different counts. I believe the methods as proposed for the round 3 splits will test this more closely. (Kent Carpenter)

Comments:

- Two particular observations of the split sample results bring into question the usefulness of Margalef's Diversity Index as a bay-wide indicator of community health at this time. First, fewer numbers of mesozooplankton species per sample (species richness) were observed in splits processed with the "new" CVS method (ODU) than in splits processed with the modified pipette method (Versar). Second, estimates of total mesozooplankton abundance obtained with the "new" CVS method are still lower than those obtained with

the pipette method. Species richness is a variable in the numerator of the index's equation and total abundance (number of organisms per sample) is a variable in the denominator. When richness is divided by abundance, as in Margalef's Diversity Index, the resulting proportion does not reflect the lower species richness and lower total abundance of the CVS counts, and the Virginia and Maryland diversity indexes are approximately the same. The Shannon-Wiener, Pielou, and Simpson indices of diversity would be similarly affected because they also rely on measures of species proportional abundance. If the two laboratories had comparable methods and similar raw counts, then the diversity indices could be reliably used (Claire Buchanan)

- Reply to Kent's second bullet: this statement needs to be supported by evidence from the data before it can be incorporated. (Claire Buchanan)

Action: No change made to text of Executive Summary or report text. Paragraph added regarding species richness vs diversity measures in new Discussion section of report.

Text about discontinuing CVS method in Virginia

"The "old" and "new" ODU counting protocols should be discontinued and a counting protocol patterned after the ICES recommended protocol (Harris et al. 2000) should be instated. Backward comparability with the pre-1998 Chesapeake Bay Program mesozooplankton data will unfortunately be lost in Virginia for most mesozooplankton taxa, but Maryland and Virginia results will become comparable and the CBP monitoring programs should be able to calculate and use multiple, Bay-wide mesozooplankton indicators." (Draft Executive Summary, page 1, first paragraph.)

3. *The "old" and "new" ODU counting protocols which are based on the Controlled Variability Sampling method (Alden et al. 1982) should be discontinued and a counting protocol patterned after the ICES recommended protocols (Harris et al. 2000) should be instated. Maintaining the "new"* Versar method and "new" ODU method will not yield results that are directly comparable and useful for Bay-wide mesozooplankton indicators. The "new" Versar counting method (Maryland program) has improved Versar's ability to measure species richness, an important Bay-wide indicator, and the "new" ODU counting method (Virginia program) has increased ODU's taxa counts per sample. However, the "new" ODU method still produces split sample results that are significantly different from Versar's results (see above). The Versar method is already very close to the ICES recommended protocols and should be maintained. (Draft Report, page 17)

Recommendations:

- One last test of the reliability and precision of the ODU CVS method and the Versar stempel pipette method should be undertaken before a final decision is made.... Recommend the CVS method is eventually adopted by both the Maryland and Virginia programs. (Kent Carpenter)
- Recommend making the following changes to #3 in Conclusions (Mary Ellen Ley):
  3. Differences between laboratories may be due to method bias or technician bias, or both. Further work is needed to determine bias. If bias is shown to be method dependent, one method will be selected for both laboratories. The method that yields comparable results, and the best precision & bias will be selected. Recommendations:
     Determine which method is truly biased, i.e., is the CVS method underestimating counts or is the Stempel pipette method overestimating?

- Check if CVS method is biased low due to sieving loss. Reanalyze one sample multiple times. Diminishing recoveries of species abundances will indicate loss.
- Check if CVS method bias due to Folsom splitter. Follow Standard Methods 19th edition procedure to verify that the splitter is unbiased and to determine the sampling error introduced by using it.
- Analyze a sample of known species identities and abundances with the CVS and Stempel pipettes each method. This comparison should be done within ODU and between ODU and Versar. (Custom made sample)
- Check the Stemple pipette method subsampling and sorting bias using procedure in section 2.1.8 of the IPB Handbook Both Versar and ODU need to do this.

Determine technician bias by comparing results from the Stempel pipette method performed by both ODU and Versar.

- Remove #3 conclusion and possibly # 4 and #5 (Rick Hoffman)
- I feel it is extremely important that you leave in your "recommendations" as stated in the original report. (Bruce Michael)

Comments:

- I come to a different conclusion than what is stated in this report, based on the available data and discussions with participants in the review. *I believe it is most logical that Versar adopt the CVS method rather than ODU begin using the stempel pipette method employed by Versar.* The main reasons for this are twofold. I will summarize these here and explain further below:

  1) The split sample tests so far have only established that the Versar method counts more of the smaller zooplankton and not whether ODU undercounts or Versar overcounts. These smaller zooplankton are not reliably counted because of the methods employed in any case and therefore should not be counted on as being important for our purposes.

  2) It was pointed out by Fred Jacobs during the 4/11/00 conference call and agreed by everyone (or at least not objected) that Versar should begin using the more common UNESCO - recommended field sampling net with a diameter of around .5 m, similar to the one currently used by ODU. *Once this new net is employed, Maryland will lose back-compatibility with its data set.* If ODU switches to the stempel pipette method, it will also loose back-compatibility with its data set. It makes more sense to loose backward compatibility in only one State. And, since the CVS method is not that much more difficult than the stempel pipette method used by Versar it would not be over-burdensome for Versar to adopt the CVS method. However, I do agree that one last test of the reliability and precision of the ODU CVS method and the Versar stempel pipette method should be undertaken before a final decision is made. I agree that ODU should switch to the Versar stempel pipette method if the new round of split sample tests indicates that the CVS method is substantially less precise than the Versar stempel pipette/folsom splitter method. (Kent Carpenter)
- *The CVS method has the advantage of being able to examine fine structure of zooplankton community structure.* If the CVS method is eventually adopted by both the Maryland and Virginia programs, as I recommend, many more possibilities exist to identify *Bay wide indicators*. The stasis or change of composition of the different sieve size classes and their taxonomic components offers many possibilities to examine

abundances and diversity at different trophic levels. This may more clearly identify components of the zooplankton that are important to other trophic levels such as juvenile fishes. With both Maryland and Virginia monitoring these components and both programs examining results, we have greater possibilities for making linkages to both upper trophic levels and water quality in general. I believe this advantage of the CVS should be considered in the report and that consideration be made that all sieve size fractions be reported to the Bay Program as part of normal data submittal. (Kent Carpenter)

- I agree with the report's conclusion (on Page 2-3) that begins with *"The "old" and "new" ODU counting protocols should be discontinued."* (Fred Jacobs)
- Kent assumes that Maryland would lose backward compatibility if a gear modification to a 0.5 m net were implemented by the Maryland program. When I brought this up on our 4/11/00 call, I meant to imply that we should *consider* making this modification. We would not make such a change until side by side field comparisons between the 0.5 and 0.2 m nets were conducted. If for some reason a systematic bias were to occur (e.g. 0.5 m net consistently gets higher counts than 0.2 m net), we would adjust our historical density estimates accordingly. We would need to ensure that any proposed change will allow for backwards compatibility. (Fred Jacobs)
- Include a section about why bay-wide indicators are important and what we need in the monitoring data in order to ensure useful indicators. (Mary Ellen Ley)
- Reply to Kent's comment # 1) above: see comment by Claire Buchanan under Text about taxa lost by CVS sieving protocol and resulting undercount (above)
- I think the #3 conclusion (i.e. DOU CVS method discontinued) and possible even 4 + 5 (though I think nobody discagrees with these) should be removed from the report for the following reason (Rich Hoffman):
    - The purpose of the report I thought was to report on the split sample study which developed and tested the success/failure of a "patch". I know you've done a lot of work and the report does a good job of achieving this objective as stated in conclusions 1+2. These final 3 (esp #3) conclusions are actually recomendations based upon your, and others, opinion but not necessarily a direct result of the split study data.
    - I guess maybe it depends on who is the "Author" of the report. If you alone and it is to represent your analysis alone, then maybe it's ok as is (esp if you move these "conclusions" to a "recommendations" section). If it is a collaborative report (with you as primary leader) then I think it should reflect the other collaborators analysis and agreement. As we know from Kent's submissions, the report does not currently reflect all collaborator opinions, and I don't think I agree with #3 as a "conclusion" supported by the data (as I say above, I think it is a recommendation).

Action: Paragraph on data needs of bay-wide indicators inserted in new Discussion section. Last three "conclusions" changed to "recommendations" (page 15) Changes recommended for #3 by MEL were made (page 15). #4 and #5 left in because there seems to be a consensus on the general ideas. Original text in #3 included in a paragraph in the discussion.

Possible useful four taxa for long-term comparisons

Recommendation: *Bosmina* is listed as one of the four taxa which may agree between the states. On Table 11, *Bosmina* have a 39.9 percent difference, and Chydorus/chydorids have -11.42 percent difference. Should chydorus/chydorids be listed as one of the four taxa with less than 20% difference instead of *Bosmina*? (Mary Ellen Ley)

Comment: There were identification problems with the chydorids and barnacle cypris in Maryland prior to 1999, making this species unsuitable for long-term trends. Further exploration of the actual monitoring data (new paragraph in Discussion) is making me rethink the choice of some of the four taxa listed on page 1. (Claire Buchanan)

Action: paragraph in new Discussion section further discussing the usefulness of the four taxa for long-term trends.

Section entitled "Split Sample Project - Round 1" (Draft Report, page 3)

Recommendation:

- I believe the general points from Round 1 should be mentioned but that discussion of data that is proven irrelevant should not be included in the main body of the report. Perhaps as an appendix? (Kent Carpenter)
- Since Round 1 data was invalidated, I would downplay quantitative data analysis and interpretation from ODU's Round 1 samples. Qualitative statements are OK, i.e., related to the presence or absence of a species. (Mary Ellen Ley)

Comment: The motorized siever malfunctioned during the first round because of the 'fix' modification, invalidating the round 1 results. The motorized siever that has been used by ODU for the CVS method previously had sieve sizes as follows: 2000, 850, 600, 300, and 200 microns. In order to sample the smaller zooplankton that the stempel pipette samples, and additional sieve chamber with a size of around 75 microns was added to the bottom of the sieve array. This appeared to function normally and the first round of splits was carried out. After the plankton summit, it was noticed that a lot of pressure was building up in the sieve array because of the additional small mesh size that was added. Upon close examination, it was noticed that a small number of the smaller zooplankton were being forced out of the sides of the smallest, added, sieve chamber. This was not readily visible and could easily have gone undetected since the operation looked normal to all who normally operate the motorized siever. When it was detected, we ran a test of the discharge water and determined that a variable number of organisms were being forced out in between the 200 and 75 micron sieve chamber seals. The normal tolerances that worked for the other sieve chambers was not working for the 200 - 75 micron chamber because of the low sieve size and increased water pressure built up in the 75 micron sieve chamber. This problem was fixed when the 75 micron chamber was detached and a 63 micron passive sieve placed underneath as a catch basin for discharge water. *However, because of this unexpected malfunction, the results of the first round are invalid and any comparisons between abundances, diversity, and taxonomic make up should be discounted.* This is not to say that the first round and the discussions at the plankton summit were fruitless and should be discounted, because many issues were addressed that went beyond the results of the first round splits. (Kent Carpenter)

Action: Condense Round 1 section of report.

Text about Versar counting method

"Versar follows a counting technique patterned after the UNESCO approved method which has been recently affirmed by the International Council for the Exploration of the Sea, ICES (Harris et al. 2000)." (Draft report, page 4, second paragraph, second bullet)

Recommendations:

- List the appropriate references. (Kent Carpenter)
- Text should be modified to state something like, "Versar follows a variation of a commonly used counting technique of subsampling using the Stempel pipette method." (Fred Jacobs)

Comments:

- There apparently is no such thing as a "UNESCO approved method." Only two UNESCO publications deal with zooplankton methodology (as far as I can tell through several bibliographic searches): UNESCO, 1968 and UNESCO, 1976. Neither of these publications mention subsampling of zooplankton samples using the stempel pipette or the folsom splitter. Therefore, the UNESCO publications do not deal with or approve of a particular subsampling technique.....

  The ICES Zooplankton Methodology Manual (2000) is a 684 page book in which one paragraph deals specifically with subsampling methodology. It superficially covers the stempel pipette, folsom splitter, and Kott splitter techniques but does not specifically recommend any one of these methods. It states the coefficient of variation for the stempel pipette and folsom splitter methods and since this coefficient appears to be wider for the Folsom splitter, it could be interpreted as one justification for choosing the stempel pipette method. And, if one examines the studies that are cited in the ICES manual you see conclusion statements such as "For fish eggs the Stempel pipette was most precise and very fast, though it is often impractical for nomral samples because of clogging" and, "For the wild sample, again the Folsom splitter was the most accurate and precise" (Guelpen et al. 1982). A recommendation is not specifically stated in the ICES manual and to state that any method mentioned in this paragraph is somehow ICES approved is making an interpretation that probably extends beyond what the authors intended. Regardless of author intent, since the Versar method employs both a stempel pipette and a folsom splitter, the coefficients of variation cannot be construed to refer to the Versar method. The Versar innovative subsampling combination is not considered in the ICES paragraph dealing with subsampling techniques.

  The Versar stempel pipette/folsom splitter combination emphasizes the use of the stempel pipette and therefore can be considered a variation on a stempel pipette method. The CVS method can also be considered a variation on the folsom splitter method since the folsom splitter technique is closely followed and the main difference is that different sieve size fractions within the split are counted. However, both the Versar and ODU methods establish and count dominants and subdominants in different subsamples of the same sample. Since both the stempel pipette and folsom splitter basic methods are mentioned in the ICES manual, both the Versar and ODU methods are more-or-less equally treated in the ICES manual. Although neither are specifically approved or recommended.

  The IBP Handbook 17 "A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters" (1984) does specifically recommend the stempel pipette

method. However, Versar does not use the recommended method since they do not follow the minimum prescribed pipette volume of 2.5 to 5.0 ml (Versar uses a 1.0 ml pipette to establish dominants) and they use a combination of stempel pipette and folsom splitter, which is not part of the recommended methodology. And, although methods used in fresh water may be useful for estuarine waters, oceanographic methods are more commonly employed. (Kent Carpenter)

- Reply to Kent's comment: Kent is correct in that UNESCO does not have an approved method for splitting and the text should be modified. We do know that the Stempel pipette has been used in a number of zooplankton programs. In addition to the IBP manual, there are also a number of other citations that can be used to support the use of the Stempel pipette. Weber (1973) describes the use of this method in a published USEPA manual for sampling in surface waters. Frolander (1968) evaluates the method and offers recommendations for improving its reliability. The ICES (2000) document that Kent mentions also discusses the Stempel pipette and indicates a relatively low coefficient of variation of 7-9% when compared to other methods.

  I also don't believe it is fair to describe the Versar method as a "hybrid stempel pipette/folsom splitter method". What happens is this - in about 99% of all cases the Stempel pipette is used exclusively. About 1% of the time, the sample is so dense that it cannot be diluted to a workable sample without splitting. In these rare cases, the sample is split with the Folsom splitter, and then the Stempel method is employed. I suggest the text be modified here to state something like, "Versar follows a variation of a commonly used counting technique of subsampling using the Stempel pipette method." (Fred Jacobs)
- Reply to Kent's comment that "Versar does not use the recommended method since they do not follow the minimum prescribed pipette volume of 2.5 to 5.0 ml." Versar seems to have *enhanced* the Stempel pipette method recommended in the 1971 and 1984 IBP Handbooks, i.e. they count 1-2 ml, 5 ml and 10 ml subsamples (see Appendix A in Report).

  The precision values given in Table 4.11 of the ICES Manual (pg 151) *can* be directly applied to the results of the Versar method when the Folsom splitter was not used because organisms were randomly distributed at the time of subsampling. (Claire Buchanan)

Action: Text modified to read "Versar follows a variation of a commonly used counting technique of subsampling using the Stempel pipette method." Relevant references for the laboratory method currently used by Versar were requested from Fred Jacobs and William Burton.

Text regarding which ODU staff counted splits in Round 1

"George Mateja, the senior ODU counter of the ODU staff, counted the 24 Virginia split samples." (Draft Report, page 3)

Recommendation: change incorrect statements regarding which ODU staff counted the Round 1split samples.

Comments:

- This is not true. It was well known at the time, and discussed during the plankton summit that the original Virginia split samples were read by the two senior counters (Miebert and Crock) and the lab supervisor (Mateja). (Kent Carpenter)

- Table 6 contains many poor assumptions, inaccuracies, and conclusions and should be removed altogether. First, it was well know at the time that Round 1 was counted by the three senior ODU counters. Some of these counts actually compare Crock versus Crock. (Kent Carpenter)
- The author of this report was under the impression that the ODU laboratory supervisor, George Mateja, was the sole counter of the Virginia split samples in Round 1. This misunderstanding was not corrected in the minutes of the "Plankton Summit" circulated in September 1998. It was not corrected in discussions of the Round 1 First Five split sample results. It was not corrected in the draft findings of "Round 2 First Ten" emailed to the zooplankton principal investigators and staff on February 1, 1999 and discussed in a subsequent conference call. This delay in correcting an important misunderstanding led directly to the author making erroneous statements and incorrect conclusions in the report (Draft Report pages 3, 8, 9). (Claire Buchanan)

Action: Text and conclusions modified.

Explanation of taxonomic differences in Round 1 and Round 2 repeat counts done by ODU

"Differences in the copepod and cladoceran species listed by the ODU Round 1 counter, the ODU Round 2 counter, and the Versar counter suggests there may be species identification discrepancies that should be investigated as well in these taxonomic groups." (Draft Report, page 8, last bullet in first paragraph).

"Visual comparison of individual taxa counts in the Round 2 first ten split samples suggest that differences may also be occurring between the senior and junior ODU counters (Table 6)." (Draft Report, page 9, fourth bullet in second paragraph)

Headers in Table 5. (Draft Report, page Table-6)

Recommendation:

- Remove erroneous conclusion and associated text (Kent Carpenter)

Comments:

- The most logical explanation for most taxonomic differences stems from the malfunction of the motorized siever in the first Round. Contamination is an extremely remote possibility, but this possibility also could have occurred during Versar counts with equal probability. The comparisons clearly show that most problems with the taxonomic differences are most likely due to small taxa being lost from the seal between the 200 micron and 72 micron chambers. (Kent Carpenter)

Action: Text in paragraphs relating to this item were changed, and conclusions revised. Footnote inserted and reiterated later.

Taxa misidentifications

- Versar technical staff previously misidentified barnacle cypris (eggs) as ostracods at high salinity stations.
- The junior ODU staff had been misidentifying *Eurytemora* as *Temora* at some freshwater stations. This error was most likely due to inexperience, and the taxonomist presently can identify the difference between the genera. (Draft Report, page 10, first paragraph)

Recommendation:

- Please add after the sentence about the Versar technical staff (first highlighted point above): "This error was most likely due to inexperience, and the taxonomist presently can

identify the difference between these two major zooplankton components." (Kent Carpenter)

- Change "junior" to "senior" staff member. (Kent Carpenter)
- I don't think we need to speculate about inexperience of ODU or Versar personnel. We can just state the taxonomic groups that Versar and ODU staff misidentified, and indicate that measures were taken to correct the problems. (Fred Jacobs)

Comments:

- First, the ODU staff that misidentified this was a senior staff member (not a junior member as interpreted here) that was not used to counting freshwater stations (we specialize counting in the lab according to salinity zones). True, it was probably due to inexperience with freshwater taxa. However, this same ODU senior staff member also was the one that pointed out that the Versar technical staff member was misidentifying barnacle cypris eggs as ostracods. If you are going to assert that the ODU staff mistake was due to inexperience, it would be unbiased to also assert the same for the Versar technical staff. (Kent Carpenter)
- The bullets were taken almost verbatim from "Appendix C: Letter from Versar to Maryland Department of Natural Resources Following March 10-12, 1999 meeting at Old Dominion University." (Claire Buchanan)

Action: Text changed according to Jacobs recommendation.

Specimen Archive

"Specimen Archive. Each laboratory would begin to assemble a reference collection of all the species encountered during regular sample analyses. Specifically, 2 or more individuals of each species (and sex if possible) would be picked and placed in a sample vial for that species. This could eventually become a long-term reference collection to be compared and shared by both laboratories." (Draft Report, page 13)

"5. A record of the mesozooplankton taxa identified in the CBP zooplankton monitoring program should be maintained in both laboratories (e.g. a type specimen collection, a photographic record). Laboratory differences in taxonomic identifications can be reconciled during side-by-side comparisons and through the assembly of a photographic or type specimen collection for Chesapeake Bay mesozooplankton." (Draft Report, page 15)

Recommendation: Allocate resources to create a taxonomic guide to zooplankton of the Chesapeake Bay, to ensure that monitoring programs identify taxa the same. (Kent Carpenter)

Comment:

- I think this is inadequate. It is important to standardize taxonomy between the ODU and Versar programs. Standardization of taxonomy should clearly be one of the most important goals of any future mesozooplankton common method between Maryland and Virginia. I disagree that a reference collection of zooplankton species should be the main component to help with this standardization. A reference collection should be made as a component of this coordination. However, the best way to ensure that this taxonomy is standardized is for both programs to use the same taxonomic guide to zooplankton of the Chesapeake Bay. I believe that one priority should be that resources be allocated to achieve this. I have offered to help and welcome any combination of ODU, Versar, or ODU and Versar collaborating to complete this guide. (Kent Carpenter)

Action: Wording was changed to better reflect these comments.

Correction factors

"Correction factors. Claire Buchanan will review all the split sample results and construct a table of conversion factors for common mesozooplankton species. These conversion factors will be used on the older, "pre-patch" ODU and Versar data for the purpose of calculating Bay-wide indicators." (Draft Report, page 13)

Comment:

- Don't like correction factors. (Mary Ellen Ley)
- This approach is looking very weak at this point. (Claire Buchanan)

Action: include some discussion of the doubtfulness of using this approach in the new Discussion section.

Quality assurance counts

"4. *Quality assurance counts within each laboratory and between laboratories should be rigorously maintained, documented, and periodically reviewed to ensure comparable, high quality mesozooplankton counts.* Quality assurance procedures should be maintained in each laboratory to ensure adequate taxonomic training of new technical staff. Quality assurance (repeated) counts for each laboratory should be regularly submitted to the states, the Chesapeake Bay Program or their designees for independent analysis. Regular site visits between the two states' technical staffs should be carried out to ensure comparable interstate taxonomy. A split sample study should be done annually for at least the next few years to ensure interstate count comparability." (Draft Report page 15)

Recommendation: Institute a common QA/QC plan. (Kent Carpenter)

Comment:

- I strongly agree... I would recommend that whatever method is commonly adopted by ODU and Versar, that common QA/QC plans be followed. This should follow a thorough QA/QC review and a plan adopted that is meaningful and practical given budgetary constraints. (Kent Carpenter)

Action: Recommendation altered to reflect comment.

General comments on Conclusions

- I agree with the conclusions, assuming the relevant wording changes I suggested earlier in this review are implemented. (Fred Jacobs)
- How about: *1. Inter-laboratory split sample comparisons between ODU and Versar indicate that the laboratories do not produce comparable abundance data for most species.* (Keep bullets the same.) (Mary Ellen Ley)

Action: Changed text of #1 to MEL's recommendation.

Field sampling method (Although not directly a part of the split sample study, this issue was discussed several times during the course of the study.)

Comments:

- *Versar should begin using a standard field sampling net which will make its future data incompatible with past data.* This point is not really a disagreement with the current report but should be included in the report as discussed in the 4/11/00 conference call. UNESCO (1968) clearly recommends a plankton net opening of around 50 cm for the size of mesozooplankton that we are intending to sample. And, as far as I can tell from

the literature, and as Fred Jacobs asserted in the 4/11/00 conference call, a 50 cm diameter net opening is the most commonly used method and the Maryland program should begin using this sampling method. This change in sampling net will make their future data incompatible with past data.. (Kent Carpenter)

- Kent is correct in that a 50 cm mouth opening net is the most common net used but there is certainly precedent for using 10 cm, 20 cm, 60 cm, 1m and 2m nets. The BLM zooplankton offshore programs of the 1970s and 1980s used 20 cm Bongos, 60 cm opening/closing Bongos, and 1 m nets, all for specific sampling objectives. In most cases, the larger the mouth opening, a higher, more accurate estimate of density will occur. This is because of reduced avoidance with larger mouth openings and presumably greater volumes of water sampled. Why don't we then just sample with 1 or 2 m nets? The answer is the: difficulty of handling such gear (especially from small boats), amount clogging in estuarine waters, and the excessive amount of laboratory time it would require to process, split and enumerate samples. It just would not be a prudent way of spending our limited resources.

  Furthermore, many of the gear studies have been done with oceanic plankton such as euphausiids and large copepods, which have greater avoidance capability than estuarine zooplankton, largely dominated by copepods in the 1mm size range, and even smaller cladocerans. Other factors such as tow speed and tow length are generally considered to be more important than size of mouth opening. For example, Wiebe (1970, 1971, 1972) conducted a series of gear studies in the 1970s. He (Wiebe 1972) concluded that increasing mouth opening from 25 cm to 1 m improved the precision of his density estimates by 15 to 19% (averaged across three tow lengths), but increasing the tow length from 500 m to 2000 m improved precision by 45% (averaged across four mouth opening sizes). There was much less of a difference in precision for nets of any size mouth opening in longer tows. Both the 0.25 and 1 m nets that were towed 2,000 m had greater average precision than either net towed at 500 m. His conclusion was that increasing tow length improves precision of replicates and provides better estimates of the relative proportions of species than does enlarging net diameter. The point is also made that it is not necessarily the volume filtered that is important but the ability to integrate across patches that can be achieved by longer tow lengths. Versar does extend their tow times in an attempt to integrate across patches.

  For these reasons, I am not convinced that Maryland would achieve much improvement in precision by switching to the larger mouth opening in the estuarine environment, although it is certainly possible. If Maryland does ultimately change to a 50 cm net, we should make sure that tow distances are relatively constant between the Versar and ODU programs.

  When we started the Plankton Monitoring Program in 1985 there was no Virginia Zooplankton program. The other large scale zooplankton monitoring program that was conducted for Chesapeake Bay (from 1971-1974) used a 20 cm Bongo and, thus, provided a good basis for comparison. Other factors we were concerned about included the high degree of turbidity in certain Maryland tributaries, and types of vessels that would be available for tributary sampling. For these reasons, the 20 cm net was selected. (Fred Jacobs)

- If Versar uses a smaller diameter net, results *could* be affected significantly. Kent assumes that they will be affected, but to really know, a side by side comparison would have to be done. (Mary Ellen Ley)

Action: None

Literature cited

APHA. 1995. Standard Methods for the Examination of Water and Wastes. 19th Edition.

Alden, R. W., III, R. C. Dahiya and R. J. Young Jr. 1982. A method for the enumeration of zooplankton subsamples. *Exp. Mar. Biol. Ecol.* 59:185-206.

Downing, J.A. and F. H. Rigler (editors) 1984. *A manual on Methods for the Assessment of Secondary Productivity in Fresh Waters.* IBP Hand Book 17 (second edition). Blackwell Scientific, London. 501 pp.

Edmondson, W. T. and G. G. Winberg (editors). 1971. IBP Handbook No. 17. A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications, Oxford, 358pp.

Frolander, H. F. 1973. Statistical variation in zooplankton numbers from subsampling with a Stempel pipette. J. Wat. Pollut. Control Fed. 40, R82-R88.

Harris, R., P. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley. 2000. ICES Zooplankton Methodology Manual. *Academic Press.* 684pp.

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

McEwen, G. F., M. W. Johnson & T. R. Folsom. 1954. A statistical analysis of the Folsom sample splitter based upon test observations. Arch. Meteorol. Geophys. Bioklimatol., Ser. A., 6:502.

Omori, M. and T. Ikeda. 1992. *Methods in Marine Zooplankton Ecology.* Krieger Publishing Co., Florida. 332 pp.

Sieburt, J. McN., V. Smetacek, and J. Lenz. 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol.Oceanog.* 23(6):1256-1263.

UNESCO. 1968. *Zooplankton sampling. Monographs in oceanographic methodology 2.* UNESCO, Paris. 174 pp.

UNESCO. 1968. *Zooplankton Fixation and Preservation. Monographs in oceanographic methodology 4.* UNESCO, Paris. 350 pp.

Van Guelpen, L., D. F. Markle, and D. J. Duggan. 1982. An evaluation of accuracy, precision, and speed of several zooplankton subsampling techniques. *J. Cons. Int. Explor. Mer*, 40: 226-236.

Weber, C. 1973. Biological field and laboratory method for measuring the quality of surface waters and effluents. Nat. Environmental Res. Center Office of Res. Development. U. S. Environmental Protection Agency. 20p.