

The 2001 Inter-laboratory Comparison of Chesapeake Bay Mesozooplankton Samples

DRAFT, 3/17/03

BACKGROUND

The Chesapeake Bay Program mesozooplankton split sample program originated in 1998 to see if modifications to different laboratory methods used by Maryland and Virginia would produce similar results. The program's current objectives are to a) document the quality and comparability of mesozooplankton monitoring data, b) identify quality assurance issues, and c) provide a forum for reviewing and refining, if needed, the field and laboratory methods and data management procedures to ensure high quality data.

Several comparisons were conducted in 1998 and 1999 (2000 report). The 2000 report concluded that Virginia's total mesozooplankton abundances were lower than Maryland's, and that the different methods produced comparable counts for only four taxa: *Eurytemora affinis* adult, *Acartia* adult, Podonidae and possibly *Bosmina*.

Because the different methods did not produce comparable results, Virginia's subcontractor, Old Dominion University (ODU), adopted the Hensen-Stemple pipet method employed by Maryland's subcontractor, Versar, Inc., beginning with samples collected April, 2000. This report covers mesozooplankton split samples enumerated in the fall of 2001, and is the first inter-laboratory comparison since ODU changed to the Hensen-Stemple method.

The 2001 comparison has revealed differences in: taxonomic identifications, nomenclature, data reduction and reporting, and level of identification (i.e., group/species/life stage). Overall, laboratory results had fewer differences and agreement has improved, however, the total counts of adult *Eurytemora* and adult *Acartia* agree less than in previous studies.

METHODS

A major emphasis of the split sample program is to assess comparability of laboratory methods and taxonomic identification. To eliminate error due to field collection, six routine samples collected and analyzed by Versar, Inc. were reconstituted and given to ODU for enumeration. Thus, the samples were "re-analyzed" rather than "split" and more accurately described as inter-laboratory replicates.

Samples were selected from six Maryland stations to represent a variety of salinity zones and seasons in the Chesapeake Bay and tidal tributaries.

Table 1. Samples Used for the Zooplankton Inter-laboratory Comparison

Station	Salinity Zone	Collection Date	Split Sample Analysis Date
CB 2.1 – Upper Bay	Oligohaline (0.5-5 ppt)	5/2000	10/2001
CB 3.3C – Mid Bay	Mesohaline (5-18 ppt)	6/2000	10/2001
CB 4.3C – Mid-Bay	Mesohaline “	9/2000	10/2001
ET 5.2 – Choptank R.	Mesohaline “	8/2000	10/2001
TF 1.5 – Upper Patuxent R.	Tidal Fresh (≤ 0.5 ppt)	12/2000	10/2001
TF 1.7 – Upper Patuxent R.	Tidal Fresh (≤ 0.5 ppt)	7/2000	10/2001

Versar, Inc. collected the samples by towing adjacent 20-cm bongo nets (202 μm mesh net) through the water column, concentrating and transferring the organisms to a one liter container preserved with 10% formalin. Taxonomists in each lab screened the samples through a 110 μm (ODU- 62 μm) sieve, rinsed off the formalin, and when necessary, diluted or split the sample to obtain a density of approximately 50-100 organisms/mL. Samples were simultaneously bubbled and subsampled with a Hensen-Stemple pipet, placed into a circular counting chamber and counted under a dissecting microscope. Both labs applied a hierarchical counting technique that first counts at least 60 individuals of the dominant species (e.g. *Acartia tonsa*) in a small subsample (1 or 2 ml), followed by 5- and 10-ml subsamples, from which all species that had counts less than 60 in the previous subsample are counted. The remaining sample was then screened through an 850 μm sieve, and those organisms not previously found were identified and counted. Total count is defined and calculated as:

Total Count (Estimate of Number in Sample) = (Number in subsample)/(Subsample volume/Dilution Volume)

Mesozooplankton total counts were reported to the Living Resources Data Manager according to the specifications in the document “*Recommended Data Submission Guidelines for Chesapeake Bay Program Living Resources Monitoring Split Samples*”, January 21, 2001, (Version 3).

PARAMETERS COMPARED

A total of 80 taxa (and eight additional life stages) were classified in the 6 samples compared. Various levels of classification occur within each lab and between labs, making direct comparisons difficult. For species routinely reported by both labs, total counts were compared. Data were also aggregated into the groups defined in Table 2 and compared.

Levels of Taxonomic Identification
4 taxa at Phylum Level
9 taxa at Class/Order Level
6 taxa at Family Level
25 taxa at Genus Level
36 taxa at Species Level

Table 2. Species and Life Stages in Mesozooplankton Parameter Groups

<u>Species Parameters</u>	<u>Definition</u>
<i>Acartia</i> adult	All NODC codes beginning with 61182901, <i>and</i> Life stage = 98
<i>Acartia</i> copepodites	All NODC codes beginning with 61182901, <i>and</i> Life stage = 12
<i>Eurytemora</i> adult	All NODC codes beginning with 61182002, <i>and</i> Life stage = 98
<i>Eurytemora</i> copepodites	All NODC codes beginning with 61182002, <i>and</i> Life stage = 12
<i>Bosmina</i>	All NODC codes beginning with 61090301 (Life stage doesn't matter)
Podonidae	NODC codes beginning with 61090501 and 61090502 (Life stage doesn't matter)
Barnacle nauplii	NODC code beginning with 6134, <i>and</i> Life stage = 11
Barnacle cypris	NODC code beginning with 6134, <i>and</i> Life stage = 17
<u>Group Parameters</u>	
Harpacticoid copepods	All NODC codes beginning with 6119, <i>and</i> Life stages = 12 and 98
Cyclopoids copepods	All NODC codes beginning with 6120, <i>and</i> Life stages = 12 and 98
Cladocera	All NODC codes beginning with 6109 (Life stage doesn't matter)
Ostracod	All NODC codes beginning with 6110 - 6113 (Life stage is not important)
Chydorids	All NODC codes beginning with 610907 (Life stage is not important)
Polychaete Larvae	All NODC codes beginning with 5001, <i>and</i> Life stage = 97

STATISTICAL ANALYSIS

The objective of the statistical analysis is to determine if the differences in the estimated sample counts differ as a result of subsampling and identification within the laboratories. Counts are compared on a sample-by-sample basis for each taxonomic group. For each sample it is possible to estimate the subsampling variance of the estimated counts, and the variance of the difference between the two labs. 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.

A z-score statistic is the basis of comparing the labs on a sample-by-sample basis. The difference between the two labs density 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. 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. Differences that result in z-scores of 3 or more are investigated further.

The Wilcoxon signed rank test may also be used to see if there is a consistent bias of one lab relative to the other based on all the samples.

The relative percent difference of the paired results is also used as a general measure of agreement, recognizing that low densities (<1,000) are expected to be affected greatly by subsampling error.

INITIAL FINDINGS AND ACTIONS

The Plankton Workgroup met at the Academy of Natural Sciences Estuarine Research Center March 27, 2002 to review and discuss the initial results. Z-scores indicated significant differences between species densities, even with both labs using the same method. Elgin determined that calculation and reporting errors occurred with the ODU dilution volumes. (The split sample data entry program is different than ODU's routine lab data entry program.) ODU corrected the calculations and reported changes to Jackie Johnson, who will repeated the statistical analysis and re-graphed the results.

The Workgroup also discussed improvements for reporting and analyzing split sample results. Although the nomenclature of taxonomic *groups* was standardized last year, it was not fully implemented. (The equivalency table from last year was distributed.) Another equivalency table for *species* is needed.

Prior to analyzing the 2002 inter-laboratory replicates, the mesozooplankton PIs agreed to meet and standardize the nomenclature for reporting species and taxonomic groups.

RECALCULATED RESULTS

Table 3 summarizes how well the reported counts agree, by species across stations. Table 4 shows the total counts reported by each lab to the lowest level classification reported. The table also gives z-scores, variances and coefficient of variance and relative percent difference between labs.

PROBLEMS IDENTIFIED AND RECOMMENDATIONS

The corrected results had much better agreement but some issues remained. A summary of the issues and recommendations follows.

1. *Acartia Tonsa* Copepedites and Adults

In three out of five samples, Versar had high copepedites and low adults relative to ODU, who had the reverse; low copepedites and high adults relative to Versar. Because the sum of the acartia life stages agree fairly well, there appears to be differences in life stage classification. It would be prudent to combine acartia counts of all life stages prior to Bay-wide data analyses until these differences are resolved.

2. *Eurytemora Affinis* Copepedites and Adults

In four samples, Versar had high copepedites and low adults relative to ODU, which had the reverse; low copepedites and high adults relative to Versar. Because the sum of eurytemora agree fairly well, there appears to be a difference in life stage classification. It would be prudent to combine counts of eurytemora of all life stages prior to Bay-wide data analyses until these differences are resolved.

3. *Polychaeta* Larvae, Juveniles and Adults

Similar to *Acartia* and *Eurytemora*, counts for the different *Polychaeta* life stages vary between laboratories, however the sums of all life stages agree fairly well. It is recommended to combine counts of all polychaeta life stages for Bay-wide data analysis until these differences are resolved.

4. Cyclops

For several of the cyclop species, one lab reports thousands, while the other reports zero. It is recommended that the Cyclops species flagged in Table 3 be compared side-by-side by the ODU and Versar taxonomists.

5. Zoea

For several of the zoea species, one lab reports thousands, while the other reports zero. It is recommended that the Cyclops species flagged in Table 3 be compared side-by-side by the ODU and Versar taxonomists.

6. Copepoda nauplii, Bosmina adult, Canuella elongata adult and Alona adult have fewer but still some differences. Taxonomic identifications of these species should be double checked in a side-by-side microscopic comparison.

7. Barnacle Nauplii

CORRECTIVE ACTION

On August 27, 2002 Forrest Crock of ODU visited the Versar, Inc. zooplankton laboratory to perform the side-by-side species comparisons. Mr. Crock, Kris Sillet and Craig Bruce of Versar, Inc., examined mounted samples to compare their classification criteria. The following topics were addressed:

- The consistency of life stage identification levels in copepods between Versar and ODU.

Versar is more conservative than ODU in their classification of adult copepods. Versar identifies copepods as adults only if they are fully developed. This includes fully developed fifth legs, and the presence of other secondary sexual characters such as geniculate antenna in males, and a distinct genital pore and/or the presence of attached spermatophores on females. It is my understanding that ODU uses the presence of fifth legs to differentiate between adult and copepodites. Using this method, late stage copepodites without fully formed fifth legs are classified as adults. These differences were apparent in our side-by-side counts of *Acartia tonsa*, and denote differences in laboratory protocol.

- Harpacticoid copepods

The most common species of Harpacticoid will be identified to genus and/or species level. Previously, Versar had lumped all harpacticoids together while ODU had identified the more common species such as *Canuella elongata* and *Euterpina acutifrons*. ODU has agreed to send copies of the keys they use to Versar to facilitate this change. Voucher specimens would also be helpful.

- Crab zoea identification

Crab zoea will be identified to genus and/or species level whenever possible. *Rhithropanopeus harrisi*, *Pinnixia*, and *Uca minax* were examined during the side-by-side investigation. Versar has the key ODU uses for their identifications, but voucher specimens would also be helpful.

- *Daphnia*

According to the 2000 split sample report, the most common species of *Daphnia* should be identified to species level. Versar never made that change, but has agreed to do so in the future.

- Polychaete larva

The 2000 split sample report indicated that specific larval stages (e.g. trochophore and spionidae) would not be differentiated. Instead they would be reported as polychaete larvae. ODU is still identifying larval stages while Versar is not.

□ Cyclops findings and resolution?

The meeting between Versar and ODU was very productive. We feel that an annual meeting to discuss taxonomy and laboratory counting techniques would be a beneficial to the continued development of a bay-wide zooplankton program. The following list of specimens were either examined or discussed during the meeting.

Acartia tonsa

A. hudsonica

A. copepodites

Barnacle cypris

B. nauplii

Canuella elongata

Centropages hamatus

Cyclops vernalis (Acanthocyclops vernalis)

Eurytemora affinis

Harpacticoids

Neomysis Americana

Oithona

Pinnixia

Podon polyphemoides

Polychaete larvae

Rhithropanopeus harrisi

Tropocyclops prasinus

Uca mina

Table 3. Agreement of Mesozooplankton Split Sample Species Counts: Versar (V2) vs. ODU (O)

SPECIES	Agreement between Versar and ODU, By Station ("good" indicates z-score < 3, "OK" means ≈ 3)						Follow-up?
	CB 2.1	CB 3.3C	CB 4.3C	ET 5.2	TF 1.5	TF 1.7	
HYDRA CARNEA_ADULT	good						
ANNELIDA_LARVAE	good		0 vs 1000				
POLYCHAETA_LARVAE & JUVEN.		0 vs 11,000	0 vs 19,000		0 vs 2000		Yes
POLYCHAETA_LARVAE	76 vs 0	21,000 vs 0	51,200 vs 0	156 vs. 0	4000 vs 0	1250 vs 0	Yes
POLYCHAETA_ADULT			0 vs 500				Yes
PODON POLYPHEMOIDES_ADULT		2250 vs 4500					
MOINA_ADULT							
OLIGOCHETA_ADULT	ok						
DAPHNIA_ADULT	3450 vs 1808						
CERIODAPHNIA_ADULT	good						
GASTROPODA				good			
DIAPHANOSOMA						good	*V1 = 1000
BRACHYURM_ADULT						3750* vs 1500	
BOSMINA LONGIROSTRIS_ADULT	518 vs 264		0 vs 1000	0 vs. 125	good	250 vs 2500	Maybe
LEPTODORA KINDTII_ADULT	good						
ALONA_ADULT	335 vs 190				4000 vs 8000		Maybe
CHYDORUS_ADULT	good						
LEYDIGIA	good						
QUADRANGULARIS_ADULT							
ALONELLA_ADULT	good						
PLEUROXUX_ADULT	good						
CAMPTOCERCUS	good						
RECTIROSTRIS_ADULT							
ILYOCRYPTUS SPINIFER_ADULT	good				good		
COPEPODA_NAUPLII		good	good	good	53,000 vs 22,000	2250 vs 500	Yes
OSTRACODA_ADULT	good	0 vs 1000					
LABDOCERA AESTIVA_COPEPIDITE							
PARACALANUS					0 vs 8000		
FIMBRIATUS_ADULT							
DIAPTOMUS_COPEPIDITE					1000 vs 0		
DIAPTOMUS_ADULT					500 vs 0		
EURYTEMORA	1400 vs 709	4000 vs 1000		156 vs. 0	170,000 vs 84,000	30,000 vs 11,500	Yes
AFFINIS_COPEPODITE							
EURYTEMORA AFFINIS_ADULT	1780 vs 3100	1000 vs 500		156 vs. 0	191,000 vs 344,000	1,250 vs 17,500	Yes
Adult + Copepedite Eurytmora Affinis	3180 vs 3810	5000 vs 1500		316 vs. 0	361,000 vs 428,000	31,250 vs 29,000	
ACARTIA TONSA_COPEPODITE	good	964,000 vs 235,000	856,000 vs 253,000	330,000 vs. 23,333		348,00 vs 114,700	Yes
ACARTIA TONSA_ADULT	good	224,000 vs 1,104,000	306,000 vs 672,000	232,500 vs. 132,000	1000 vs 2000	173,000 vs 480,000	Yes
Adult + Copepedite Acatia Tonsa		1,188,000 vs 1,339,000	1,162,000 vs 925,000	562,500 vs. 155,333		521,000 vs 594,000	
SAPHIRELLA_ADULT			235 vs. 0				
OITHONIA_ADULT		good	good				
ARGULUS_ADULT							
HARPATICOIDA_ADULT					13,000 vs 0	250 vs 0	Yes
EUTERPINA ACUTIFRONS						0 vs 500	

SPECIES	Agreement between Versar and ODU, By Station ("good" indicates z-score < 3, "OK" means ≈ 3)						Follow-up?
	CB 2.1	CB 3.3C	CB 4.3C	ET 5.2	TF 1.5	TF 1.7	
CANUELLA ELONGATA_ADULT	good				0 vs 10,000		Yes
ERGASILUS VERSICOLOR_ADULT	good				0 vs 2000	8000 vs 4000	
HALICYCLOPS					4000 vs 0	250 vs 0	Yes
MAGNACEPS_ADULT							
CYCLOPS_COPEPODITE	82 vs 0			156 vs. 0	3000 vs 0		Yes
CYCLOPS_ADULT	53 vs 0						
MESOCYCLOPS EDAX_ADULT	good						
EUCYCLOPS AGILIS_ADULT	good						
EUCYCLOPS SPERTUS_ADULT					2000 vs 0		Maybe
PARACYCLOPS_ADULT	good						
MACROCYCLOPS_ADULT					500 vs 0		Maybe
TROPOCYCLOPS					2000 vs 0		
PRASINUS_ADULT							
ACANTHOCYCLOPS VERNAL_ADULT	good				0 vs 2000		Maybe
DIACYCLOPS_COPEPODITE	good				0 vs 4000		Yes
DIACYCLOPS_COPEPODITE	good				0 vs 4000		Yes
DIACYCLOPS THOMASI_ADULT	good					0 vs 1000	
BALANIDAE_NAUPLII	good	good		17,344 vs. 4,000		good	
BALANIDAE_CYPRIS LARVAE	good	good	471 vs 0	2,344 vs. 500			
AMPHIPODA_ADULT	good						
UCA_ZOEA	good						
CHIRONOMIDAE_LARVAE	good						
NEOMYSIS AMERICANA_ADULT		ok	good	Good			
AEGATHOA MEIALIS_ADULT						good	
PALAEONETES PUGIO ZOEAE		good	good	Good		0 vs 500	
HEXAPANOPEUS							
ANGUSTIFRONS_ZOEA		0 vs 4000	good	0 vs. 125	0 vs 48	0 vs 5500	Yes
RITHROPANOPEUS							
HARRISII_ZOEA		2750 vs 0		156 vs. 0		3500 vs 0	Yes
PISIDIIDAE_EGG		ok					
SAGITTA_ADULT			good				
Rel. % Difference of Total Zoo.	20.4 %	8.5 %	24.5 %	113.6 %	15.9 %	10.8%	
# species detected by Versar V2	23	12	7	11	16	14	
# species detected by ODU	25	15	12	10	15	15	

CONCLUSIONS

- **The use of the Hensen-Stempel Pipet method by ODU has significantly improved the bias and precision of total mesozooplankton abundances and thus the utility of this metric.**

In the 1999 comparison study, ODU's total mesozooplankton counts were consistently lower than Versar's, on average by a factor of 2. This bias did not occur in the 2001 comparison. In three samples the ODU total mesozooplankton abundances were higher than Versar's, and in three samples they were lower. Therefore, the bias between the laboratories appears to be gone with respect to total zooplankton.

Inter-laboratory precision has also improved significantly for total mesozooplankton. When the 2001 total mesozooplankton counts are pooled, there is a 1.5 % relative percent difference between labs, which is excellent. In the 1999 study, there was a 67.4 % relative percent difference of total mesozooplankton in the pooled results of 10 sample pairs. In five out of six samples, the inter-laboratory total mesozooplankton precision was < 25% (See Table 3.) In future comparisons, it would not be unreasonable to apply this as an interim "control limit" for total mesozooplankton.

Total zooplankton abundance is directly used in the Chesapeake Bay Program Juvenile Striped Bass Indicator, hence the comparability of this indicator in Maryland and Virginia waters will also improve. Other indicators currently being developed by Chesapeake Bay Program scientists will also benefit from this work.

- **Some differences in identification and classification between Maryland and Virginia taxonomists remain.**

Now that sample preparation is the same in both laboratories, inter-laboratory differences in taxonomy and level of classification are easier to see. The effect of misidentification is that counts of two taxa are incorrect. Species agreement has improved greatly, particularly if the life stages are combined. This comparison study suggests there may be acceptable inter-agency agreement in the following species/groups:

- Total Acartia Tonsa
- Total Eurytemora
- Copepoda Nauplii
- Bosmina (sometimes)

For 2001 and 2002 mesozooplankton data sets, quantitative interpretation by life stages (e.g., adult or juveniles) is not possible at this time. The effort to consistently separate life stages needs to be weighed against the benefits of having quantitative data.