

COMPARATIVE ZOOPLANKTON SAMPLING EFFICIENCY OF A RING NET AND BONGO NET WITH COMMENTS ON POOLING OF SUBSAMPLES

MARK D. OHMAN

Integrative Oceanography Division
Scripps Institution of Oceanography
La Jolla, California 92093-0218
mohman@ucsd.edu

BERTHA E. LAVANIEGOS¹

Departamento de Ecología
Centro de Investigación Científica y Educación Superior de Ensenada
Apartado Postal 2832
Ensenada, B.C., 22800 México

ABSTRACT

We analyzed the comparative sampling efficiency of CalCOFI 1 m diameter ring nets and bongo nets in a series of paired comparisons from the California Current. Seventeen major taxa of holozooplankton were enumerated and species-specific analyses were carried out for hyperiid amphipods. The only consistent difference detected between these two nets was a significant increase in abundance of salps in the bongo net. In contrast with comparisons of abundance, the species diversity of hyperiid amphipods was higher when estimated from ring net than from bongo net collections, at higher numbers of individuals enumerated. Analysis of a sample pooling procedure revealed that the average abundance of the more numerous taxa was generally similar, whether determined by enumerating plankton samples individually or from a pooled sample comprising quantitative splits of individual samples. The average abundance of rare taxa was not well represented in the pooled sample. Sample pooling is particularly inappropriate for studies of pelagic species diversity.

INTRODUCTION

There is growing recognition that low-frequency perturbations in the ocean can have significant consequences for marine populations and ecosystems (e.g., Ebbesmeyer et al. 1991; Roemmich and McGowan 1995; Mantua et al. 1997; Planque and Taylor 1998; Lavaniegos and Ohman 1999; Hare and Mantua 2000; Rebstock 2001). Such studies depend upon measurement methods that document ocean properties in a consistent manner over many tens of years. Against this need for analytical consistency is the need for the development of new measurement methods that improve upon the accuracy, precision, or temporal/spatial resolution of traditional methodologies. As old methodologies give way to new, it becomes of particular importance to understand and intercalibrate measurements taken by different methods if a long temporal history is to be reconstructed.

Our interest in understanding decadal-scale variations in pelagic ecosystem structure in the California Current

System has led us to examine the effects of changes in zooplankton sampling methods over the course of the CalCOFI time series. Historical variations in CalCOFI zooplankton sampling methods have been summarized (Ohman and Smith 1995). Here, we turn our attention to the possible influence of a change in net types on the measured abundances of different zooplankton taxa. In December 1977 the bridled 1-m diameter ring net used by CalCOFI since 1949 was replaced by a 0.71-m bridleless bongo net (McGowan and Brown 1966) for standard CalCOFI zooplankton collections. The two nets are illustrated in Brinton and Townsend (1981), who also explored the relative catchability of 12 species of euphausiids by the two nets. Hewitt (1980) compared the differential avoidance of the two nets by larval anchovy, Rebstock (2001) analyzed the capture efficiency of 28 species of calanoid copepods, and Ohman and Smith (1995) compared the relative efficiency of the two net designs for collection of total zooplankton biomass (measured as displacement volume). Here, our objective is to understand the catchability of all major holozooplankton taxa censused by the two nets, in order to improve our ability to detect long-term variability in pelagic ecosystem structure. In addition to our attention to major taxa, we analyzed hyperiid amphipods catches by the two nets because of our interest in reconstructing the temporal history of hyperiids and their gelatinous hosts in the water column (Lavaniegos and Ohman 1999).

Accurate taxonomic identification of zooplankton is a labor-intensive process and means to reduce the time spent in such identifications would be broadly welcomed. We sought to evaluate the feasibility of combining quantitative splits of a group of individual zooplankton samples into one pooled sample for enumeration, in order to reduce the amount of sample processing needed to obtain measures of mean abundance. Here, we present the results of our comparisons of pooled sample counts in relation to abundance estimates derived from the analysis of individual plankton samples.

METHODS

We based our analysis on samples collected by CalCOFI at selected stations in Alta California and Baja California waters. Paired, metered tows were carried out

¹Also Integrative Oceanography Division, Scripps Institution of Oceanography, La Jolla, California 92093-0218.

using a 1-m diameter ring net with a preceding bridle in comparison to a bridleless bongo net. The diameter of the bongo frame was 0.60 m in 1975 and 0.71 m in 1978. Both nets were of 0.505 mm Nitex mesh and were towed obliquely from the surface to 210 m and back to the surface, maintaining a wire angle close to 45°. The net catch was preserved in buffered formaldehyde and a total of 62 samples analyzed by microscope (tab. 1). Only nighttime samples were considered, to avoid issues of light-mediated net avoidance and differential vertical migration. Sample pairs were collected within 30–40 min of each other (for further sampling details see Brinton and Townsend 1981; and Ohman and Smith 1995).

Zooplankton groups were counted and measured from the complete sample when individual body size exceeded 25 mm in length. After removal of these large specimens, samples were split with a Folsom splitter and one-eighth, one-sixteenth, or one-thirty-second of the original sample enumerated. Specimens less than 25 mm in length were identified to taxon and recorded in intervals of length class (0.5 mm or 1.0 mm, depending on the taxon). In the case of hyperiid amphipods the complete sample was analyzed and all specimens identified to species following Brusca (1981) and Vinogradov et al. (1996).

Counts were standardized to individuals per square meter of sea surface. To estimate mean abundance and 95% confidence intervals, data were first Log (x+1) transformed. Diversity of hyperiid amphipods was estimated using modified rarefaction curves as proposed by Hurlbert (1971):

$$E(S_n) = \sum_{i=1}^S \left[1 - \frac{\binom{N-N_i}{n}}{\binom{N}{n}} \right]$$

where $E(S_n)$ is the expected number of species in a sample of n individuals from a collection containing N individuals and S species, and N_i is the number of individuals of the i th species ($i = 1, 2, \dots, S$).

The effect of pooling samples from different stations compared to samples counted individually was analyzed using 13 spring nighttime samples from CalCOFI cruise 9804 (i.e., Apr. 1998). The pooled sample was constituted with 10.5–12.1% of each sample. The fraction removed from each corresponded to 50 m³ of water filtered in the ocean (cf. Rebstock 2001). Zooplankton groups were counted and measured from complete individual samples when individual body size exceeded 25 mm in length. After removal of these large specimens, subsampling was done with a 5 ml Stempel pipette until one-eighth of the original sample had been enumerated. In the case of hyperiids, the complete sample was analyzed

TABLE 1
 Zooplankton Samples Used in the Net Comparison
 Analysis and Portion of Each Sample Analyzed

Cruise	Stations	Number of paired tows	Portion analyzed	
			1 m ring net	Bongo net
7501	73.60	5	1/32	1/16
	103.60	5	1/16	1/16
7507	70.60*	4	1/32	1/16
	103.65	5	1/32	1/16
7804	83.60	1	1/8	1/8
	87.60	1	1/8	1/8
	90.30	1	1/8	1/8
	90.60	1	1/8	1/8
	103.30	1	1/8	1/8
	103.90	1	1/8	1/8
	7805	73.60	1	1/8
	80.90	1	1/8	1/8
	83.60	1	1/8	1/8
	87.60	1	1/8	1/8
	90.30	1	1/8	1/8
	103.60	1	1/8	1/8

*Only copepods and amphipods were counted.

and all individuals identified to species. In the pooled sample, subsampling was done with a 5 ml Stempel pipette for small-sized individuals, but the complete sample was counted for large size categories and for hyperiid species.

RESULTS

Bongo Versus Ring Net

Comparisons of the capture of 15 different major taxa of zooplankton by the bongo net and ring net in nighttime collections are illustrated in Figure 1, accompanied by a 1:1 line. Analysis of the catches of matched pairs of zooplankton samples by the Wilcoxon signed-rank test revealed no consistent bias in collection of any major taxon except salps ($p < 0.0001$, $N = 205$; see tab. 2) and pteropods (thecosomes plus gymnosomes, $p < 0.05$, $N = 154$). The alpha level has not been corrected for multiple testing. In both cases the bongo net collected more individuals than the ring net. For many taxa there was considerable scatter in the relationship between the two nets; this arises partly because of the finely subdivided length categories in which organisms were enumerated, leading to fewer individuals per category and a consequent greater susceptibility to subsampling error. In the case of the salps, where the variability was considerable, we combined all size classes from each net sample and again compared abundances (fig. 2). As with the results from individual size classes, combined abundances were significantly higher in the bongo net than in the ring net ($p < 0.02$, Wilcoxon signed-rank). In some comparisons for every taxon, specimens of a given length class were captured by one net but not by the other. A higher proportion of such instances generally occurred

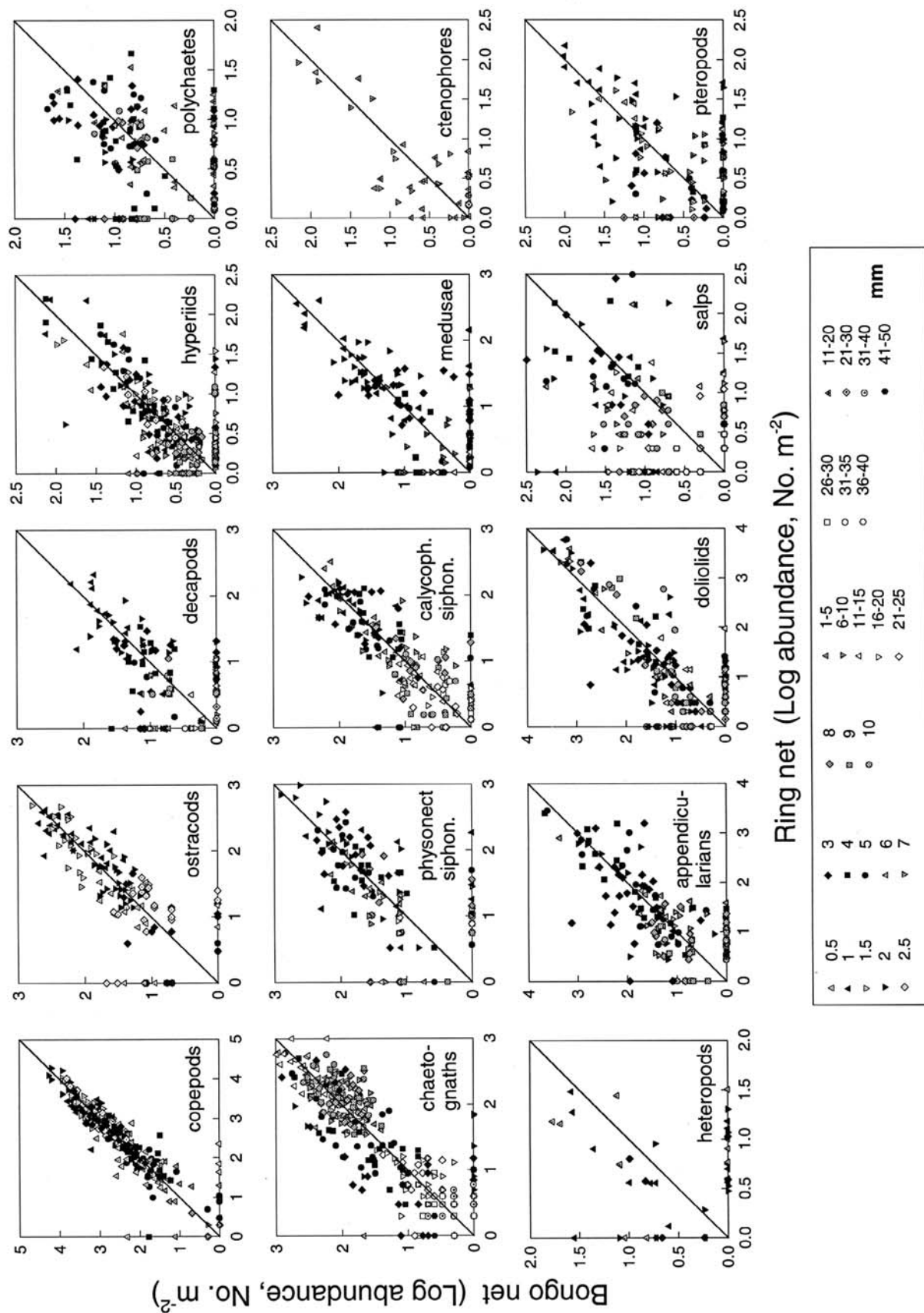


Figure 1. Comparative abundances of different zooplankton taxa captured by the bongo net and ring net. Comparisons are made by size classes of organisms, ranging from 0.5–1 mm to 41–50 mm. Diagonal is a 1:1 line. Note differences in scales among taxa.

TABLE 2
 Comparison of Bongo and Ring Net Catches

Taxon	Wilcoxon matched-pairs signed-rank tests ^a		Ratio of bongo to ring net ^b		
	N ^c	p value	N ^c	Median	95% C.I. ^d
Copepoda	284	> 0.10	265	1.02	0.93–1.11
Ostracoda	154	> 0.10	124	0.97	0.84–1.16
Decapoda	180	> 0.10	78	1.39	0.93–1.68
Hyperiid Amphipoda	335	> 0.10	215	1.13	1.00–1.23
Polychaeta	214	> 0.10	108	1.23	0.98–1.47
Chaetognatha	462	> 0.10	390	0.99	0.89–1.11
Physonect Siphonophora	141	> 0.10	101	1.12	0.86–1.23
Calycophoran Siphonophora	198	> 0.10	159	1.06	0.86–1.15
Medusae	132	> 0.10	72	1.14	0.94–1.35
Ctenophora	49	> 0.10	25	1.29	0.75–1.65
Heteropoda	52	> 0.10	17	2.01	1.26–3.10
Appendicularia	173	> 0.10	122	1.07	0.87–1.47
Doliolida	234	> 0.10	140	1.30	0.97–1.54
Salpa	205	< 0.0001	91	2.68	1.66–3.38
Pteropoda (Gymnosomata + Thecosomata)	154	< 0.05	75	1.09	0.90–1.24
Radiolaria	92	> 0.10	72	1.07	0.83–1.40
Cladocera	33	> 0.10	22	0.95	0.44–1.67

^aExcludes cases where a size class was missing from both net types.

^bExcludes cases where a size class was missing from either net type.

^cNumber of paired comparisons including all available length classes within a taxon.

^dConfidence intervals, based on the binomial distribution (Zar 1999).

with the rarer taxa (heteropods, gymnosomes and thecosomes, polychaetes, hydromedusae, decapods) and with taxa that were not necessarily rare but tend to have highly patchy distributions (salps, doliolids) or are associated with patchily distributed hosts (hyperiid amphipods). Such scatter did not occur in the copepods, which were generally abundant in all size classes.

The average ratio of bongo to ring net catch of each major taxon is also reported in Table 2. The median catch ratio did not differ significantly from 1.0 for any taxa except salps and heteropods. For calculations of the median catch ratio, cases where the catch of either net was zero had to be eliminated. This resulted in a different sample size from the comparisons with the Wilcoxon matched-pairs signed-rank test. After elimination of these cases, the average catch of pteropods was no longer different between nets. All heteropods in the 3 mm size class or below were members of the genus *Atlanta*, and all larger heteropods were carinariids and pterotracheids. Heteropods were generally too rare to make a conclusive assessment of catch ratios.

The shapes of the size frequency distributions of the major taxa enumerated did not differ appreciably between the two net types (fig. 3) for most taxa. However, in the case of salps the bongo net caught consistently higher numbers in all size classes but one, although not all such comparisons were significantly different (at $\alpha < 0.05$) in a Wilcoxon matched-pairs test. Significant differences at $\alpha < 0.05$ were detected for single length classes of ostracods, hyperiid amphipods, polychaetes, physonect siphonophores, and calycophoran siphon-

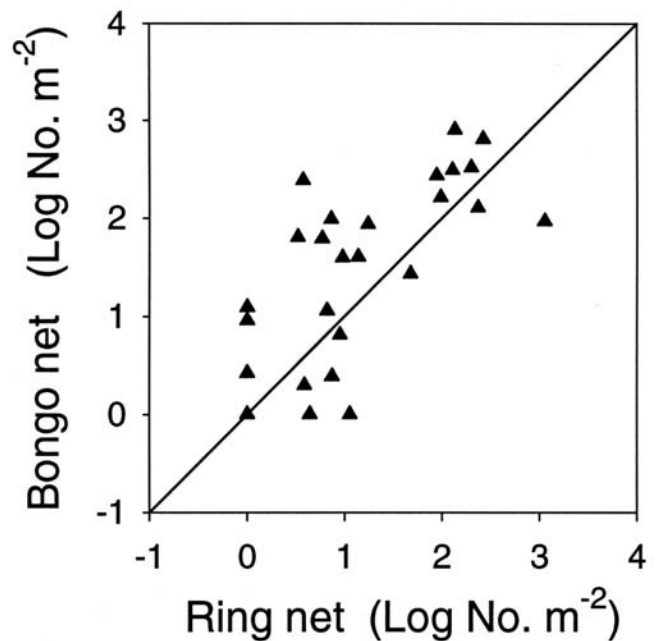


Figure 2. Comparative abundance of salps captured by the bongo net and ring net. All size classes from fig. 1 have been combined from each sample in the present plot. Diagonal is a 1:1 line.

ophores, and for two length classes of copepods and chaetognaths. Such differences in isolated length categories or in noncontiguous length classes, which are not upheld when corrected for multiple testing by the Bonferroni criterion, suggest that these results may be artifacts of large numbers of statistical tests. Note that the test results reported with asterisks in Figures 3 and

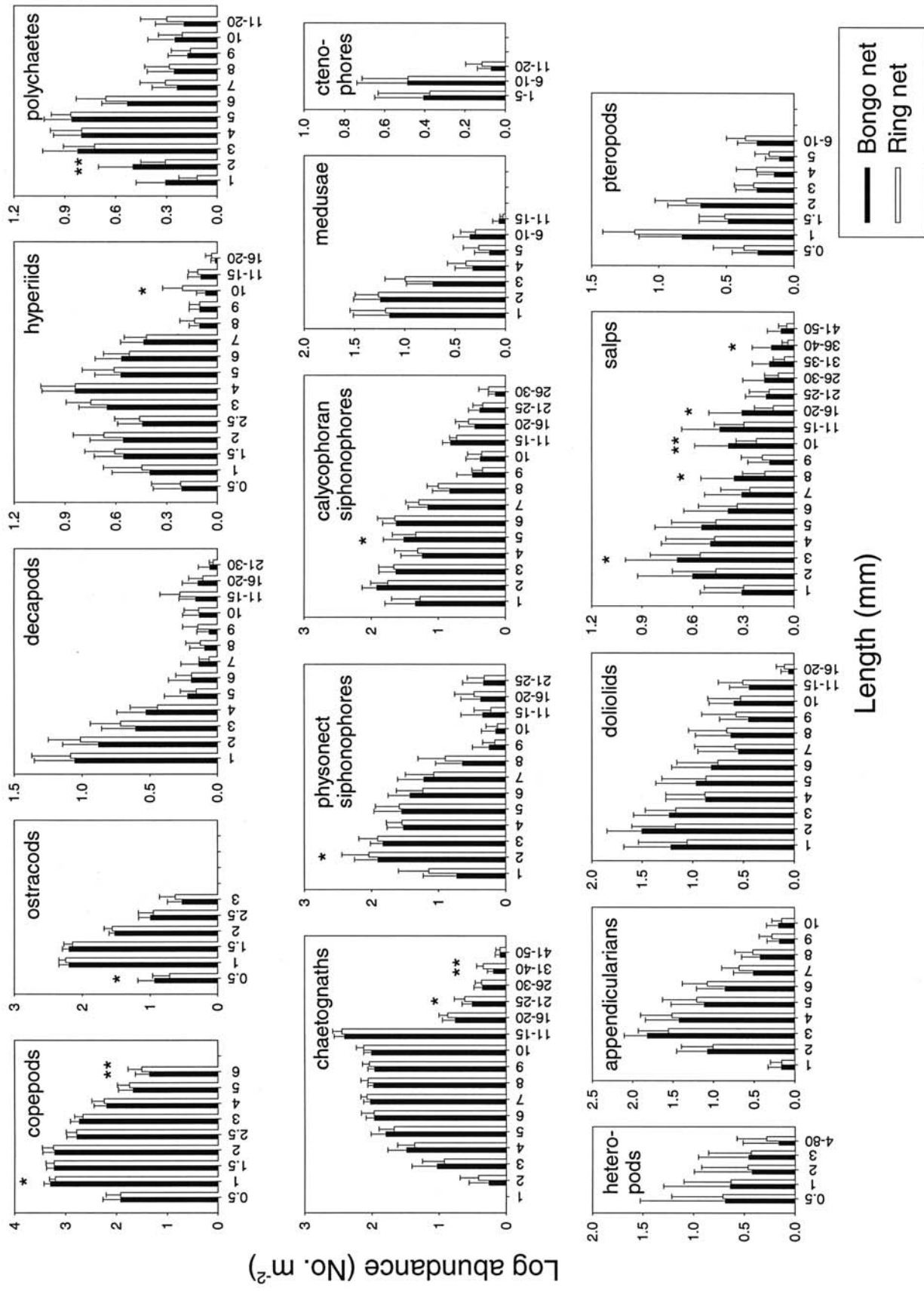


Figure 3. Comparative size-frequency distributions of different zooplankton taxa captured by the bongo net (filled bars) and ring net (open bars), \pm 95% confidence intervals. Illustrated distributions are based on all samples combined without regard to specific pairing of bongo and ring net samples taken adjacent in time. Note the differences in scales among taxa. Asterisks indicate significant differences ($p < 0.05$) or $p < 0.01$ (*) from a separate paired-comparisons test (Wilcoxon matched pairs), which utilizes the specific pairing of individual bongo and ring net samples and is not based on the combined frequency distributions shown here.

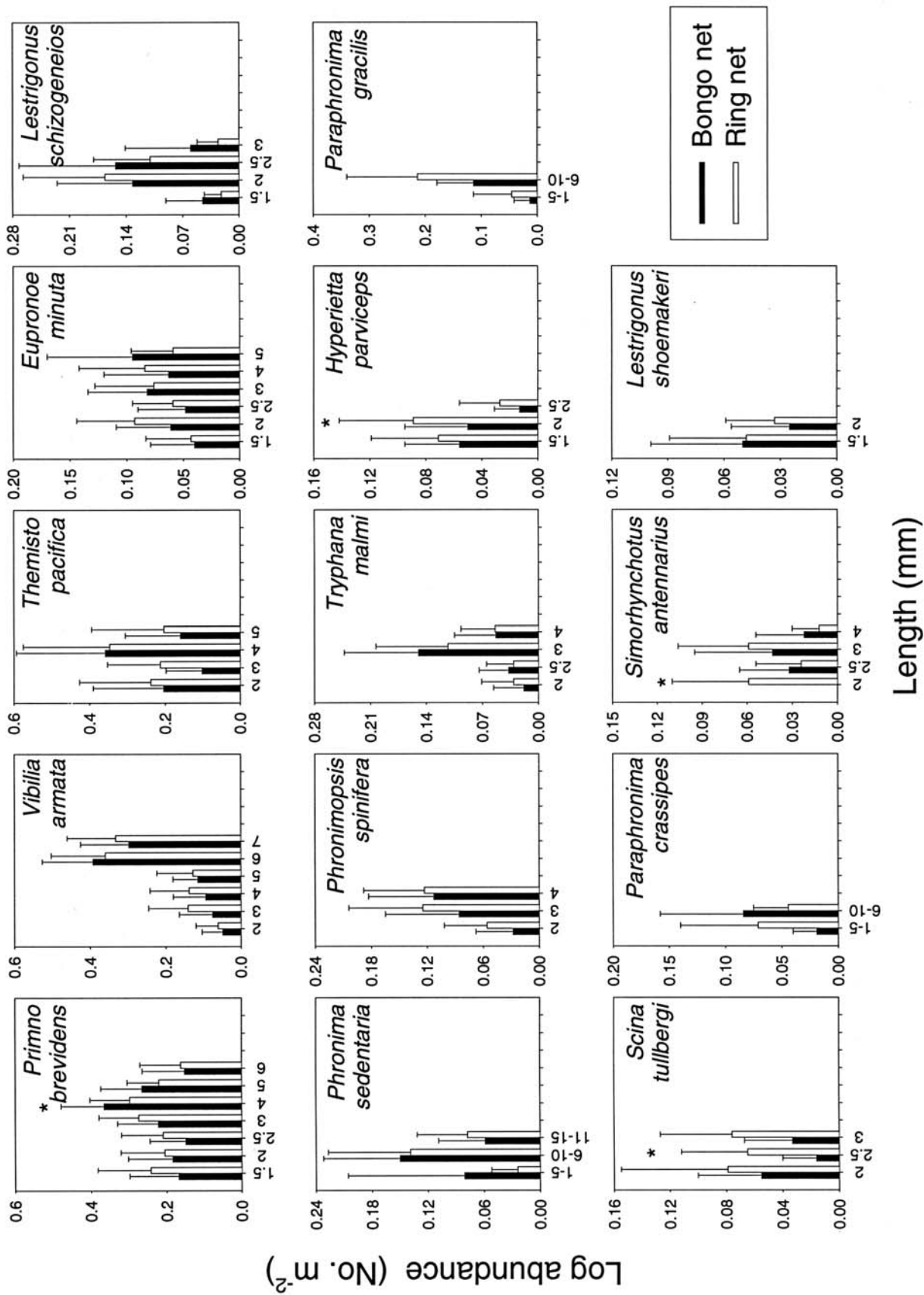


Figure 4. Comparative size-frequency distributions of different hyperiid amphipod species captured by the bongo net (filled bars) and ring net (open bars), \pm 95% confidence intervals. Illustrated distributions are based on all samples combined without regard to specific pairing of bongo and ring net samples taken adjacent in time. Note the differences in scales among taxa. Asterisks indicate significant differences ($p < 0.05$ *) from a separate paired-comparisons test (Wilcoxon matched pairs), which utilizes the specific pairing of individual bongo and ring net samples and is not based on the combined frequency distributions shown here.

TABLE 3
 Comparison of Bongo and Ring Net Catches of Hyperiid Amphipods

Taxon	Wilcoxon matched-pairs signed-rank tests ^a		Ratio of bongo to ring net ^b		
	N ^c	p value	N ^c	Median	95% C.I. ^d
<i>Primno brevidens</i>	141	> 0.10	66	1.27	1.13–1.66
<i>Vibilia armata</i>	90	> 0.10	48	1.35	1.00–1.71
<i>Themisto pacifica</i>	41	> 0.10	23	1.28	0.70–1.65
<i>Eupronoe minuta</i>	71	> 0.10	19	1.66	1.00–3.08
<i>Lestrigonus schizogeneios</i>	40	> 0.10	16	1.49	1.00–2.38
<i>Phronima sedentaria</i>	29	> 0.10	15	1.10	0.52–1.51
<i>Phronimopsis spinifera</i>	31	> 0.10	15	1.51	0.84–2.38
<i>Tryphana malmi</i>	35	> 0.10	10	1.22	0.50–2.05
<i>Hyperietta parviceps</i>	29	< 0.05	10	0.91	0.38–1.71
<i>Paraphronima gracilis</i>	25	> 0.10	7	1.69	0.75–3.31
<i>Scina tullbergi</i>	34	> 0.05	6	1.70	0.59–2.48
<i>Paralycaea gracilis</i>	8	> 0.10	4	1.30	—
<i>Paraphronima crassipes</i>	22	> 0.10	3	1.55	—
<i>Scypholanceola aestiva</i>	8	> 0.10	3	2.21	—
<i>Vibilia propinqua</i>	7	> 0.10	3	1.03	—
<i>Phrosina semilunata</i>	4	> 0.10	3	1.54	—
<i>Vibilia chuni</i>	6	> 0.10	3	3.42	—
<i>Simorhynchotus antennarius</i>	27	> 0.10	2	1.47	—
<i>Lestrigonus shoemakeri</i>	18	> 0.10	2	2.12	—
<i>Hyperoche medusarum</i>	10	> 0.05	2	0.96	—
<i>Lycæopsis themistoides</i>	8	> 0.10	2	3.59	—
<i>Vibilia australis</i>	8	> 0.10	2	1.78	—
<i>Phronima stebbingi</i>	7	> 0.10	2	1.25	—
<i>Euthamneus rostratus?</i>	6	> 0.10	2	1.44	—
<i>Vibilia stebbingi</i>	5	> 0.10	2	1.63	—
<i>Dairella californica</i>	8	< 0.05	1	0.68	—
<i>Streetsia challengerii</i>	12	> 0.10	1	2.44	—
<i>Hyperoche martinezi</i>	9	> 0.05	1	1.51	—
<i>Parascelus edwardsi</i>	9	> 0.10	1	3.38	—
<i>Hyperoides longipes</i>	8	> 0.10	1	2.13	—
<i>Hyperia medusarum</i>	7	> 0.10	1	2.38	—
<i>Phronima pacifica</i>	7	> 0.10	1	0.44	—
<i>Vibilia gibbosa</i>	6	> 0.10	1	0.77	—
<i>Parapronoe parva</i>	5	> 0.10	1	2.05	—
<i>Phronima atlantica?</i>	5	> 0.10	1	0.55	—
<i>Hyperoche mediterranea</i>	4	> 0.05	1	1.19	—
<i>Oxycephalus clausi</i>	4	> 0.05	1	0.51	—
<i>Primno latreillei</i>	4	> 0.10	1	2.21	—
<i>Lycæa pulex</i>	8	> 0.10	0	—	—
<i>Scina borealis</i>	7	> 0.10	0	—	—
<i>Phronima bucephala</i>	6	> 0.10	0	—	—
<i>Lycæa pachypoda</i>	4	> 0.05	0	—	—

^aExcludes cases where a size class was missing from both net types.

^bExcludes cases where a size class was missing from either net type.

^cNumber of paired comparisons including all available length classes within a taxon.

^dConfidence intervals, based on the binomial distribution; too few comparisons were available to estimate nonparametric confidence limits of the median for most species.

4 are from the Wilcoxon matched-pairs test of paired comparisons, whereas the overall length–frequency distributions in those figures are based on means and 95% confidence limits of combined abundances in each length class from all samples combined.

We considered 42 individual species of hyperiid amphipods. Many were too rare to obtain valid pairwise comparisons. The only species for which there were detectable overall differences in abundance between the two net types were *Hyperietta parviceps* (Wilcoxon matched pairs, $p = 0.019$, $N = 29$ pairs) and the much rarer

Dairella californica ($p = 0.012$, $N = 8$ pairs). Neither of these comparisons would be considered statistically significant if corrected for multiple testing. Analysis of the bongo to ring net ratio showed that for only one species (*Primno brevidens*) was this ratio significantly greater than 1.0 (tab. 3), although the lower confidence limit for this ratio was exactly 1.00 for three other species. Notably, the results from the Wilcoxon test and the net ratios did not agree. The size frequency distributions of the 14 most abundant hyperiid species, for which sufficient counts were obtained to make size-based comparisons,

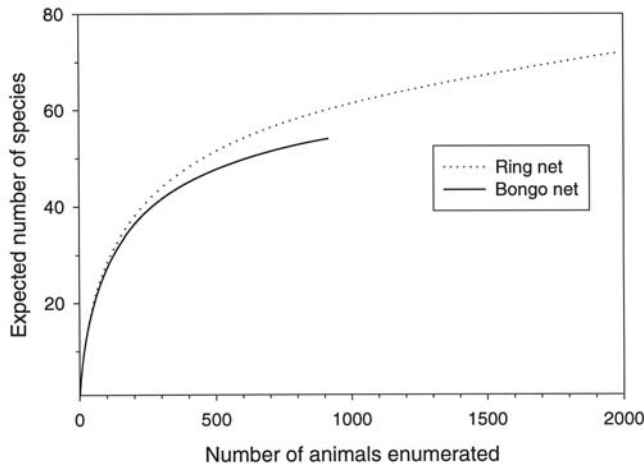


Figure 5. Rarefaction curves for hyperiid amphipods captured by the bongo net (solid line) and 1 m ring net (dotted line), for all net comparison samples combined.

showed similar distributions. When the matched ring-bongo net pairs were compared with a Wilcoxon test, we detected only isolated differences in size composition between the two net types (asterisks in fig. 4). In one case (*Simorhynchotus antennarius*) the smaller size class was not detected in the bongo net, probably due to the rarity of these individuals.

Comparison of the hyperiid amphipod species diversity as assessed by the two nets is shown in the rarefaction curves in Figure 5. At lower numbers of individuals counted (< 100 per sample) the two curves are virtually indistinguishable, but the increased species diversity as assessed by the ring net becomes clear at higher abundances (> 200–300 individuals counted). The total num-

ber of hyperiid individuals collected by the two nets differs because of the two-fold greater mouth area of the 1 m ring net than the 0.71 m bongo net, hence double the volume of water filtered for a tow of the same duration.

Sample Pooling

The mean abundance of all major taxa was determined on one cruise by two methods. One involved enumeration of each of the nighttime samples from the study region individually. The other involved subsampling a pooled sample containing a quantitative split of each of the preceding samples. For most (17 out of 21) taxa, the average abundance assessed from the pooled sample was within the 95% confidence limits of the mean of the samples analyzed individually (fig. 6). The exceptions were pteropods, radiolarians, appendicularians, and foraminifera. We attribute the differences in mean abundance of foraminifera and radiolaria to a tendency to adhere to other particulate matter and thus to clump, which makes completely randomized subsampling difficult. The differences found for pteropods (tecosomes and gymnosomes) and appendicularians appear to be a consequence of random processes in the subsampling procedure that led to the presence of slightly more individuals in some subsamples than in others.

Consideration of the effect of pooling on the estimated abundance of individual species of hyperiid amphipods yielded rather different results. Generally, the six most common species were reasonably represented by the abundance estimates from the pooled sample (fig. 7a). However, numerous rare species were found only in the individual samples and not detected at all in

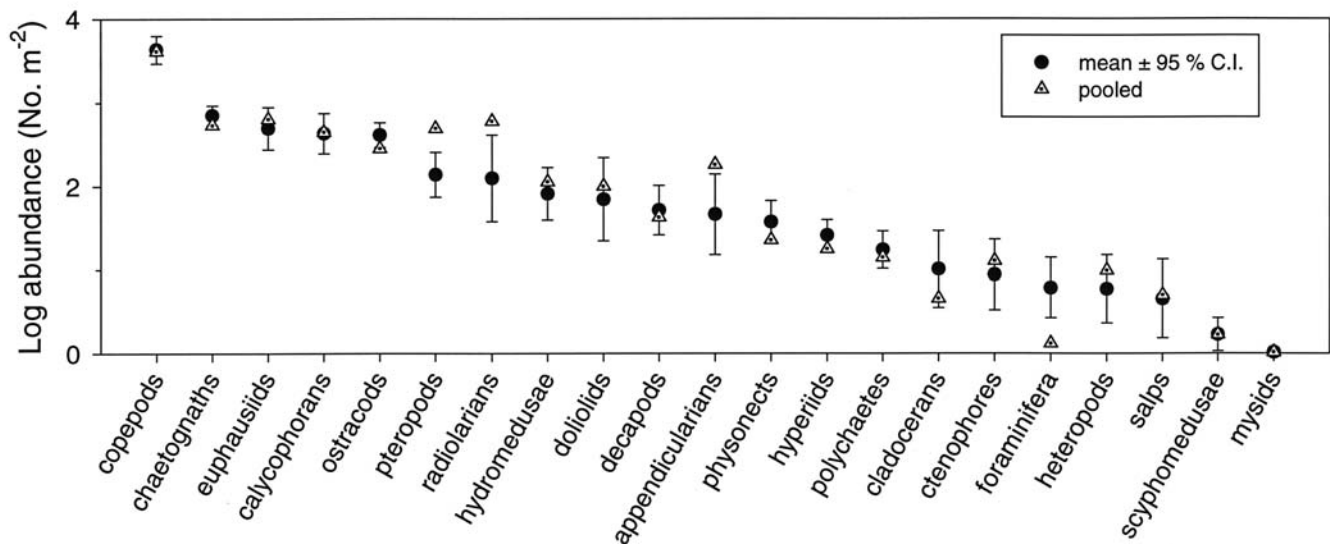


Figure 6. Pooling experiment from cruise 9804. Comparative abundances of different zooplankton taxa following enumeration of aliquots from all samples individually (circles, mean \pm 95% confidence interval) and from a single pooled sample (triangles).

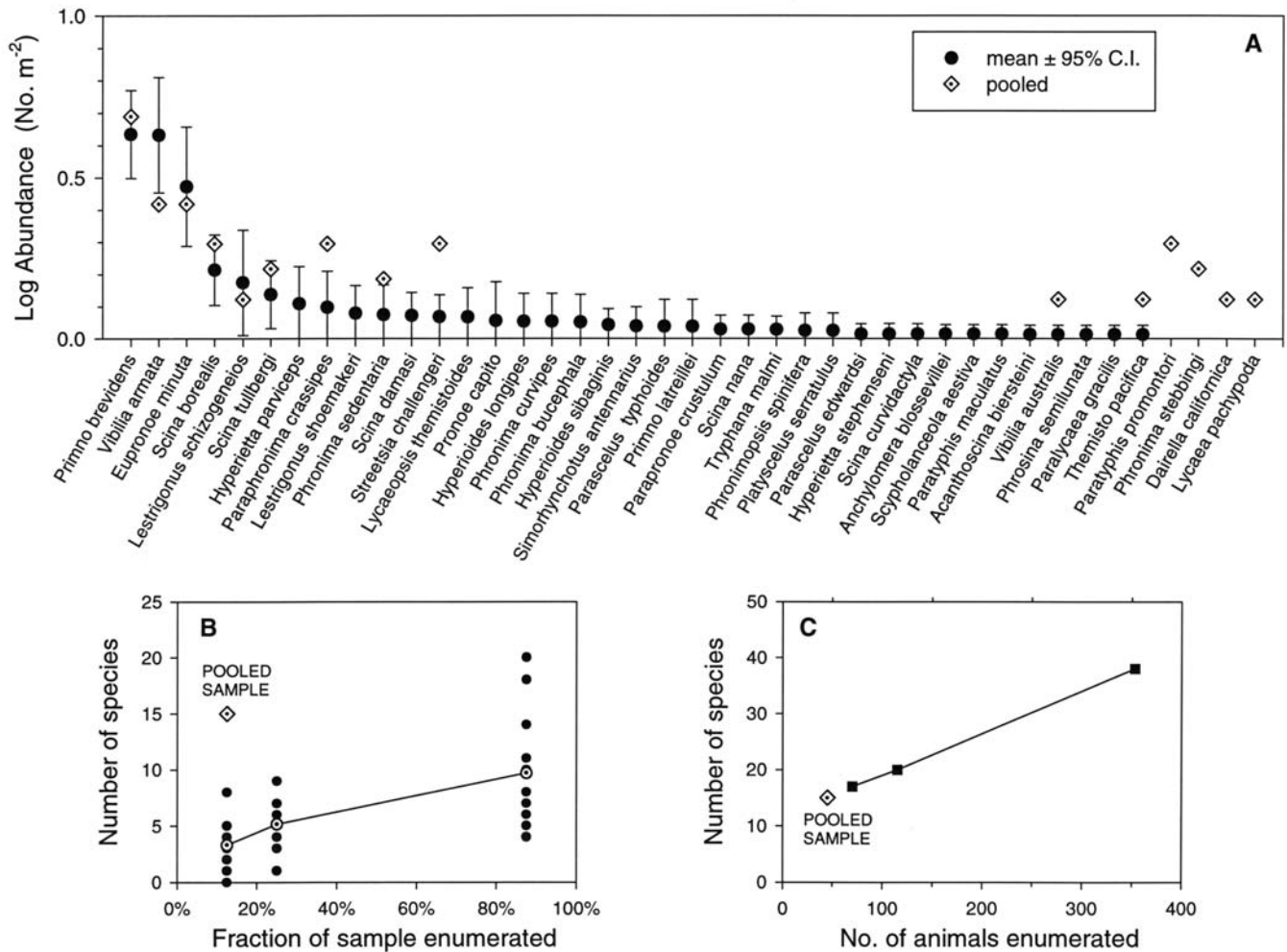


Figure 7. Pooling experiment from cruise 9804. A, comparative abundances of different species of hyperiid amphipods following enumeration of aliquots from all samples individually (circles; mean \pm 95% confidence interval) and from a single pooled sample (diamonds). B, number of hyperiid amphipod species detected as successively larger fractions of individual plankton samples are enumerated (filled circles; open circles and solid line connect the mean of 13 samples); also indicated is number of species detected in the pooled sample. C, number of hyperiid amphipod species detected as a function of the number of individuals enumerated, for all samples in the pooling experiment combined; also indicated is number of species detected in the pooled sample.

the pooled sample. In addition, four species were found in the pooled sample that were not detected in any of the individual samples. The latter result reflects the fact that 10–12% of each sample was removed for pooling before enumerating each individual sample. Thus, by chance alone, rarer species could have been transferred into the pooled sample, leaving none in the 90% of the original sample that remained. Analysis of successively larger fractions of each sample, from 12.5% to 87.5%, resulted in an increase in the mean number of species detected (fig. 7b). The corresponding number of species detected in analysis of 12.5% of the pooled sample was appreciably higher than in the average of individual samples. The total number of species recognized was directly proportional to the number of individuals enumerated (Fig. 7c).

DISCUSSION

Comparison of the capture efficiency of different nets is complicated by the perennial difficulty in sampling precisely the same parcels of water and associated plankton patches. However, paired samples were usually taken within half an hour, which is as almost as closely spaced in time as was practicable with these nets. In instances where more abundant taxa are enumerated, such differences in time/space should have relatively little consequence, provided individuals do not have highly aggregated spatial distributions when abundant. For rarer taxa, or others with aggregated distributions, the consequence can easily be the absence of an individual in a net of one type and its presence in another. Such an effect was frequently seen with salps, doliolids, appendicularians, and pteropods. Overall, the primary consequence of slight

temporal/spatial differences in waters sampled will be increased variability in the catch ratios of the two nets, but the median catch ratios should remain unbiased provided that a sufficient number of comparisons are made.

A statistical consequence of such variability in catch ratios is that the power of a test such as the Wilcoxon matched-pairs signed-rank test will be lessened. Rebstock (2001) analyzed the power efficiency of the Wilcoxon test for comparisons of calanoid copepods collected in these same samples. She found that the power of the test is relatively low, with power to detect differences between net types of a factor of 1.5 to vary between about 5% and 90%, depending on the species considered and its variability. This result suggests that caution be used before accepting the null hypothesis of no difference between nets. Our comparison of copepod collections in the two net types differs from that of Rebstock (2001), in that she counted primarily adult calanoids, whereas we counted and sized all copepods collected. This may account for the bongo to ring net ratio she observed (0.93), which was not significantly different from 1.0 (0.82–1.02 bootstrapped confidence intervals), whereas we observed a ratio of 1.02 (0.93–1.11, confidence interval based on the binomial distribution).

Another component of variability in such net comparisons is within-laboratory splitting and subsampling error. Our comparisons between pooled and separately enumerated samples is affected by this bias alone. For all of the abundant categories of higher taxa, and for the most abundant hyperiid species, the consequences of subsampling were relatively minor. We can conclude for the more abundant taxa that the mean abundance from the pooled sample generally approximates the mean abundances that would result from analysis of numerous replicate samples. However, for rare taxa, and most notably the rarer species of hyperiid amphipods, sample pooling is quite unsatisfactory. Sample pooling in the manner conducted here should not be carried out in studies of species diversity, in particular, where the presence or absence of rare taxa materially affects the outcome of the analysis.

We have not explored the possibility of differences in sampling efficiency by year, season, geographic area, or diameter of bongo net (0.60 m vs. 0.71 m). There were not sufficient paired comparison samples available to explore any of these factors in detail and we expect that such differences will be relatively minor. Concerning differences in the diameter of the bongo net, Ohman and Smith (1995) found no difference in the biomass ratio of 0.60 m diameter bongo net to 1 m ring net versus 0.71 m bongo to 1 m ring net.

The most consistent difference detected in the comparisons between net types was the improved collection of salps by the bongo net. The bongo net was a more

efficient collector of many salp size classes. We infer that this difference is attributable to the lack of a three-point wire bridle and hydrowire immediately in front of the bongo net mouth, unlike the 1 m ring net. The hydro-wire and bridle preceding the ring net would serve to break up and disperse salp chains and probably generate lower abundances inside the net itself. Hydrodynamic disturbances generated by the hydrowire could also lead to enhanced net avoidance, although it does not seem likely that the escape responses of salps are sufficiently rapid to permit avoidance (Wiebe et al. 1979). Salps were identified to species only on cruises 7501 and 7507. The dominant species present was *Thalia democratica*, and other species identifiable were *Salpa fusiformis*, *Iasis zonaria*, *Cyclosalpa bakeri*, *Salpa aspera*, and *Ritteriella picteti*. We note that net sampling is to be avoided altogether if the objective is to collect salp specimens in optimal condition for physiological and behavioral research (Madin and Kremer 1995), but this was not the purpose of the present study.

The extreme variability in the bivariate plot of salp abundances collected by the two nets illustrates, in part, the notoriously patchy distributions of these organisms (Andersen 1998). This complicates the assignment of an average catch ratio between the two nets that could be used to correct the ring net catches for equivalent bongo catches. However, assuming that the median value is an unbiased measure of central tendency, and that a comparable collection bias against salps collected by a ring net exists for other salp species, the average correction factor of 2.68 could be applied to ring net abundances to make them correspond approximately to bongo collections for time series studies.

The apparent difference in abundance of gymnosomatous and thecosomatous pteropods between bongo and ring nets is not robust to correction for multiple testing and was not sustained in the analysis of catch ratios, for which missing values from either net had to be excluded. Conversely, for heteropods, there were no significant between-net differences by the Wilcoxon test, in which only double zeroes were excluded, whereas the median catch ratios were significantly different from 1.0. However, since only 17 paired comparisons with positive heteropod abundance remained after elimination of cases of zero abundance for either net, the power of the test was low and this result must be considered inconclusive.

Of the hyperiid amphipod species that were sufficiently abundant to test for net differences, only *Hyperietta parviceps* suggested a significant change, but this comparison does not withstand correction for multiple testing. Most hyperiids utilize gelatinous zooplankton as hosts (Laval 1980; Harbison et al. 1977; Lavaniegos and Ohman 1999) or are associated with marine snow par-

ticles in the water column (Laval 1980). If hosts are (or are not) collected equally by both types of nets, one would expect the pattern of amphipod collections to follow. It is not clear whether *H. parviceps* has well-defined hosts, although members of the genus have been found to be associated with radiolarians (Laval 1980; Lavaniegos and Ohman 1999). We found no difference between net types in catches of radiolarians. In light of the lack of overall significant difference in collections of total hyperiids by the two net types, as well as the preponderance of individual species where no such bias was detectable, we conclude in general that there was little detectable effect of net changes on the collection of hyperiid amphipods.

The difference in species diversity, as estimated by rarefaction curves from all individuals collected by the two nets, is somewhat surprising. This difference is negligible at low total numbers of individuals compared. The time series analysis of hyperiid diversity by Lavaniegos and Ohman (1999) was based on the expected number of hyperiid species in a sample of 88 individuals (E_{88}), and the results above indicate that the difference between net types would have contributed a 3.9% bias in expected number of species at E_{88} . The long-term changes observed by Lavaniegos and Ohman were considerably larger than this, and thus their conclusions are not affected by the change in nets. However, at much larger numbers of individuals enumerated, an appreciably higher expected number of hyperiid species are found in the ring net samples. This may be related to either the bongo net's tendency to collect more salp specimens of all size classes or the weak (but nonsignificant) tendency of the bongo to catch more doliolids, and the importance of such gelatinous organisms as hosts for hyperiids. If a tendency existed for more individuals of a given host species to be collected in a bongo net tow, this could result in more individuals of the associated parasitoid species, and thus fewer species present for the same cumulative number of individuals counted.

The present results, together with the study of Brinton and Townsend (1981) help explain the findings of Ohman and Smith (1995) that the total zooplankton biomass collected by the bongo net was 1.366 times that collected by the ring net. Brinton and Townsend found that the bongo net generally caught more juvenile and adult euphausiids (due to diminished avoidance of the bongo), but the ring net usually caught more larval euphausiids. Considering 12 euphausiid species, and addressing biomass rather than abundance, the average difference was 1.6 times greater biomass collected by the bongo than the ring net (range 0.7–3.1, depending on the species). Here, the bongo net collected approximately 2.7 times greater abundance of salps but probably due to decreased disruption of colonies by the bongo net

rather than to diminished avoidance of the bongo. Little difference in overall abundance or size classes of copepods (results above) or in individual copepod species (Rebstock 2001) has been found, or in other holozooplankton taxa. Hewitt (1980) found that the bongo net was a preferred collector for larger larvae of the northern anchovy, but larval fish were removed from the samples prior to our analyses, so they were not a contributor to the augmented biomass. Thus, the previously documented 36.6% increased biomass collected by the bongo net is principally attributable to its increased collection efficiency of salps and euphausiids.

The results of sample pooling suggest that the mean abundances of the more abundant taxa are generally relatively well approximated by enumerating the combined sample. The abundances of the rare taxa, however, and, in particular assessments of the diversity of rarer species, are not represented in a satisfactory manner in the pooled sample. The results from Rebstock's (2001) and Venrick's (2002) studies are consistent with this conclusion. Sample pooling is to be avoided where measures of species richness are required.

In summary, we have detected few consistent differences in collection efficiency of holozooplankton between CalCOFI collections by the 1 m diameter ring net and the bongo net. We must temper this conclusion with the recognition that the statistical power of such comparisons is limited. The bongo net is the preferred collector of adults and juveniles of most species of euphausiids (Brinton and Townsend 1981) and of salps. Appropriate correction factors can be found in Brinton and Townsend (1981), Ohman and Smith (1995), and in the present study.

ACKNOWLEDGMENTS

We wish to express our thanks to Annie Townsend and Christopher Martin for their assistance and our appreciation to the seagoing personnel of the CalCOFI program who took the samples we have analyzed here. Contribution number 227 from the U.S. GLOBEC Northeast Pacific Program, supported by the National Science Foundation and by NOAA, and a contribution from the Pelagic Invertebrates Collection of the Scripps Institution of Oceanography.

LITERATURE CITED

- Andersen, V. 1998. Salp and pyrosomid blooms and their importance in biogeochemical cycles. In *The biology of pelagic tunicates*, Q. Bone, ed. Oxford: Oxford University Press, pp. 125–137.
- Brinton, E., and A. W. Townsend. 1981. A comparison of euphausiid abundances from bongo and 1-m CalCOFI nets. *Calif. Coop. Oceanic Fish. Invest. Rep.* 22: 111–125.
- Brusca, G. J. 1981. Annotated keys to the Hyperidea (Crustacea: Amphipoda) of North American coastal waters. Allan Hancock Found. Tech. Rep. 5:1–76.

- Ebbesmeyer, C. C., D. R. Cayan, D. R. McLain, F. H. Nichols, D. H. Peterson, and K. T. Redmond. 1991. 1976 step in the Pacific climate: forty environmental changes between 1968–1975 and 1977–1984. *In* Proceedings of the Seventh Annual Pacific Climate (PACCLIM) Workshop, April 1990. Calif. Dept. Water Res., Interagency Ecological Studies Program Tech. Rept. 26:115–126.
- Harbison, G. R., D. C. Biggs, and L. P. Madin. 1977. The associations of Amphipoda Hyperidea with gelatinous zooplankton. II. Associations with Cnidaria, Ctenophora, and Radiolaria. *Deep-Sea Res.* 24: 465–488.
- Hare, S. R., and N. J. Mantua. 2000. Empirical evidence for North Pacific regime shifts in 1977 and 1989. *Progr. Oceanogr.* 47:103–145.
- Hewitt, R. 1980. Distributional atlas of fish larvae in the California Current region: northern anchovy, *Engraulis mordax* Girard, 1966–1979. *Calif. Coop. Oceanic Fish. Invest. Atlas* 28:1–101.
- Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* 52:577–586.
- Laval, P. 1980. Hyperiid amphipods as crustacean parasitoids associated with gelatinous zooplankton. *Oceanogr. Mar. Biol. Ann. Rev.* 18:11–56.
- Lavaniegos, B. E., and M. D. Ohman. 1999. Hyperiid amphipods as indicators of climate change in the California Current. *In* Crustaceans and the biodiversity crisis. Proceedings of the fourth international crustacean congress, 20–24 July, 1998, Amsterdam, F. R. Schram and J. C. von Vaupel Klein, eds., vol. I. Leiden: Brill, pp. 489–509.
- Madin, L. P., and P. Kremer. 1995. Determination of the filter-feeding rates of salps (Tunicata, Thaliacea). *ICES J. Mar. Sci.* 52:583–595.
- Mantua, N. J., S. R. Hare, Y. Zhang, J. M. Wallace, and R. C. Francis. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. *Bull. Amer. Meteorol. Soc.* 78:1069–1079.
- McGowan, J. A., and D. M. Brown. 1966. A new opening-closed paired zooplankton net. *Univ. Calif. Scripps Inst. Ocean. Reference no.* 66–23, pp. 1–56.
- Ohman, M. D., and P. E. Smith. 1995. A comparison of zooplankton sampling methods in the CalCOFI time series. *Calif. Coop. Oceanic Fish. Invest. Rep.* 36:153–158.
- Planque, B., and A. H. Taylor. 1998. Long-term changes in zooplankton and the climate of the North Atlantic. *ICES J. Mar. Sci.* 55:644–654.
- Rebstock, G. A. 2001. Long-term changes in the species composition of calanoid copepods off Southern California. Ph.D. diss., University of California, San Diego, 240 p.
- Roemmich, D., and J. McGowan. 1995. Sampling zooplankton: correction. *Science* 268:352–353.
- Venrick, E. L. 2002. Floral patterns in the California Current System off southern California: 1990–1996. *J. Mar. Res.* 60:171–189.
- Vinogradov, M. E., A. F. Volkov, and T. N. Semenova. 1996. Hyperiid amphipods (Amphipoda, Hyperidea) of the world oceans. D. Siegel-Causey, Scientific ed. Lebanon, N.H.: Science Publishers, 632 p.
- Wiebe, P. H., L. P. Madin, L. R. Haury, G. R. Harbison, and L. M. Philbin. 1979. Diel vertical migration by *Salpa aspera* and its potential for large-scale particulate organic matter transport to the deep-sea. *Mar. Biol.* 53:249–255.
- Zar, J. H. 1999. *Biostatistical analysis*. 4th ed. Upper Saddle River, N.J.: Prentice-Hall, 929 p.