Using an optical plankton counter to determine the size distributions of preserved zooplankton samples

Stace E. Beaulieu1,3, Michael M. Mullin2, Van T. Tang2, Solana M. Pyne2, Andrew L. King2 and Benjamin S. Twining2,4

1Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla, CA 92093-0202 and 2Marine Life Research Group, Scripps Institution of Oceanography, La Jolla, CA 92093-0218, USA

3Present address: MS #9, Applied Ocean Physics and Engineering Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

4Present address: Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000, USA

Abstract. Long time series and/or spatially extensive observations of zooplankton are needed in order to examine fluctuations in community composition and biomass. We tested the utility of a laboratory optical plankton counter (OPC) in determining the size spectra and biomasses of a large number of preserved zooplankton samples. OPC size measurements of a variety of taxa from the California Current region were well correlated with visual measurements ($r^2 = 0.80$). Although organisms preserved for long time periods may fragment, trends in total biovolumes of ~800 samples that were >10 years old were consistent with displacement volumes measured just weeks after collection. A laboratory test in which euphausiids were damaged manually indicated that OPC counts of large zooplankton are robust to moderate fragmentation. The greatest effect of formalin preservation on crustacean zooplankton was increased opacity, resulting in an increase of 3–25% over the digital sizes measured when alive. Formalin preservation significantly reduced the biovolume of gelatinous zooplankton measured visually, but did not change the size measured by the OPC due to the compensating increase in opacity. As examples of the advantage of using the OPC to analyze a large number of preserved samples, we examined (i) the positive relationship of biomass to chlorophyll $a$ in three size classes at >100 stations occupied during a 1984 cruise and (ii) the longshore pattern of size-fractionated zooplankton biomass in ~800 samples from the California Current region in 1981 and 1984.

Introduction

Due to the temporal and spatial variability of zooplankton populations, long time series and/or extensive collections are needed in order to examine fluctuations in biomass and community composition. The California Cooperative Oceanic Fisheries Investigations (CalCOFI) program has collected samples of zooplankton, along with physical, chemical and other biological data, within the highly productive California Current System (CCS) since 1949. Recently, Roemmich and McGowan (1995a,b), using the CalCOFI time series, reported a significant (70%) decrease in total zooplankton biomass within the CCS over the last 45 years. However, not all size classes or taxa within the CCS zooplankton may be responsible for this decline. Mullin (1998) did not detect a decrease in the biomass of Calanus pacificus, an abundant component of the zooplankton, in CalCOFI samples from winters and springs of the late 1950s to the early 1990s. Similarly, the dominant euphausiid in the CCS, Euphausia pacifica, was as abundant in 1996 as in 1957, the peak year for this species in the 1950s (E. Brinton, unpublished data). Since some, but not all, kinds of zooplankton are food for fish, especially
larval and juvenile fish, determining whether the decrease in total zooplankton biomass was manifested in various size classes of zooplankton will suggest how fish populations may have been affected in the CCS over the past 45 years.

Because the microscopic analysis of zooplankton samples is labor intensive and requires taxonomic expertise, we are currently analyzing curated CalCOFI zooplankton samples with an optical plankton counter (OPC; described by Herman, 1988, 1992). Although the OPC does not allow the identification of species, it permits determination of the size distributions of zooplankton samples in considerably less time than do conventional methods. Size categorization of pelagic organisms can be nearly as instructive as categorization by species or trophic level [reviews in Rodríguez and Li (1994), Heath (1995) and references therein].

Although OPCs have been deployed in situ in lakes (e.g. Stockwell and Sprules, 1995; Sprules et al., 1998), fjords (Heath, 1995) and the ocean (e.g. Herman et al., 1991; Huntley et al., 1995), nets remain the typical instruments for sampling zooplankton. Data from nets generally are limited to total displacement volume as a measure of biomass (or, more appropriately, biovolume) and time-consuming sorting of samples into taxa. Most researchers who have reported results from a laboratory OPC used it only to calibrate a field-deployed OPC (with some exceptions, e.g. Gallienne et al., 1996; Gallienne and Robins, 1998). Here we report the use of a laboratory-based OPC and circulation system in retrospective analyses of a large number of preserved zooplankton samples.

Our objective for the work described here was to test the effectiveness of a laboratory OPC in determining size spectra of preserved zooplankton. We first describe an OPC circulation system developed to minimize coincident counts, bubbles and sample damage. We then compare conventional methods, including visual measurement of individual taxa and total displacement volume of whole samples, to the OPC analysis of preserved CalCOFI zooplankton. We discuss several experiments intended to test the effects of preservation on OPC-sensed sizes, including analyzing zooplankton with the OPC while alive and after preservation. Finally, we present two applications of the use of OPC-generated data in elucidating patterns for a large number of preserved samples. We utilized OPC counts for CalCOFI samples to test two ecological hypotheses: (i) zooplankton biomass in all size classes is correlated positively with phytoplankton standing stock; (ii) size classes exhibit the same pattern in biomass over a large geographic scale (~1000 km). Some of our methods and findings can be applied to any set of zooplankton samples, collected from lakes or from the ocean.

**Method**

**OPC circulation system**

We used a laboratory OPC (Model OPC-1L; Focal Technologies Inc., Dartmouth, Nova Scotia, Canada) connected to a specially designed circulation system. The sensing zone of the OPC-1L is a parallel light beam of 2 cm × 4 mm cross-section with a 2.5 cm path length. When a zooplankter passes through the sensing zone,
the OPC reports a digital size unit (DSU) proportional to the amount of light blocked by the organism. Raw data are recorded in 4096 DSU size categories. Each DSU is converted to an equivalent spherical diameter (ESD) using a non-linear equation determined by Herman (1992) based on calibration with spheres of known diameters. The dimension measured by the OPC is better termed the equivalent circular diameter (ECD), i.e. the diameter of a circle with the same area as the silhouette of a zooplankter passing through the sensing zone (Sprules et al., 1998). The laboratory OPC, set at normal gain, is effective in measuring plankton with ECDs from 0.25 to ~16 mm.

Because the OPC measures a projected cross-section of particles, non-spherical organisms produce a range of ECD values depending on their orientation as they pass through the light beam. Inaccurate measurement of ECD can also occur due to coincidence, when two or more particles pass through the light beam simultaneously and generate a single count with a larger DSU. Inaccuracy in determining ECD also can result from translucency of zooplankton. Because of the imprecision in measurement of zooplankter size, we combined data into a smaller number of size categories for most analyses (12 bins; see below).

We designed a circulation system that (i) dilutes a concentrated zooplankton sample, (ii) transports zooplankters through the OPC so as to minimize coincidence, bubbles and breakage of preserved animals, and (iii) permits recovery of the sample (Figure 1). When analyzing preserved samples, the system is filled with deionized water sterilized with bleach. The hydrostatic head in the upper reservoir is adjustable to maintain a steady flow rate of ~20 l min⁻¹ through the OPC. A concentrated sample is placed in the removable acrylic chamber, which is then lowered into the glass tube. The operator adjusts the secondary flow through the Tygon tubing so that zooplankters are gradually washed out of the acrylic chamber and dispersed into a larger volume of water in the glass tube before being carried through the OPC. The open top of the glass tube allows bubbles to be released rather than entering the OPC where they would be counted as particles. The diluted sample flows through the OPC at a rate of ~5 counts s⁻¹ and is re-concentrated onto a mesh filter in the lower reservoir, from which the plankton-free water is pumped back to the upper reservoir.

**Calibration to microscope measurements**

For our tests of the effectiveness of the OPC in counting and sizing preserved zooplankton, we calibrated the OPC with respect to visual measurements of marine taxa that varied in size, shape (e.g. conical euphausiids, cylindrical pyrosomes, discoid ostracods) and transparency. Length and width of individual organisms were measured under a dissecting microscope. We calculated the geometric mean of these dimensions [(length × width) ^ 0.5] as the expected ECD of the area that would be sensed by the OPC, assuming that the long axis of the organism was perpendicular to the light beam. This calculation is based on setting the area of an ellipse equal to the area of a circle.
Comparison to original displacement volume

We also wanted to determine whether the biovolumes calculated for decade-old samples analyzed by the OPC were equivalent to the displacement volumes measured just after collection. Zooplankton used in this study were sampled during CalCOFI cruises in 1981 and 1984 by towing a bridleless (Bongo) net with 505 µm mesh obliquely from the surface to 210 m (or near bottom). Samples were preserved in 5% buffered formalin, and the displacement volume of organisms <5 ml in individual size was determined in the laboratory several weeks after preservation (methods in Scripps Institution of Oceanography, 1984). In order to minimize the amount of time required for examining a large number (~800) of the CalCOFI samples, we split each sample with a Folsom plankton splitter so that fractions analyzed with the OPC contained ~2000–4000 particles.

From the raw data reported by the OPC, we considered only the counts with DSU ≥ 50 (corresponding to ECD > 0.75 mm) as equivalent to cylindrical organisms that had been retained by the net. We combined the raw data into 12 DSU size bins with the following endpoints: 50, 50 + 20, 50 + 20 + 21, and so on (see bins in Figure 5). We chose this scheme because real samples are likely to be
dominated by small animals, and significant changes in the numerical importance of large, rare animals might be difficult to detect. The exponential (base 2) increase in width of size bins accentuates the numerical importance of large animals, but also smooths minor differences in their OPC-sensed sizes. We then calculated the total biovolume for each sample as the sum over all bins of the number of counts per bin times the volume of the appropriate sphere, calculated from the geometric mean ESD for that bin (i.e. the geometric mean of the upper and lower endpoints of the bin).

Fragility of preserved samples

Since the CalCOFI zooplankton samples are returned to an archive for future researchers, we needed to determine how stressful the OPC circulation system was to the preserved samples. A reference sample was constituted from several CalCOFI zooplankton samples and sorted to remove detritus. Approximately once per month for 1 year, this reference sample was split twice and run through the OPC. The binned OPC counts from different time points were compared using paired \( t \)-tests.

We also wanted to ascertain the effects of destructive handling of the preserved samples on size spectra. For example, observations of decade-old preserved euphausiids suggested that eyes and swimming appendages can break off when the samples are handled. We tested this observation by running a sample of ~300 euphausiids, cleaned of debris, through the OPC five times. Then we manually damaged the sample by separating eyes and limbs from the bodies in order to mimic a naturally damaged sample, and ran the damaged sample through the OPC five times. We compared binned size spectra from the ‘clean’ and ‘damaged’ runs visually and with paired \( t \)-tests.

Effects of formalin on transparency

Formalin, the preservative used for CalCOFI zooplankton samples, may affect sizes sensed by the OPC due to its tendency to reduce volume and increase opacity of zooplankters. Three experiments were conducted in which zooplankton were run through the OPC alive and then after preservation in formalin. In the first two experiments, live zooplankton were collected with a 505-\( \mu \)m-mesh net in oblique tows to a depth of 60 m over the La Jolla Canyon (water depth > 300 m) in May and June 1998. The zooplankters were resuspended in sea water, taken to the laboratory alive in cooled containers, divided into aliquots (~2000 organisms per aliquot) and passed through the OPC in filtered sea water. The aliquots were then preserved in 5% buffered formalin. Based on previous studies of biovolume decrease due to formalin preservation (summarized in Beers, 1976), we re-analyzed these samples with the OPC after ~5 weeks.

We determined the percentage difference in the average size (in DSU) of the zooplankters for each aliquot before and after preservation, and checked for significant differences in the means using \( t \)-tests (one-tailed; \( \alpha = 0.05 \)). The spectra for the live and preserved runs were plotted for comparison without binning the
counts. To gain a better understanding of modal shifts in the histograms from the live to preserved state, we subsampled three numerically dominant taxa from the preserved samples. We measured the subsampled specimens under a dissecting microscope and then analyzed them with the OPC.

For the third experiment, moon jellyfish (*Aurelia aurita*) ephyrae, acquired from the Birch Aquarium at Scripps, were raised in a 5 l chamber filled with filtered sea water. When the ephyrae had matured to medusae that were similar in size to other gelatinous zooplankters in CalCOFI samples, we measured the diameter and height of each medusa's relaxed bell under a dissecting microscope. After the visual measurements, each medusa was introduced individually into the OPC in a flow of filtered sea water. Then, each was preserved in a separate vial with 5% buffered formalin. After 5 weeks, we measured each medusa again with the same techniques. We calculated biovolume for the visual measurements by assuming that the shape of a medusa was half of an ellipsoid. The biovolume for each medusa sensed by the OPC was estimated using $0.5 \times \text{ESD}$ as the radius of a sphere. We used paired $t$-tests to determine whether biovolume estimates were significantly different between the live and preserved specimens (one-tailed and two-tailed; $\alpha = 0.05$).

**Retrospective analyses of CalCOFI samples**

To test the hypothesis that zooplankton biomass in all size bins is correlated positively with phytoplankton biomass, we examined data for the first CalCOFI cruise in which chlorophyll $a$ measurements were made at the same stations as zooplankton collections (Cruise 8401, January 1984). Chlorophyll $a$ was measured in the seston of water samples collected at ~10 m depth (methods described in Scripps Institution of Oceanography, 1984).

To determine whether all size bins exhibit the same pattern in biomass over a large geographic scale (~1000 km), we combined data from four winter/spring cruises during 1981 and 1984, and plotted size-fractionated biovolume in a long-shore swath from San Francisco, California, to Punta Baja, Baja California Sur, Mexico (~8° of latitude).

**Results**

**Calibration to microscope measurements**

Individuals from nine taxa ranging in mean length from 1.4 (*Metridia* sp.) to 22.5 mm (pyrosomes) were used to compare visual counts and measurements with analogous properties measured by the OPC (Table I and Figure 2). The number of organisms detected by the OPC agreed well with the number supplied for analysis, and the expected ECDs for the taxa were well correlated with the average ECDs recorded by the OPC ($r^2 = 0.80; P < 0.01$). Non-parametric regression of these data using Theil’s method (Sprent, 1993) yielded a much greater slope ($y = 0.77x + 0.41$). This lack of agreement between the parametric and non-parametric regressions was likely due to the large, translucent pyosome colonies; when we did not include the data point for pyrosomes, the least squares
regression yielded \( y = 0.83x + 0.30 \) \( (r^2 = 0.84) \). The slope for the relationship of OPC ECD to expected ECD for the 11 crustacean groups was approximately unity in both least squares and non-parametric regressions.

**Comparison to original displacement volume**

Estimates of OPC-determined biovolume for 820 CalCOFI samples from 1981 and 1984 were compared to original displacement volumes. Although the OPC

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Microscope Counts</th>
<th>Mean ± SD (mm) Width</th>
<th>Mean ± SD (mm) Length</th>
<th>Optical plankton counter Counts No. of runs</th>
<th>Mean ECD (mm)</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Metridia</em> sp.</td>
<td>72</td>
<td>0.55 ± 0.02</td>
<td>1.38 ± 0.05</td>
<td>2</td>
<td>68</td>
<td>1.00</td>
<td>[0.74, 1.34]</td>
</tr>
<tr>
<td><em>Calanus pacificus</em></td>
<td>91</td>
<td>0.69 ± 0.04</td>
<td>2.18 ± 0.09</td>
<td>2</td>
<td>91</td>
<td>1.23</td>
<td>[0.83, 1.64]</td>
</tr>
<tr>
<td>Ostracod</td>
<td>113</td>
<td>1.06 ± 0.20</td>
<td>1.94 ± 0.29</td>
<td>3</td>
<td>108</td>
<td>1.32</td>
<td>[0.75, 1.95]</td>
</tr>
<tr>
<td><em>Pleuromamma</em> sp.</td>
<td>184</td>
<td>0.85 ± 0.05</td>
<td>2.36 ± 0.14</td>
<td>2</td>
<td>170</td>
<td>1.61</td>
<td>[0.80, 2.46]</td>
</tr>
<tr>
<td><em>Eucalanus</em> sp.</td>
<td>126</td>
<td>0.98 ± 0.11</td>
<td>4.57 ± 0.26</td>
<td>1</td>
<td>126</td>
<td>1.71</td>
<td>[1.03, 2.18]</td>
</tr>
<tr>
<td><em>Rhincalanus</em> sp.</td>
<td>16</td>
<td>0.84 ± 0.09</td>
<td>3.83 ± 0.28</td>
<td>2</td>
<td>17</td>
<td>1.74</td>
<td>[1.34, 2.12]</td>
</tr>
<tr>
<td>Chaetognath</td>
<td>136</td>
<td>0.65 ± 0.10</td>
<td>9.54 ± 1.14</td>
<td>3</td>
<td>122</td>
<td>1.79</td>
<td>[0.83, 2.62]</td>
</tr>
<tr>
<td>Euphausiid (small)</td>
<td>170</td>
<td>1.14 ± 0.08</td>
<td>3.08 ± 0.23</td>
<td>2</td>
<td>170</td>
<td>3.16</td>
<td>[0.75, 4.89]</td>
</tr>
<tr>
<td>Euphausiid (larger)</td>
<td>128</td>
<td>2.15 ± 0.18</td>
<td>4.95 ± 0.29</td>
<td>3</td>
<td>152</td>
<td>3.62</td>
<td>[0.75, 8.64]</td>
</tr>
<tr>
<td>Pyrosome</td>
<td>15</td>
<td>7.67 ± 0.98</td>
<td>22.47 ± 4.64</td>
<td>6</td>
<td>15</td>
<td>5.96</td>
<td>[0.77, 15.65]</td>
</tr>
</tbody>
</table>

**Fig. 2.** Regression of mean equivalent circular diameter (ECD) determined by the OPC against expected ECD (geometric mean of length and width) from visual measurements of various preserved planktonic organisms. Filled circles are the taxa in Table I, and filled squares are the taxa in Tables III and IV. Lines indicate the least squares regressions for all data points and for solely the crustacean taxa.
biovolume estimates generally were less than the displacement volumes, the overall increase in biovolume from January through May within each year was conserved in the OPC estimates (Figure 3A). Positive correlations between the OPC biovolume estimates and the original displacement volumes were significant for all cruises ($P < 0.05$; e.g. $r^2 = 0.81$ for the April 1981 cruise in Figure 3B), except for Cruise 8405 in which samples were notably gelatinous and contained many salps. We note that the displacement volumes and the OPC estimates were not distributed normally but were log-normal (Lilliefors's test at $\alpha = 0.05$ level; SYSTAT 7.0.1). We present the least squares regression in Figure 3B because non-parametric regression (using Theil’s method; Sprent, 1993) and weighted linear regression (weighted to the reciprocal of the square of the OPC estimates) had slopes similar to least squares regression (0.87 and 0.64, respectively, compared to 0.86). The non-parametric correlation coefficients for Cruise 8104, Kendall’s $\tau$ and Spearman’s $\rho$, were 0.77 and 0.93, respectively, both significant at $P < 0.01$.

Fragility of preserved samples

The OPC circulation system appeared to minimize breakage of samples. The reference sample was split and analyzed 10 times with the OPC between May 1997 and April 1998. Although the estimated total biovolume for the sample decreased by ~15% between the first and tenth run, the histograms of counts in the 12 bins did not differ significantly (two-tailed, paired t-test; $P = 0.43$). Some of the decrease in the OPC biovolume estimate may be explained by loss of particles during handling (while splitting or reconstituting the sample).

However, we did see evidence for the breakage of preserved euphausiids, occurring most likely due to turbulence in the PVC sample ‘catcher’ (indicated in Figure 1). We detected breakage between the first and second runs of the ‘clean’ euphausiid sample as a nearly 50% increase in particle counts, mainly in the smaller size categories. Apparently, the first run damaged all of the fragile specimens; between the second and third runs of the ‘clean’ sample, there was only a 2% difference in total counts.

Figure 4 presents a ‘worst-case scenario’ for sample handling. We show the most ‘clean’ euphausiid size spectrum (Run 1) and the manually damaged euphausiid size spectrum (Run 4) that was the most dissimilar (two-tailed, paired t-test; $P = 0.03$). The increase in the number of small particles in the ‘damaged’ sample was probably a result of the eyes and swimming appendages that had been detached intentionally. The size classes most affected were <128 DSU (ECD < 1.2 mm; note that only nine bins were used in this comparison and 8 DSU was the lowest size limit). The number of counts in the larger size classes (i.e. the number of euphausiids detected) did not decrease between the ‘clean’ and ‘damaged’ runs, probably because the eyes and appendages comprise a small portion of the cross-sectional area of the large animals.
A.

![Graph](image)

CalCOFI Cruise

- Lab OPC
- Displacement volume

B. Cruise 8104

- Regression
- 95% C.I.
- 95% P.I.

Fig. 3. Comparison of laboratory OPC biovolume estimates with measurements of displacement volume for CalCOFI samples. (A) Mean (± SE) biovolume for samples obtained during eight cruises (January, February, April and May) in 1981 and 1984. (B) Regression of OPC estimates against the corresponding displacement volume for all 119 samples from the April 1981 cruise. A weighted least squares fit falls within the 95% prediction interval.
Effects of formalin on transparency

For the first two experiments, a total of 10 samples of live zooplankton was analyzed with the OPC in order to compare size distributions before and after preservation in formalin. These samples consisted mainly of crustaceans, with few gelatinous organisms other than chaetognaths. The six samples collected in Set 1 (May) contained many crab zoeae as well as copepods, while the four samples from Set 2 (June) were mostly copepods. We determined the mean DSU size before and after preservation for all organisms \( \geq 24 \) DSU (ECD > 505 µm) and \( \geq 50 \) DSU (the lowest size limit for binning counts from CalCOFI samples). The mean DSU for the samples increased by as much as 25% after preservation in formalin (Table II). The increase in mean DSU was significant for eight of the 10 samples \((P < 0.05; \text{one-tailed } t\text{-tests})\). Probably due to organisms that were smaller than the threshold DSU values or were transparent when analyzed alive, the total number of counts after preservation increased, on average, by 10% and by as much as 29% (for DSU \( \geq 24 \)).

Size spectra for individual samples before and after preservation were plotted on the same axes for direct comparison (Figure 5). The example from Set 1 shows a shift from a mode at \(~30\) DSU when alive to a mode at \(~40\) DSU after preservation (Figure 5A). This shift may be due to an increase in the mean digital size of crab zoeae, abundant in this particular sample (mean size 43.1 DSU after preservation; Table III). The apparent shift in abundance of counts from Bin 6

Fig. 4. The number of counts in nine size class bins for a sorted euphausiid sample when it was initially run through the OPC (‘clean’) and after it had been damaged manually (‘damaged’). The lower and upper limits for each bin in terms of digital size unit (DSU) and equivalent circular diameter (ECD) are indicated next to horizontal axes. The bins are of increasing widths, each bin being twice as wide as the preceding one. Note that this analysis includes particles smaller than the minimum (50 DSU) recorded for CalCOFI samples.
while alive to Bin 7 after preservation (in Figure 5A) was possibly due to copepods that were detected as larger objects after preservation. The average size of the preserved copepods from Set 1 samples was 111.8 DSU (Table III).

The example from Set 2 shows two clear modal shifts between the live and preserved runs: from 55 to 60 (with the decrease in counts likely contributing to the peak at 80) and from 90 to 110 DSU (Figure 5B). The maximum number of counts after preservation of this sample occurred at 24 DSU, reflecting the abundance of *Acartia* sp. (mean size 21.3 DSU after preservation; Table III). The mean ECDs for the three taxon groups specifically examined in these experiments were remarkably similar to the ECDs expected from visual measurements (Table III).

In the third experiment, the estimate of mean biovolume based on visual measurements of 62 specimens of *A. aurita* decreased by 41% after preservation in formalin (one-tailed, paired t-test, *P* << 0.001; Table IV). However, the biovolumes calculated for the OPC measurements of individual medusae did not differ after preservation (two-tailed, paired t-test, *P* = 0.61). For both living and preserved medusae, the microscopic and OPC estimates of ESD were significantly correlated (r² = 0.70 and 0.64, respectively; *P* < 0.01). However, even though the preservation increased the opacity of the medusae, the OPC still underestimated biovolume by about half.

### Table II. Percentage increase in mean digital size (DSU) from the live to preserved state for mostly crustacean zooplankton (after 5 weeks in buffered formalin). Top row: all counts with DSU ≥ 24, corresponding to equivalent circular diameter (ECD) > 0.51 mm. Bottom row: all counts with DSU ≥ 50 (ECD > 0.75 mm)

<table>
<thead>
<tr>
<th></th>
<th>Set 1 Range (n = 6)</th>
<th>Set 2 Range (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSU ≥ 24</td>
<td>4.6–11.3%</td>
<td>4.3–17.2%</td>
</tr>
<tr>
<td>DSU ≥ 50</td>
<td>2.8–9.5%</td>
<td>11.3–24.6%</td>
</tr>
</tbody>
</table>

Retrospective analyses of CalCOFI samples

During the January 1984 cruise, zooplankton tows were conducted at 118 stations at which chlorophyll *a* was measured. We determined that total zooplankton biovolume, estimated from binned OPC counts, was correlated positively to chlorophyll *a* (as a proxy for phytoplankton biomass) at these stations (Figure 6A). Biovolumes in three of the size categories—Bin 2 (758–774 mm ECD), Bin 6 (969–1157 µm ECD) and Bin 10 (2.7–3.8 µm ECD)—were also correlated to chlorophyll *a* (Figure 6B–D). Although the biovolume data were not distributed normally (log-normal at the α = 0.05 level; Lilliefors’ test), we show the least squares linear equations because the slopes determined for non-parametric regressions were virtually identical (e.g. 41.4 using Theil’s method versus 41.9 for the total biovolume to chlorophyll *a* relationship). We also calculated weighted regressions and found similar positive slopes. All of the relationships were significantly positive (*P* < 0.01; Spearman’s non-parametric correlations).
Fig. 5. Spectra for two zooplankton samples analyzed with the OPC first while alive and then after preservation in formalin. For DSUs in the range [24, 200], each plot was smoothed with a five-point running mean. The last three points on the right of each plot are pooled results for the following DSUs: 201–400, 401–700 and 701–1000. The scale showing the logarithmic size class bins used to examine the results further (same scale for CalCOFI samples) is aligned below. (A) A sample from Set 1. (B) A sample from Set 2.

Table III. Details for the most abundant taxa in the net tow samples used to test the effect of formalin on transparency of zooplankton. Crab zoeae and unidentified calanoids were most abundant in Set 1; *Acartia* sp. was most abundant in Set 2. Expected ECD was determined from the geometric mean of length and width (width:length ratio 0.9 for crab zoeae, 0.35 for the unidentified calanoids and 0.22 for *Acartia*). Data were collected only for preserved specimens.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Microscope</th>
<th>Optical plankton counter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Counts</td>
<td>Length (mm)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Crab zoeae</td>
<td>150</td>
<td>0.67 ± 0.17</td>
</tr>
<tr>
<td>Calanoid copepods</td>
<td>150</td>
<td>1.89 ± 0.31</td>
</tr>
<tr>
<td><em>Acartia</em> sp.</td>
<td>80</td>
<td>1.08±</td>
</tr>
</tbody>
</table>

*Standard deviation not available.
To examine geographic patterns in total biomass and biomass of size classes in a longshore swath off California, we created stacked-area plots for average OPC estimates for all stations on each CalCOFI line (Figure 7). As expected from previous studies (e.g. Reid, 1962; Chelton et al., 1982), samples with the largest biovolume (in which total biovolume exceeded 500 mm$^3$ m$^{-3}$) were in the northern half of the CCS region. The three size classes that roughly correspond to Joint

![Fig. 6. Regression of (A) total OPC biovolume and biovolume for three size class bins representing (B) small (Bin 2 as in Figure 5), (C) medium (Bin 6) and (D) large (Bin 10) individuals against chlorophyll a during CalCOFI Cruise 8401, January 1984.]

| Table IV. Biovolume (mm$^3$; mean ± SD) for *A. aurita* cultured in the laboratory and measured while alive and after 5 weeks in formalin (n = 62). Volume for the microscope measurements was calculated assuming a half-ellipsoid shape, and volume for the OPC measurements was calculated using 0.5 × ECD as the radius of a sphere |
|-------------------------------|-------------------|-------------------|
|                               | Microscope        | Optical plankton counter |
| Live                          | 174 ± 129         | 57.5 ± 38.1        |
| Preserved                     | 102 ± 69          | 55.1 ± 34.0        |
Global Ocean Flux Study (JGOFS) standard size classes (Gallienne and Robins, 1998) exhibited dissimilar geographic patterns in biovolume. For example, in 1984, the medium and small size classes had maxima at Lines 77 and 80, near Point Conception, while the large size class had about the same biovolume from Point Conception northward. Large samples tended to have a greater dominance of large organisms: the ratio of large (arbitrarily defined as >1.96 mm ECD; Bins 9–12) to small (750–860 µm ECD) organism biovolume was positively correlated with total biovolume. The few circumstances in which the ratio of the biovolume of large organisms to small ones was exceptionally large (>150) were also in the northern half during both years.

Fig. 7. Stacked-area plots for size-fractionated biovolume on a longshore swath of the California Current System in Winter and Spring of 1981 and 1984. CalCOFI 'lines' are transect lines running WSW orthogonally to the coast; Line 60 reaches shore at San Francisco (38°N), line 80 at Point Conception (34°27'N) and Line 110 at Punta Baja (30°N). Means are plotted for stations at distances up to 550 km offshore during the four cruises for each year (number of stations per line ranging from 13 to 43). The three size classes roughly correspond to the following Joint Global Ocean Flux Study (JGOFS) standard size classes: 500–1000 µm (Bins 1–5), 1000–2000 µm (Bins 6–8) and >2000 µm (Bins 9–12).
Discussion

One of our initial concerns in using the OPC to analyze preserved samples was the accuracy to which size could be measured in comparison to the conventional method of visual measurements. Previous research has demonstrated that the OPC accurately measures copepods, cylindrical or conical shaped organisms that are abundant marine zooplankters (Herman and Mitchell, 1981; Herman, 1988). Wieland et al. (1997) also reported that an OPC accurately measures copepods, although the range of OPC-sensed sizes was larger than for microscopic measurements. However, zooplankton samples are most often a mixture of organisms with many shapes and a wider range of sizes than represented by copepods. Sprules et al. (1998) found that an OPC accurately matched expected ECDs for preserved freshwater zooplankton of various shapes. For CalCOFI taxa that differed in shape and transparency, we found size measured by the OPC to be well correlated to size measured under the microscope. Euphausiids, Aurelia and pyrosomes deviated most from the linear fit of observed to expected ECD (Figure 2); however, this result is likely due to the inclusion of the data point for pyrosomes. The average length and width of the pyrosomes were greater than the dimensions of the beam width in the sensing zone of the OPC (Table I). Herman et al. (1993) describe a calibration for large (~2 cm length) euphausiids that overlap the beam width of the OPC. Taking a similar calibration into account, we would calculate a lower value for the expected ECD for pyrosomes, increasing the slope of the linear relationship. When the linear regression was performed without any gelatinous zooplankton, the relationship of OPC to expected size was ~1:1, likely due to the opaque nature of preserved crustaceans. Note, in our calibration for crustaceans, the euphausiids were, on average, <5 mm in length and not likely to overlap the beam width.

Another concern was whether our estimates of total biovolume based on OPC spherical diameters would be similar to displacement volume measured when the samples were first preserved. The relationship was significantly positive for samples from seven of the eight CalCOFI cruises. Although the slopes of the linear regressions were slightly less than 1.0 (e.g. Figure 3B), we expected OPC biovolume to underestimate displacement volume for two reasons. First, the threshold we selected for the lowest size class limit (DSU ≥ 50) resulted in some cases, in the exclusion of particles that contributed significantly to displacement volume. Our choice of 50 DSU was only an approximation to the retention by a 505-µm-mesh net. For example, OPC biovolume estimates for samples with large numbers of pteropod shells (which measured <50 DSU) gave poor agreement to displacement volume. Second, particle sizes as detected by the OPC depend both on actual volume and on transparency of the particles, as well as the orientation of each particle in the OPC’s sensing zone. Beers (1976) reviewed studies on the effects of formaldehyde preservation on the wet weight and volume of zooplankton. Zooplankters with high water content, such as gelatinous organisms, lose a large percentage of their original volume, especially within the first few days after preservation in formalin, but rates of volume loss are minimal after several weeks. Recently, Wieland and Köster (1996) also found that most of the shrinkage of cod...
eggs due to formalin fixation occurred within the first 3 weeks. Because the original displacement volumes were measured several weeks after preservation, the underestimates of biovolume by the OPC are most likely due to transparency and/or orientations of the zooplankters, not to further shrinkage.

A third major concern was whether our circulation system would damage the preserved zooplankton, creating spurious counts from fragments and rendering the samples less useful for future examination. Results from a reference sample run through the OPC once per month for a year indicated that our OPC set-up minimizes breakage of preserved specimens. However, damage did occur during the first run of the ‘clean’ euphausiid sample. This damage to euphausiids can be reduced by filling the lower reservoir to a level above the mesh of the sample catcher (see Figure 1), a practice we now follow. The damage did not affect the OPC counts for the large organisms; the greatest increase in counts occurred in size classes less than our threshold 50 DSU value used for CalCOFI data analysis (Figure 4).

Fourth, we were concerned about the effects of the preservative, formalin, on the size distributions measured by the OPC. We found only a few studies that specifically examined the changes in transparency of zooplankton after preservation. Checkley et al. (1997) and Wieland et al. (1997) found a significant increase in the OPC-sensed size of fish eggs after preservation in formalin (44 to 102 DSU and 0.55 to 0.8 mm ECD, respectively). Herman (1988), however, reported little difference in OPC-sensed sizes between live and preserved zooplankton samples. Wieland et al. (1997) also did not detect a difference in size between live and preserved copepods.

For our two sets of net tow samples analyzed with the OPC while alive and after preservation in formalin, we found significant increases in the mean sizes sensed by the OPC. Crustaceans lose only ~8% of their original volume after formalin preservation, and preservation of crustaceans in formalin tans protein (Steedman, 1976). Thus, our laboratory tests basically quantified the effect of increased opacity on measurements of size spectra by the OPC. After preservation, the crustaceans became more opaque so that the ECD expected from visual measurements was approximately equal to the diameter reported by the OPC (Table III).

Although the gelatinous zooplankton (A.aurita medusae) increased in opacity after preservation, the OPC biovolume still underestimated true volume by about half. These results explain why the estimates for total biovolume based on OPC counts for Cruise 8405 were not significantly correlated to original displacement volumes. Cruise 8405 was the only cruise (of the eight cruises examined) in which most samples were notably gelatinous. Gelatinous organisms such as salps and siphonophores are often components of high-biomass samples.

We presented two examples to illustrate that basic ecological questions concerning zooplankton biomass can be refined, using curated and archived material, when information on size composition is available. We first tested the hypothesis that zooplankton biomass in all size classes is correlated positively with phytoplankton standing stock. Using OPC counts for samples from the January 1984 cruise, we showed that total biovolume of zooplankton was correlated to chlorophyll $a$ and
that this positive correlation held true for small, medium and large sized zooplankters (Figure 6). These findings suggest that tight coupling between trophic levels existed in the CCS plankton—energy from primary producers was translated through the primary grazers to predators in the planktonic food web.

As a second example, we plotted size-fractionated biovolume for CalCOFI samples collected during eight cruises that each covered a longshore swath in the order of 900 km in length. Gallienne and Robins (1998) present a plot similar to our Figure 7 for a 1000 km transect across the Atlantic. Using an OPC-1L in conjunction with surface underway sampling, they were able to determine that the proportions of total biovolume represented by specific size fractions differed greatly along the transect. In our analysis, we confirmed earlier findings that total biovolume of zooplankton in the CCS is greatest and exhibits greatest variability north of Point Conception. OPC analysis showed that samples with large total biovolume also tended to have relatively more biovolume of organisms of individual size > 2 mm ECD. Using the OPC allowed us to determine size class contributions to the total biovolume and gave us information we could only have obtained through time-consuming sorting or sieving of all 820 samples.

Zooplankters in the size range measured by the OPC are a trophic link between primary production and predatory fish in the CCS (Huntley et al., 1995). Since fish are visual predators and often feed on a specific size of zooplankton, rather than on specific species, the OPC can refine the assessment of availability of food to a particular fish species. Our long-term goal for CalCOFI sample analysis is to create a single, long time series linking the size distributions of preserved samples collected over the past 50 years with in situ size distributions measured on cruises now and in the future. On current CalCOFI cruises, an in situ OPC is mounted in a plankton net that collects the organisms that passed through the sensing zone. However, problems inherent to the field-deployed OPC include avoidance of the opening (reducing the counts of larger, more agile organisms) and coincident counts (common in concentrated patches). For ongoing studies, collecting samples with wide-mouthed nets and then analyzing them with the laboratory OPC and circulation system eliminates the problem of coincidence, and is more time and cost effective than microscopic counts and measurements. For analysis of preserved samples in retrospective studies, the advantages of using an OPC over conventional microscopic methods can be substantial.

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