Evaluation and correction of subresolved particles by the optical plankton counter in three Australian estuaries with pristine to highly modified catchments

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[1] High concentrations of subresolved particles smaller than the 250 μm equivalent spherical diameter (ESD) detection limit of the optical plankton counter (OPC) have hampered its use in turbid estuarine waters. Coincidence of these subresolved particles produced erroneous counts of up to 58 L^{-1} for 100 μm mesh filtered water samples from three subtropical east Australian estuaries using the laboratory OPC-1L. The influence of these erroneous counts on in situ OPC-2T measurements was assessed by comparison with measurements of simultaneously collected net zooplankton using the laboratory OPC-1L. The total zooplankton abundance from the in situ OPC-2T measurements could be corrected for erroneous counts of subresolved particles using OPC-1L measurements of 100 μm mesh filtered water sampled from the same site but with large error. No such corrections were possible for OPC-2T measurements of total zooplankton biomass or normalized biomass size spectra (NBSS). No meaningful or significant correlations were found between the abundance or biomass of subresolved particles and in situ light attenuance, probably due to tannin-rich waters with low subresolved particle concentrations. NBSS of simultaneously collected net zooplankton from OPC-1L measurements indicated higher biomass in the disturbed Manning and Wallamba rivers, whose catchments support intensive livestock agriculture and some residential development, compared to the forested Wallingat River. NBSS may therefore be a useful indicator of nutrient enrichment in estuaries. The slope of NBSS may respond to both production of small particles and the predation and loss of large particles.

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1. Introduction

[2] The optical plankton counter (OPC [*Herman*, 1988, 1992]) has been extensively used to study zooplankton from lakes and oceans [*Huntley et al.*, 2000, 1995; *Nogueira et al.*, 2004; *Rissik et al.*, 1997; *Stockwell and Sprules*, 1995; *Suthers et al.*, 2004], by counting and sizing particles over large temporal and spatial scales. For zooplankton communities dominated by few species, taxonomic information can be retained by relating peaks in the size frequency distribution, or spectra, to individual taxa. Biological size is a useful determinant of population dynamics and ecological rates [*Cyr and Pace*, 1993; *Edvardsen et al.*, 2002; *Heath*, 1995; *Zhou and Huntley*, 1997], including grazing by zooplankton which may assimilate phytoplankton blooms. By examining certain characteristics of linear zooplankton size spectra, such as the slopes and intercepts of log normalized models, important information can be obtained on trophic state, productivity, and total biomass [*Rodriguez and Mullin*, 1986; *Sprules et al.*, 1983; *Sprules and Munawar*, 1986; *Zhou and Huntley*, 1997]. In general, steeper slopes indicate an increase in zooplankton community productivity with smaller size classes responding more rapidly to increased phytoplankton abundance compared to larger size classes, and greater intercepts at the smallest size class indicate higher total biomass.

[3] The Achilles' heel of this method is the issue of "coincidence" where multiple particles are simultaneously detected by the instrument as they pass through the sampling tunnel of the OPC. Coincident counts are more likely to occur when particle concentrations are high and pass through the detection area simultaneously, or because new particles enter the detection area of the OPC before the electronics return to baseline voltage. At this instant the OPC counts one large particle with a size equivalent to the sum of the projected area of the particles. The result is an overestimation of the abundance of larger particles and an underestimation of smaller and total particle concentrations.

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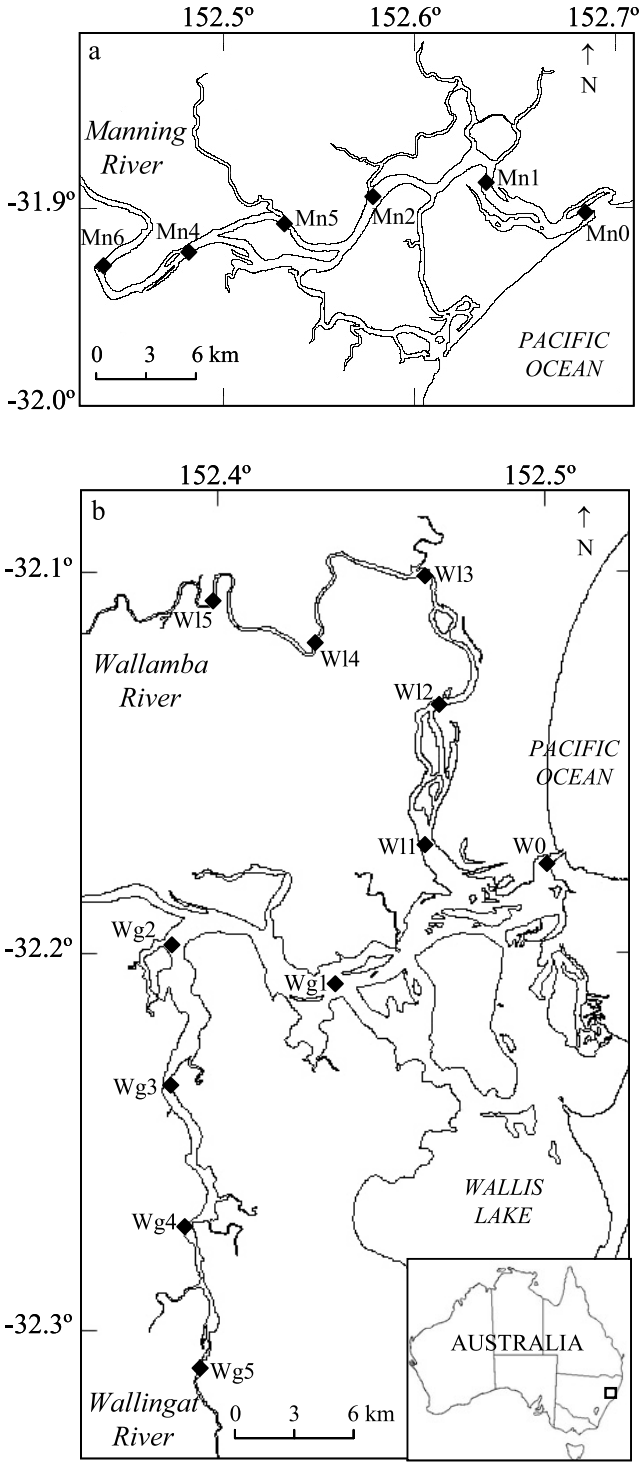

Figure 1. (a) Manning River sites (Mn0 to Mn6) and (b) Wallis Lake region showing site W0 at the entrance to the estuary and Wallamba (Wl1 to Wl5) and Wallingat (Wg1 to Wg5) River sites.

[4] Coincidence of particles larger than the 250 μm detection limit can be minimized in the field by using an acrylic insert to reduce the width of the sampling tunnel for the OPC-1T from 25.1 to 6.2 cm. Alternatively, the mini in situ OPC (OPC-2T) with a sampling tunnel of only 10 cm wide can be used. The smaller tunnel width reduces the volume of water and hence the number of particles passing through the instrument.

[5] Particles <250 μm equivalent spherical diameter (ESD) are too small to be detected by the OPC individually, yet coincidence of these subresolved particles can result in a projected area large enough to be detected. This may lead to an apparent overestimation of particle concentrations. In contrast, coincidence of resolved particles >250 μm ESD leads to an underestimation of particle concentrations and an overestimation of resolved particle biomass [*Sprules et al.*, 1998]. In estuarine waters, high amounts of subresolved particles are often present as organic and inorganic detritus. Subsequently, few studies have been conducted in estuaries using the in situ OPC [see *Edvardsen et al.*, 2002; *Roman et al.*, 2005]. The influence of these subresolved detrital particles on the zooplankton size spectra measured by the OPC is therefore unknown.

[6] Accurate estimates of zooplankton concentrations can be obtained by the laboratory OPC (OPC-1L) after correcting for the influence of background coincident counts of subresolved detrital particles [*Zhang et al.*, 2000]. Unfortunately this correction method cannot be applied to in situ OPC measurements of zooplankton communities in high detritus waters. This is because the longer path length of in situ OPCs (>6.2 cm) compared to the OPC-1L (2 cm) substantially increases the likelihood of coincident counts over a large range of particle sizes. Therefore a simple subtraction of background detritus counts obtained from the OPC-1L from the particle counts obtained from in situ OPCs is not possible.

[7] The primary objectives of this study were to determine the abundance of subresolved particles in estuaries using OPC-1L measurements of 100 μm mesh filtrate, and investigate the influence of these particles on the in situ zooplankton size spectra obtained from the OPC-2T by comparison with OPC-1L measurements of net collections. Secondly, normalized biomass size spectra (NBSS) from the OPC-1L is used to compare the zooplankton biomass and productivity of three southeast Australian estuaries with differing levels of anthropogenic disturbance.

2. Methods

2.1. Study Area

[8] The Manning, Wallamba and Wallingat rivers (Figure 1) are subtropical east Australian estuaries on the mid-north coast of New South Wales, Australia. The region generally experiences a summer rainfall regime with the wettest month often in March and the driest often in September. All three estuaries have a semidiurnal tidal cycle that extends to approximately 54 km and 20 km for the Manning and Wallingat rivers, respectively. The Wallamba River is tidal to 28 km at which point it is restricted by a weir. The mean tidal ranges of the Manning, Wallamba, and Wallingat rivers are 1.18, 0.27, and 0.13 m, respectively.

[9] The Manning River has the largest catchment area of the estuaries covering 8927 km^2. The lower catchment has been extensively modified for livestock agriculture, urban and residential purposes. The estuary has two entrances to the ocean: a main northern entrance with a secondary entrance ~12 km south.

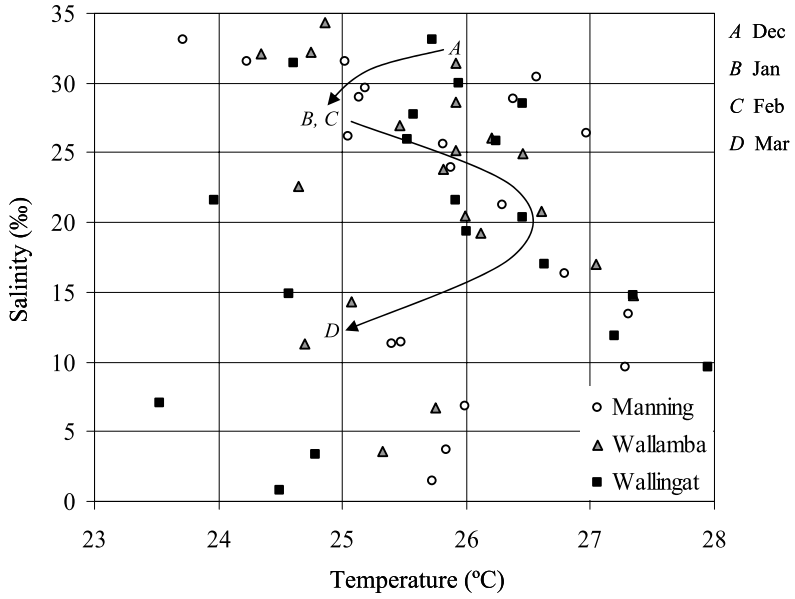

Figure 2. Temperature and salinity measurements at sites in the Manning, Wallamba, and Wallingat rivers. The arrowed lines show progression from December 2002 (line A) to January 2003 (line B) to February 2003 (line C) to March 2003 (line D).

[10] The Wallamba and Wallingat rivers flow into Wallis Lake, a permanently open coastal lagoon. The Wallamba River has a subcatchment covering approximately 500 km^2, or one third of the entire Wallis Lake catchment (1440 km^2). The subcatchment has been extensively cleared and is used for livestock agriculture. The Wallingat River subcatchment is the smallest of the estuaries covering approximately 185 km^2 and remains mostly forested.

[11] Sampling occurred at monthly intervals from December 2002 until March 2003 at five sites approximately 4 km apart in each of the Manning, Wallamba, and Wallingat rivers with additional sites at the mouths of the systems (Figure 1). Patterns in surface water temperature and salinity observations were similar within each estuary, with temperatures showing a seasonal decrease from December to March and salinities fresher at upstream sites and also decreasing following rain events in December 2002 and February 2003 (Figure 2). Because no distinct clustering of estuaries was observed within any one sampling month, physicochemical properties resulting from tidal mixing and freshwater inputs to the estuaries are likely to be similar.

2.2. Subresolved Counts

[12] Experiments were conducted using estuarine water to determine any possible background counts detected by the OPC-1L (Focal Technologies, Inc., Dartmouth, Canada) due to subresolved particles following *Zhang et al.* [2000]. The OPC-1L counts and classifies particles greater than 250 μm into several hundred digital size bins as they pass through a light beam of 4 × 20 mm cross section. The area of light blocked by the particle as it passes through the sensing zone is converted into an equivalent spherical diameter (ESD) using a calibration table, which is the diameter of a sphere with the same projected area as the particle [*Herman*, 1988, 1992]. Light attenuance is simultaneously measured so that the intensity of the light beam is automatically adjusted to ensure that area measurements are independent of small variations in the amount of light absorbed by the water. Particles between 250 to 1600 μm are effectively measured using the OPC-1L set at normal gain.

[13] Estuarine water (20 L) was collected from the surface of the Manning, Wallamba and Wallingat rivers using a large container at all sites and on all four months. Unpreserved water was gently filtered using 100 μm mesh,

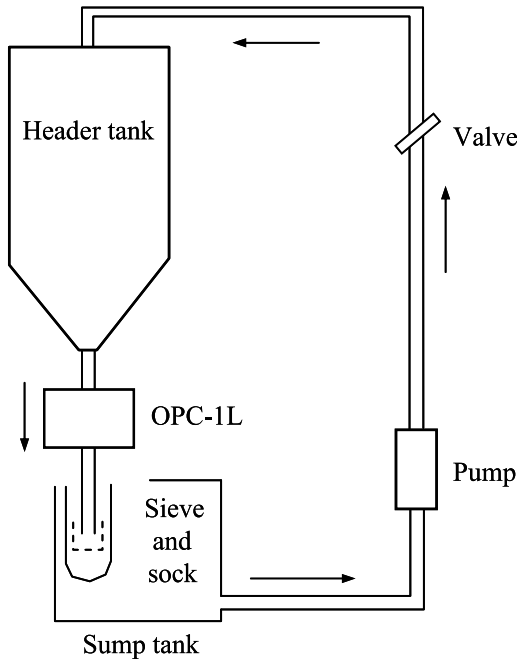

Figure 3. Schematic diagram of the OPC-1L circulation system. The sump and header tanks are polyethylene, and all tubing is PVC with a diameter of 1.9 cm. Arrows indicate the direction of flow.

Table 1. Particles Passing Through 100 μm Mesh and Detected by the OPC-1L Using a 20 L Estuarine Water Sample From Each Site[a]

Site	3 to 5 Dec 2002	7 to 9 Jan 2003	4 to 9 Feb 2003	8 to 10 Mar 2003
		Manning River		
Mn0	0.8 (0.2)	0.8 (0.1)	0.8 (0.3)	3.6 (0.8)
Mn1	11.2 (3.0)	0.9 (0.5)	0.6 (0.1)	7.0 (0.6)
Mn2	6.9 (2.0)	1.5 (0.4)	0.4 (0.2)	7.7 (2.1)
Mn5	18.7 (3.5)	2.3 (0.4)	3.4 (1.0)	4.2 (0.4)
Mn4	2.5 (0.9)	1.8 (0.2)	6.2 (3.4)	1.3 (0.3)
Mn6	0.9 (0.3)	2.6 (0.6)	2.1 (0.7)	0.6 (0.2)
		Wallis Lake Mouth		
W0	1.2 (0.2)	9.0 (2.4)	0.3 (0.1)	0.4 (0.3)
		Wallamba River		
Wl1	3.9 (1.4)	4.9 (0.9)	0.3 (0.1)	1.4 (0.2)
Wl2	5.1 (1.6)	55.2 (6.8)	0.9 (0.4)	20.1 (3.2)
Wl3	0.6 (0.1)	33.0 (6.7)	0.9 (0.3)	2.9 (0.6)
Wl4	2.4 (0.6)	11.1 (2.2)	0.5 (0.1)	3.9 (0.6)
Wl5	2.3 (0.4)	1.2 (0.7)	3.6 (1.2)	3.6 (1.1)
		Wallingat River		
Wg1	57.9 (6.5)	0.5 (0.2)	0.1 (0.1)	0.4 (0.1)
Wg2	3.0 (0.6)	0.3 (0.1)	0.4 (0.2)	0.4 (0.2)
Wg3	0.2 (0.2)	0.8 (0.2)	0.5 (0.1)	0.3 (0.1)
Wg4	1.1 (0.7)	2.9 (0.7)	0.3 (0.1)	0.4 (0.1)
Wg5	0.3 (0.1)	5.8 (2.3)	0.3 (0.1)	0.2 (0.2)

[a]Particles are in number of counts L^{-1}; values in parentheses are mean ± SE with $n = 3$. These counts are caused by coincidence of subresolved particles. Standard error values represent measurement error of the OPC-1L rather than variability in natural concentrations of subresolved particles.

transferred to the header tank and allowed to flow through the OPC-1L at a rate of approximately 25 L min^{-1}. Any counts measured by the OPC-1L are therefore a result of coincidence of subresolved particles. Three measurements were made of each estuarine water sample on the same day of collection.

[14] The OPC-1L was also used to determine the contribution of subresolved particle counts from different size classes by sequential filtration treatments using three different mesh sizes. Estuarine water collected from the surface during February 2003 from sites in the Manning, Wallamba and Wallingat rivers and was analyzed by the OPC-1L. The sample was then separately filtered and analyzed through 264 and 100 μm mesh and finally with a felt sock normally used for aquaculture purposes to remove phytoplankton with an effective pore size of less than 1 μm. Three measurements were made sequentially on the unpreserved unfiltered and filtered water on the same day of collection as above.

2.3. In Situ OPC and Net Zooplankton Counts

[15] Experiments were conducted using the OPC-2T (Focal Technologies, Inc., Dartmouth, Canada) in the Manning, Wallamba and Wallingat rivers at monthly intervals from December 2002 until March 2003. The path length of the light beam is 10 cm and the sampling aperture is 10 × 2 cm. The OPC-2T and digital flowmeter (General Oceanics Model 2031) were mounted to a pole fixed to the transom of a 5 m aluminum vessel with a 60 hp outboard motor, using a modified outboard motor bracket and lowered to 0.7 m depth when in operation. The sampling tunnel of the OPC-2T was ahead and to the left of the propeller and 0.4 m below the draft of the vessel avoiding any flow disturbance. The instrument was attached to the pole at a single pivot point so it presented the tunnel squarely to the flow. At each site one tow ~1 km long was conducted at a speed of ~1.5 m s^{-1}.

[16] Simultaneous net tows at 0.5 m depth were conducted with each OPC-2T tow using a 100 μm mesh net

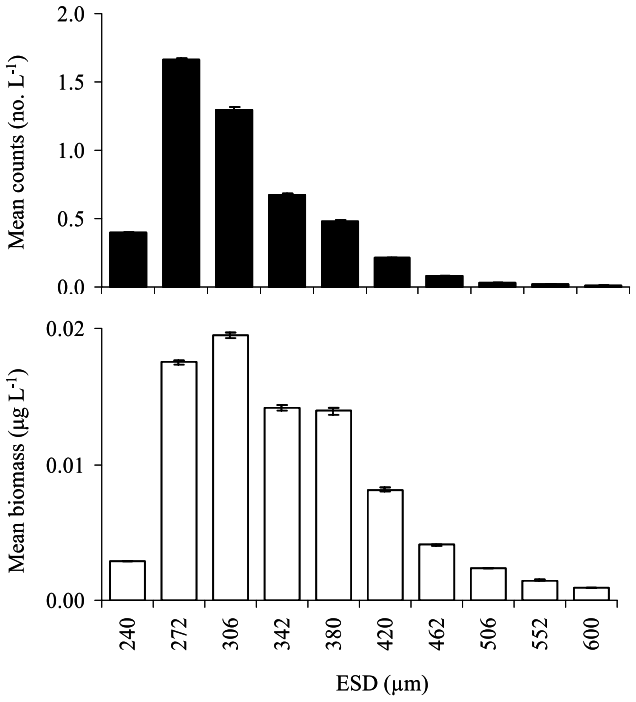

Figure 4. Size frequency and size biomass distributions (mean ± SE) of filtrate from 20 L estuarine water samples passed through 100 μm mesh for all three estuaries. Subresolved particles are those passing through the mesh and detected by the OPC-1L.

Table 2. Two-Factor ANOVA to Assess the Effects of Time and River on the $\log_{10}$ of Subresolved Counts Using the OPC-1L Using the Five Sites Within Each Estuary as Replicates[a]

Source of Variation	MS	df	*F*	*p*
Time	1.3	3	5.0	0.004
River	2.3	2	9.2	<0.001
Time times river	0.6	6	2.3	0.046
Error	0.3	56		

[a]Subresolved counts are the sums of counts L^{-1} over all size classes for 20 L estuarine water passing through 100 μm mesh.

with a 20 cm diameter opening. The length and speed of net tows was therefore the same as for OPC-2T tows, and the average volumes of water sampled by the net and OPC-2T were approximately 30 and 2 m^3, respectively. Ideally, an 80 μm mesh net would be used to avoid the extrusion of ellipsoid particles, such as copepods, that are large enough to be detected by the OPC if presented to the light beam on the longest axis based on a 3:1 length-to-width ratio [*Gallienne and Robins*, 2001; *Hopcroft*, 2001]. Nonetheless, the 100 μm mesh used in this study is an acceptable compromise. Net zooplankton samples were preserved in 5% formalin until processing.

[17] Net zooplankton was rinsed of formalin using a 100 μm mesh sieve and all large detritus particles such as leaves and twigs removed with fine tweezers before being diluted into 1 L of water and slowly added to a 70 L header tank, flowing through the sampling tunnel of the OPC-1L at a rate of 35 L min^{-1} and 5 to 20 counts s^{-1}. At the sump, zooplankton was collected onto a 70 μm mesh sieve positioned inside a felt aquaculture sock. The felt sock also prevented bubbles from the outflow being pumped back into the upper header tank with zooplankton-free water (Figure 3). Large zooplankton samples were split using a Folsom plankton splitter and only 1/2 to 1/4 were processed to reduce the amount of processing time, and counts were multiplied back up before analysis. Zooplankton in samples mostly contained calanoid copepods and large nauplii.

2.4. Data Processing

[18] The digitally counted and sized particles from the OPC-1L and OPC-2T were classified into 24 size classes from 240 to 1482 μm ESD based on the geometric mean of the squares of two consecutive whole numbers from 15 to 39. This classification scheme was used to represent the projected areas measured by the OPC. Concentrations of subresolved particles measured by the OPC-1L are expressed as number per liter (number L^{-1}). Particles measured by the OPC-2T and net zooplankton measured by the OPC-1L are expressed as number per cubic meter (number m^{-3}).

[19] For measurements of subresolved particles, the initial 10 s of counts, equivalent to 4 L of water passing through the OPC-1L, were removed from the processed data to ensure any possible bubbles were flushed through avoiding erroneous counts. A procedural control of reverse osmosis water repeated three times produced zero counts by the OPC-1L after the data were processed using this technique.

[20] Biomass of zooplankton measured by the OPC-2T and from net zooplankton using the OPC-1L was estimated using the method of *Suthers et al.* [2004]. ESD values were converted to biomass assuming the volume of a sphere and the density of water, with densities of zooplankton expressed in mg m^{-3} for each body mass size class. Normalized biomass (m^{-3}) was calculated by dividing the biomass of each mass class by its mass interval or range [*Platt*, 1985; *Platt and Denman*, 1978]. The estimated slopes (regression coefficients) and zero intercepts from ordinary least squares regression of $\log_{10}$-normalized biomass (m^{-3}) against $\log_{10}$ body mass (mg) were used to assess zooplankton productivity and zooplankton total biomass in the Manning, Wallamba and Wallingat rivers.

3. Results and Discussion

3.1. Subresolved Particle Counts and Biomass

[21] Up to 58 subresolved particle counts L^{-1} were detected by the OPC-1L using 20 L of 100 μm mesh filtered estuarine water from each site (Table 1). Zero counts were expected because the ESD of individual particles passing through the mesh were below the detection limit of the OPC. *Zhang et al.* [2000] suggest that some large, flat detrital particles may fold and pass through the filtration process and then unfold to project an area large enough to be detected by the OPC. These large detrital particles may have contributed to the "subresolved" counts determined in

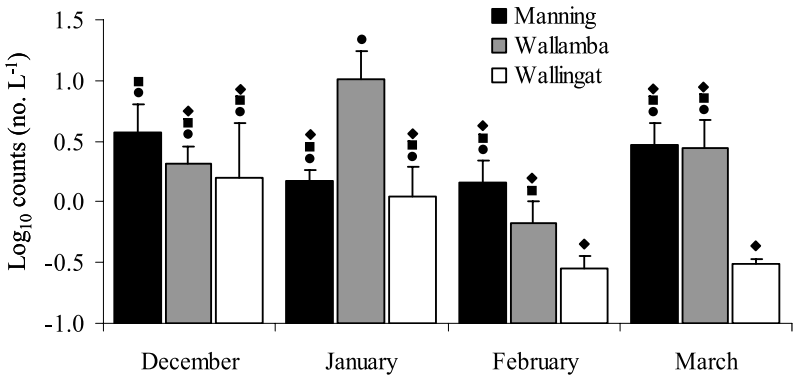

Figure 5. $\log_{10}$ total subresolved particle counts (mean ± SE) detected by the OPC-1L in 100 μm mesh filtered estuarine water samples from the Manning, Wallamba, and Wallingat rivers from December 2002 to March 2003. Columns with different symbols are significantly different.

Table 3. Unfiltered Estuarine Water (20 L) Collected on 4 to 9 February 2003 and for Particles Passing Through 264 and 100 μm Mesh and the Felt Sock, Measured Using the OPC-1L[a]

Site	Unfiltered	264 μm	100 μm	Felt Sock
		Manning River		
Mn0	10.9 (0.7)	2.6 (0.2)	0.8 (0.3)	0.3 (0.1)
Mn1	1.3 (0.1)	1.7 (0.2)	0.6 (0.1)	0.4 (0.2)
Mn2	3.8 (2.2)	5.3 (0.9)	0.4 (0.2)	0.5 (0.1)
Mn5	3.1 (0.7)	2.3 (0.3)	3.4 (1.0)	1.3 (0.2)
Mn4	4.2 (1.4)	2.1 (0.2)	6.2 (3.4)	0.5 (0.1)
Mn6	1.0 (0.1)	1.9 (0.4)	2.1 (0.7)	0.5 (0.1)
		Wallis Lake Mouth		
W0	29.9 (2.6)	1.6 (0.4)	0.3 (0.1)	0.3 (0.1)
		Wallamba River		
Wl1	3.0 (1.0)	1.3 (0.2)	0.3 (0.1)	0.3 (0.1)
Wl2	1.7 (0.4)	1.7 (0.1)	0.9 (0.4)	0.5 (0.3)
Wl3	1.6 (0.4)	0.9 (0.2)	0.9 (0.3)	0.8 (0.2)
Wl4	1.0 (0.5)	1.0 (0.5)	0.5 (0.1)	1.0 (0.5)
Wl5	3.3 (1.1)	3.2 (0.1)	3.6 (1.2)	0.4 (0.1)
		Wallingat River		
Wg1	1.2 (0.4)	0.5 (0.1)	0.1 (0.1)	0.9 (0.2)
Wg2	3.0 (1.2)	1.2 (0.3)	0.4 (0.2)	0.9 (0.1)
Wg3	1.7 (0.5)	1.3 (0.2)	0.5 (0.1)	0.7 (0.4)
Wg4	0.3 (0.1)	0.5 (0.1)	0.3 (0.1)	0.7 (0.1)
Wg5	1.5 (0.7)	1.2 (0.3)	0.3 (0.1)	0.6 (0.1)

[a]Unfiltered estuarine water is in number of counts L^{-1}; values in parentheses are mean ± SE, with $n = 3$. Standard error values represent measurement error of the OPC-1L rather than variability in natural concentrations of subresolved particles.

this study from experiments using filtrate. Further, the filtration process may break apart larger detrital particles into smaller fragments and contribute more subresolved counts. In spite of this, the influence of larger detrital particles folding or breaking apart and contributing to subresolved counts is not expected to be significant because large particles are less abundant than small particles [see *Zhang et al.*, 2000, and references therein].

[22] Most of the coincident subresolved particle counts detected by the OPC-1L occurred in the 272 to 420 μm ESD size classes. These size classes contained 88 and 74% of the subresolved particle abundance and biomass, respectively, when averaged over all samples (Figure 4). The larger size classes above 600 μm ESD contained less of the subresolved particle abundance compared to biomass (i.e., <1% compared to 15%, respectively), as expected from the disproportionate increase in biomass with increasing ESD. The influence of subresolved particles on OPC particle abundance is less than that on OPC particle biomass measurements.

[23] The total subresolved particle counts in all size classes were averaged for each site and compared using two-factor Analysis of Variance (ANOVA) to determine any effects of time and river. Data were $\log_{10}$ transformed to satisfy the assumption of homogeneity of variance prior to analysis. The effect of time and river and their interaction were significant (Table 2). Tukey's pairwise multiple comparisons revealed that subresolved counts were significantly greater by ~20 counts L^{-1} in the Wallamba River in January compared to the Wallamba and Wallingat rivers in February and the Wallingat River in March, and subresolved counts measured in the Manning River in December were significantly greater by ~8 counts L^{-1} compared to the Wallingat River in February and March (Figure 5). Although not consistently significant, water from the forested Wallingat River generally produced less subresolved particle counts compared to water from the disturbed Manning and Wallamba rivers with cleared and livestock farmed catchments.

[24] Subresolved particle counts were detected following each sequential filtration treatment of the 20 L estuarine water through three different mesh sizes (Table 3). Average total subresolved particle counts in all size classes from three replicate measurements using the OPC-1L were between 0.3 and 6.2 L^{-1} for filtrate. Unfiltered water from the entrances to the estuarine systems had the highest number of particle counts of up to ~30 L^{-1}, likely a result of high concentrations of the red tide forming dinoflagellate, *Noctiluca scintillans*, observed at these sites during this study and previous studies [*Dela-Cruz et al.*, 2003]. Factor analysis was used to determine if there were groupings of particle counts from unfiltered water and filtrate. Factor analysis is a more objective method of drawing inferences from a correlation table. Factor loadings were estimated from $\log_{10}$ transformed data and rotated using the orthogonal varimax method so that the four filtration treatments were aligned with either factor 1 or factor 2. Particle counts from unfiltered water loaded highly on factor 1, whereas subresolved particle counts from the 264 and 100 μm mesh filtrate loaded highly on factor 2 (Table 4). The two rotated

Table 4. Rotated Loading Matrix From the Factor Analysis of the $\log_{10}$ of Particle Counts per Liter in Unfiltered 20 L Estuarine Water and Following Filtration Through 264 and 100 μm Mesh and the Felt Sock, Measured Using the OPC-1L

Rotated Loading Matrix	Factor 1	Factor 2
Unfiltered	0.83	0.21
264 μm	0.55	0.74
100 μm	−0.08	0.95
Felt sock	−0.88	0.06
Total variance explained	44%	38%

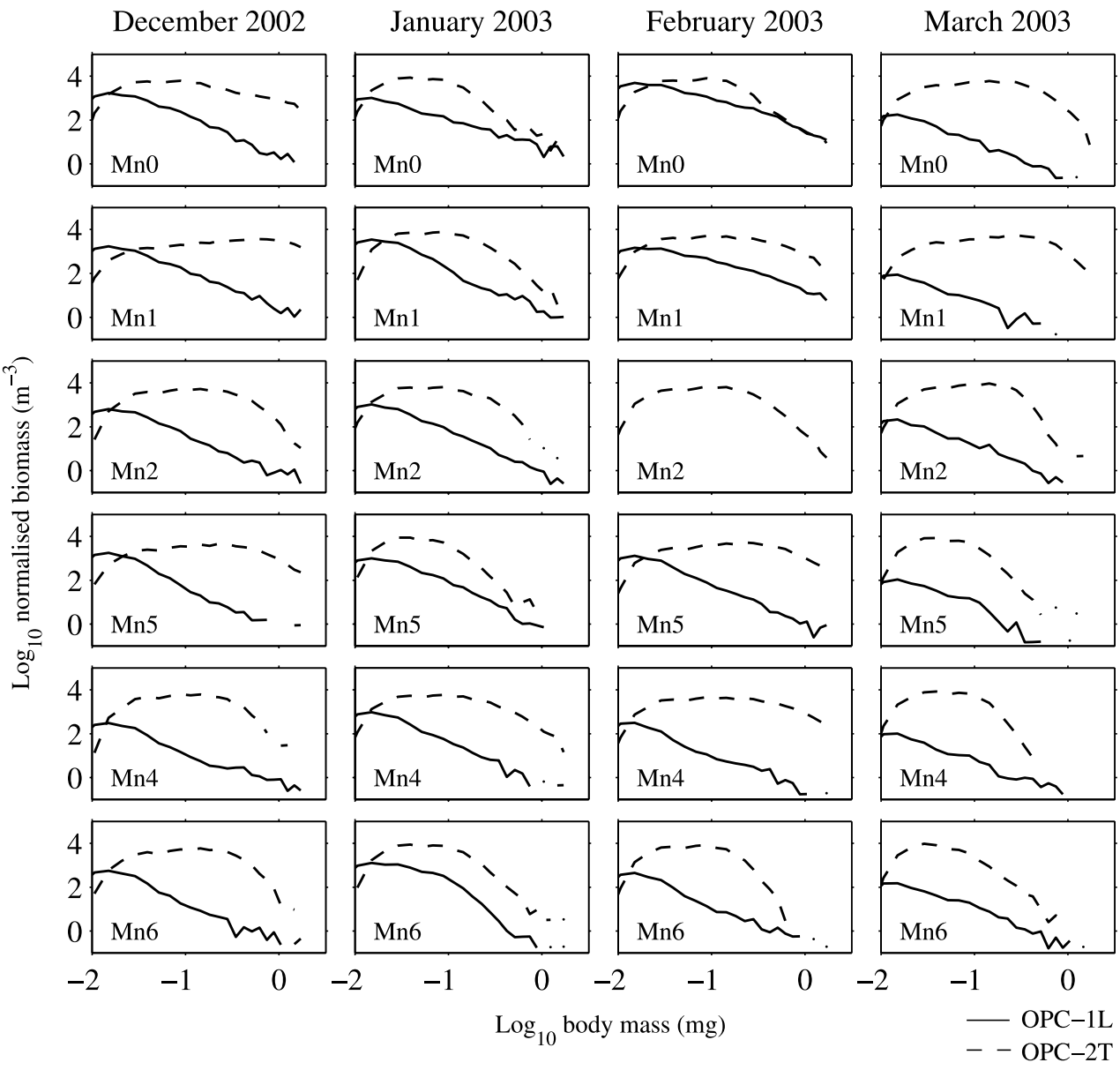

Figure 6. Normalized biomass size spectra determined from in situ OPC-2T measurements and from OPC-1L measurements of simultaneously collected net zooplankton for sites Mn0 to Mn6 in the Manning River from December 2002 to March 2003.

factors together explained 82% of the total variance. Therefore coincident subresolved particle counts detected using the smaller 100 μm mesh filtered estuarine water adequately represent the range of subresolved particles (i.e., <250 μm ESD), because they are closely related to subresolved counts from the 264 μm mesh filtered estuarine water.

[25] In some cases, subresolved counts detected in filtrate using the felt sock were of similar magnitude or greater than for other filtration treatments (Table 3). This is likely due to repeated filtration treatments increasing the breakage of detrital particles such that they are smaller than the effective pore size of the felt sock and in increased abundance, resulting in increased subresolved counts. Therefore, although useful in determining the size characteristics of subresolved particles and the size classes most influenced by their coincidence, subresolved particle counts detected

Table 5. Three-Factor ANOVA to Assess the Effects of OPC Type, Time, and River on the Coefficients of Determination, r^2, From Ordinary Least Squares Regression of $\log_{10}$ Biomass Against $\log_{10}$ Size Class

Source of Variation	MS	df	*F*	*p*
OPC	1.4	1	33.8	<0.001
Time	0.1	3	2.1	0.111
River	0.4	2	9.2	<0.001
OPC times time	0.1	3	2.2	0.094
OPC times river	0.3	2	6.5	0.002
Time times river	0.1	6	0.4	0.852
OPC times time times river	0.1	6	0.4	0.847
Error	0.1	96		

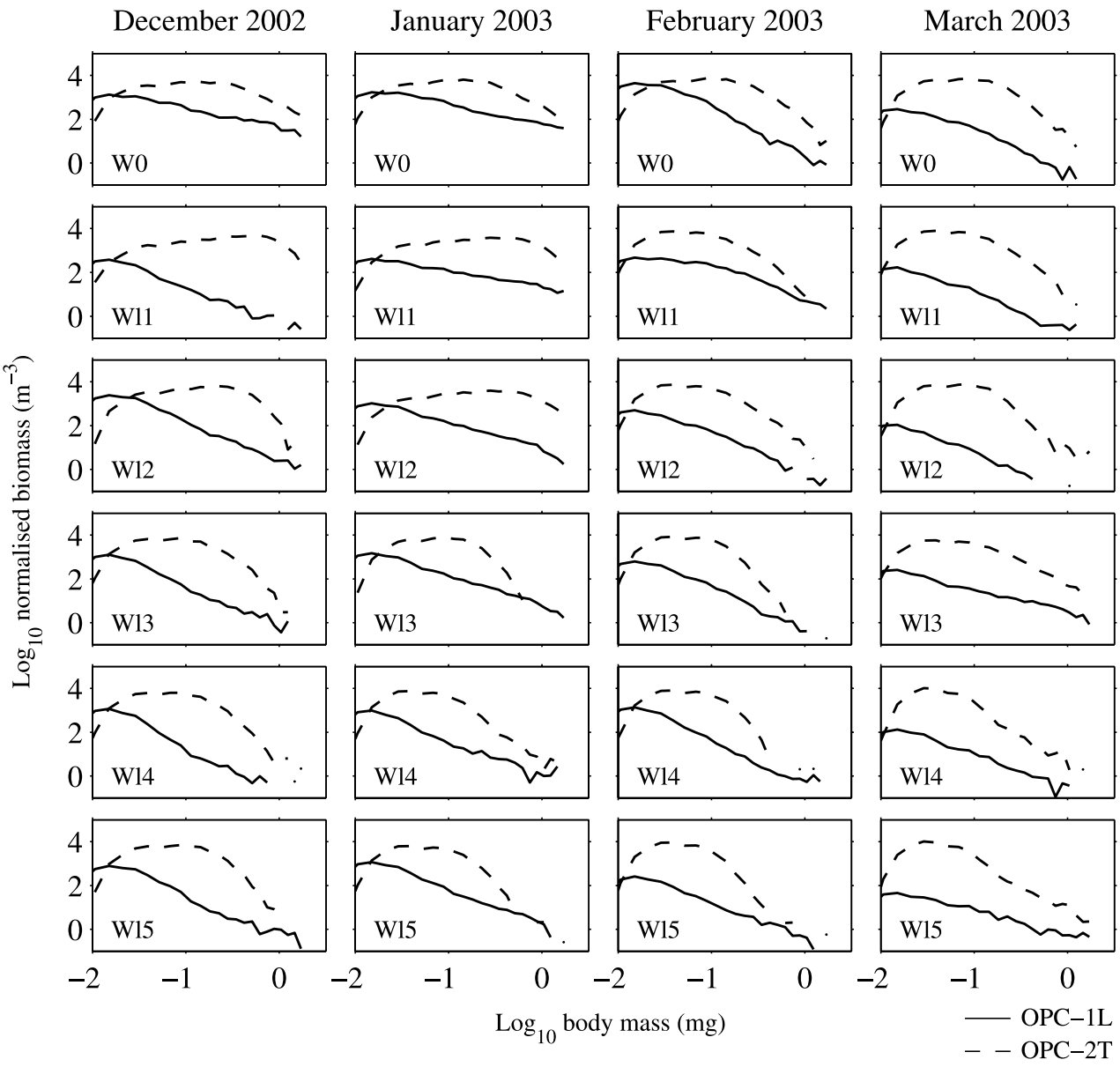

Figure 7. Normalized biomass size spectra determined from in situ OPC-2T measurements and from OPC-1L measurements of simultaneously collected net zooplankton for site W0 at the entrance to Wallis Lake and sites Wl1 to Wl5 in the Wallamba River from December 2002 to March 2003.

by the OPC-1L may also include fragments due to breakup and extrusion during the repeated filtration process despite our best efforts to minimize this. This would alter the particles from their natural state and so OPC-1L subresolved particle counts may differ from those detected in situ by the OPC-2T.

3.2. Influence of Subresolved Particles in Situ

[26] Only body mass size classes between 380 and 992 ESD were included in the regressions because size classes outside this range were inadequately sampled by the gear types used in this study. By inspection of NBSS, the lower 380 μm ESD was a more realistic detection limit for the in situ OPC-2T, and particles >992 μm ESD were too rare in the volumes of water sampled by the in situ OPC-2T or the 100 μm mesh net to provide reliable estimates of biomass. Further, ellipsoid particles <300 μm on the longest axis may not have been adequately retained by the 100 μm mesh net due to extrusion.

[27] The NBSS obtained in situ using the OPC-2T were highly nonlinear for all three estuaries, with greater biomass of particles in midrange and larger size classes compared to NBSS from simultaneously collected net zooplankton using the OPC-1L (Figures 6 to 8). Doming of the NBSS obtained using the OPC-2T and OPC-1L was assessed by comparing the coefficients of determination, r^2, of NBSS from ordinary least squares regression of $\log_{10}$-normalized biomass against $\log_{10}$ body mass using three-factor ANOVA (OPC times time times river). Coefficients of determination of NBSS obtained in situ using the OPC-2T were significantly less than those obtained from net zooplankton using the OPC-1L, but the difference

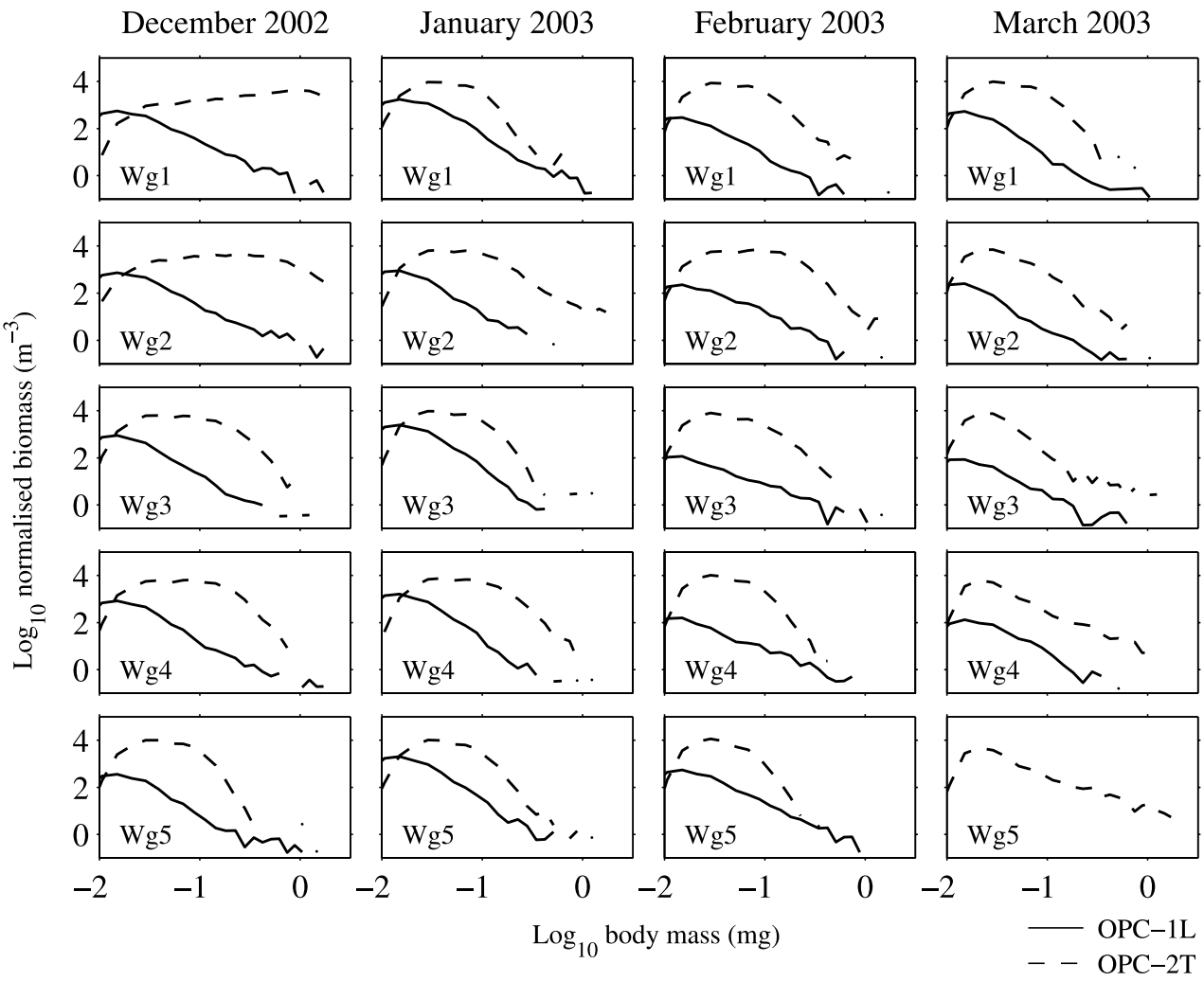

Figure 8. Normalized biomass size spectra determined from in situ OPC-2T measurements and from OPC-1L measurements of simultaneously collected net zooplankton for sites Wg1 to Wg5 in the Wallingat River from December 2002 to March 2003.

in r^2 values was greater for the Manning River compared to the other estuaries producing a significant interaction (Table 5; i.e., Manning River NBSS were the most nonlinear). Coefficients of determination from NBSS obtained in situ using the OPC-2T were less than those from net zooplankton using the OPC-1L by an average of 0.41 for the Manning River, compared to 0.14 and 0.11 for the Wallamba and Wallingat rivers, respectively.

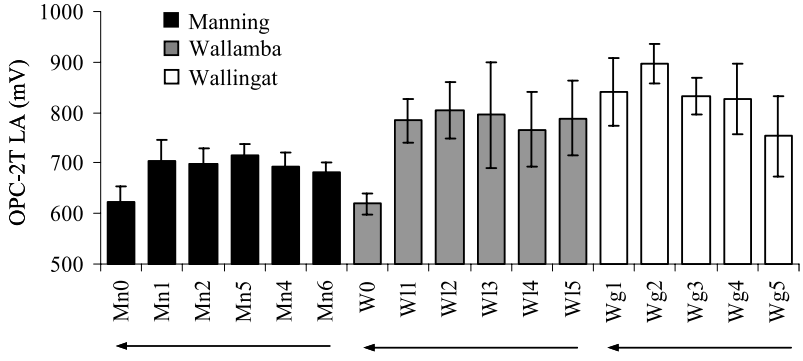

Figure 9. Light attenuance (mean ± SE) measured in situ by the OPC-2T at each site in the Manning, Wallamba, and Wallingat rivers for all sampling months. Note that the relatively undisturbed Wallingat River has the highest light attenuance. This is likely a result of tannins in the water rather than detritus or suspended particulates. Arrows point downstream for each estuary.

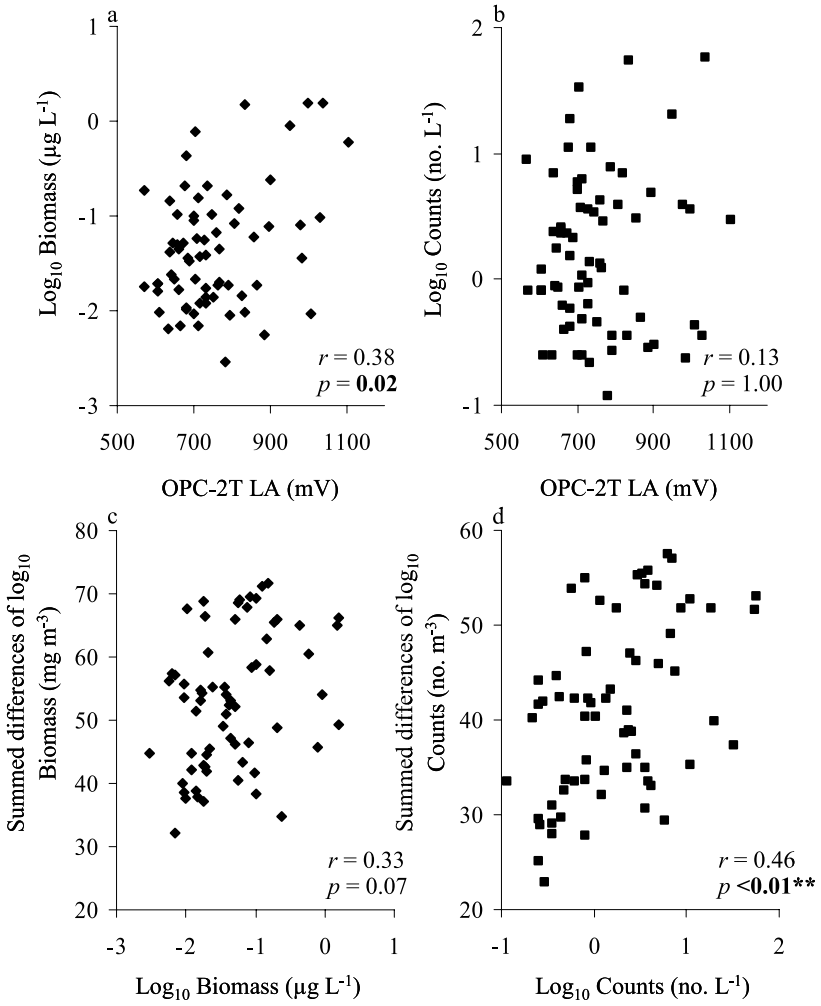

Figure 10. Correlations between light attenuance measured by the OPC-2T and (a) $\log_{10}$ subresolved particle biomass and (b) $\log_{10}$ subresolved particle counts and between (c) the summed differences in $\log_{10}$ particle biomass between OPC-1L and OPC-2T measurements and $\log_{10}$ subresolved particle biomass and (d) the summed differences in $\log_{10}$ particle counts between OPC-1L and OPC-2T measurements and $\log_{10}$ subresolved particle counts.

[28] In spite of their simultaneous collection, some discrepancies between in situ OPC-2T measurements and OPC-1L measurements of net zooplankton are expected due to the different collection methods. Both methods have limitations including coincidence and counting of detritus and phytoplankton aggregates by in situ OPCs, and extrusion of ellipsoid copepods through net mesh and clogging (see *Remsen et al.* [2004] for review). Nets can also damage fragile gelatinous zooplankton, however few such species exist within our samples at this time. Nonetheless, the 100 μm mesh net employed in this study should have produced agreeable net-OPC intercomparisons by adequately retaining ellipsoid copepods with a length-to-width ratio of 3:1 while minimizing clogging [*Gallienne and Robins*, 2001; *Hopcroft*, 2001].

[29] Coincidence of resolved particles is known to become problematic at particle densities of ~10,000 m^{-3} for in situ measurements using the OPC-1T with a path length of 20 cm [*Herman et al.*, 2004]. Use of the mini in situ OPC-2T reduces the likelihood of coincidence by half at this particle density because of its shorter path length (i.e., 10 cm). The maximum concentration of zooplankton in this study, determined from net zooplankton using the OPC-1L, was ~8400 m^{-3}. This is considerably less than the ~20,000 m^{-3} particle density required to produce problematic coincident counts of resolved particles by the OPC-2T. Therefore coincidence of resolved particles could not have produced the large discrepancies between in situ OPC-2T measurements and OPC-1L measurements of net zooplankton here (Figures 6, 7 and 8). It is more likely that coincidence of subresolved particles produced erroneous counts by the in situ OPC-2T, artificially enhancing zooplankton biomass in midrange and larger size classes. Particles <100 μm ESD are not retained by the net, so the OPC-1L measurements of net zooplankton are not confounded by coincident subresolved particle counts. The OPC-1L measurements of net zooplankton therefore best represent actual concentrations of zooplankton compared to in situ OPC-2T measurements.

[30] OPC measurements are susceptible to variation in the light attenuance of water because particles are detected and sized by the amount of light blocked as they pass through

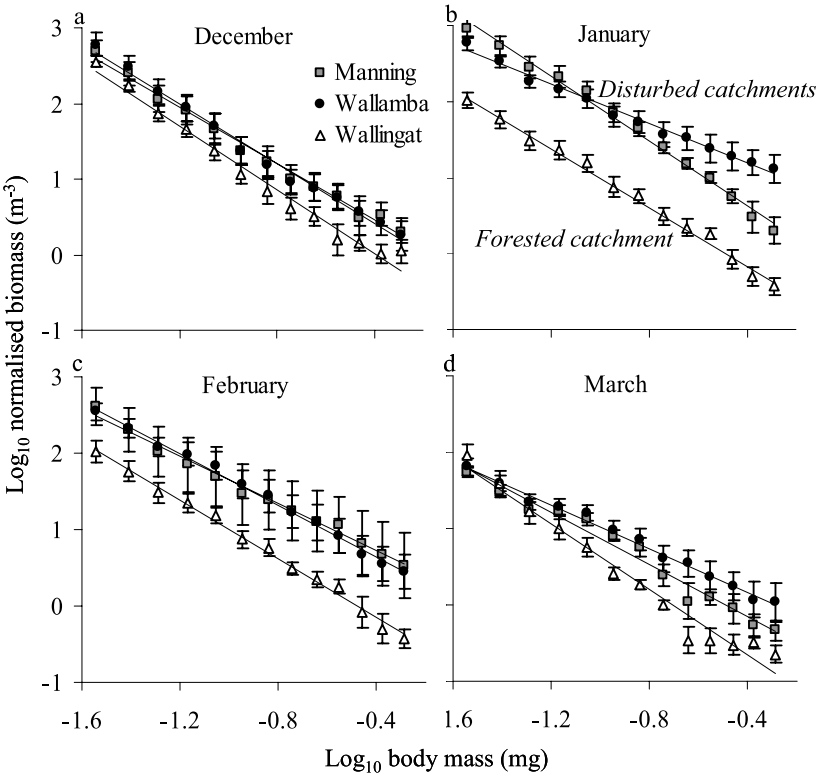

Figure 11. Normalized biomass size spectra (mean±SE) determined from OPC-1L measurements of net zooplankton for the Manning, Wallamba, and Wallingat rivers averaged over all sites in December 2002, January 2003, February 2003, and March 2003 showing greater biomass in the disturbed Manning and Wallamba rivers compared to the forested Wallingat River.

the sampling tunnel. To compensate for this the intensity of light projected by the OPC increases as the light absorbed by the water increases, ensuring that particles of the same size always block the same amount of light. Water absorbs more light when concentrations of subresolved particles are high, therefore light attenuance may indicate the contribution of subresolved particles to total counts or biomass measured by the OPC [*Zhang et al.*, 2000]. In this study, light attenuance measured in situ by the OPC-2T was lowest in the disturbed Manning River and highest in the forested Wallingat River (Figure 9), and yet the Wallingat River had fewer subresolved counts by the OPC-1L of 100 μm mesh filtered water compared to the Manning and Wallamba rivers (Figure 5). This was likely due to higher concentrations of tannins observed in the Wallingat River from its densely vegetated catchment and foreshore rather than higher concentrations of detritus or suspended particulates (S. K. Moore, personal observation, 2005).

[31] *Zhang et al.* [2000] found a significant relationship using light attenuance allowing OPC-1L measurements of zooplankton abundance and biomass to be corrected for background subresolved particles. In this study, a significant relationship was found between in situ light attenuance measured by the OPC-2T and subresolved particle biomass measured by the OPC-1L (Figure 10a; $r = 0.38$, $p = 0.02$, $n = 65$). However, this relationship cannot be used to correct in situ OPC-2T measurements of zooplankton biomass because the longer path length of the in situ OPC-2T increases the likelihood of coincidence of subresolved particles by an unknown factor. For resolved particles this relationship is linear, however it is unknown how subresolved particles overlap with each other and with resolved particles for longer path lengths, thus generating a variety of possible sizes. Therefore the subresolved particle biomass measured by the OPC-1L cannot be simply subtracted from in situ measurements by the

Table 6. Intercepts and Slopes of $\log_{10}$ Normalized Biomass Size Spectra of Net Zooplankton in the Manning, Wallamba, and Wallingat Rivers From December 2002 to March 2003

	NBSS Intercepts				NBSS Slopes			
	Dec	Jan	Feb	Mar	Dec	Jan	Feb	Mar
				Manning River				
Mn1	0.40	0.05	1.47	−0.83	−1.70	−2.14	−1.09	−1.53
Mn2	−0.26	−0.09	–	−0.48	−1.89	−1.89	–	−1.62
Mn5	−0.47	−0.08	−0.46	−0.86	−2.17	−1.95	−1.52	−1.57
Mn4	−0.41	−0.33	0.00	−1.49	−1.57	−1.96	−1.82	−2.23
Mn6	−0.75	−0.68	−0.63	−0.59	−2.02	−2.70	−1.78	−1.62
				Wallamba River				
Wl1	−0.40	1.35	1.17	−0.75	−1.71	−0.72	−1.05	−1.79
Wl2	0.30	0.98	−0.01	−1.07	−1.89	−1.16	−1.65	−1.80
Wl3	−0.29	0.82	−0.36	0.54	−1.95	−1.34	−1.97	−0.95
Wl4	−1.02	0.03	−0.60	−0.58	−2.33	−1.57	−2.22	−1.57
Wl5	−0.54	0.16	−0.40	−0.38	−2.07	−1.67	−1.62	−1.23
				Wallingat River				
Wg1	−0.45	−0.85	−1.42	−1.61	−1.90	−2.58	−2.27	−2.50
Wg2	−0.47	−0.98	−0.95	−1.62	−1.97	−2.19	−2.07	−2.13
Wg3	−1.09	−1.53	−0.78	−1.39	−2.35	−3.06	−1.61	−1.83
Wg4	−1.03	−1.52	−0.82	−1.54	−2.31	−2.82	−1.67	−2.22
Wg5	−1.29	−1.14	−0.72	–	−2.17	−2.62	−2.06	–

Table 7. Two-Factor ANOVA to Assess the Effects of Time and River on the Intercepts and Slopes of Normalized Biomass Size Spectra From OPC-1L Measurements of Net Zooplankton, Using the Five Sites Within Each Estuary as Replicates

Source of Variation	MS	df	*F*	*p*
	Intercept			
Time	1.2	3	6.8	0.001
River	3.8	2	22.5	<0.001
Time times river	0.4	6	2.1	0.065
Error	0.2	48		
	Slope			
Time	0.2	3	3.7	0.018
River	1.0	2	19.0	<0.001
Time times river	0.3	6	5.7	<0.001
Error	0.1	48		

OPC-2T to obtain corrected zooplankton biomass. No relationship was found for in situ light OPC-2T attenuance and OPC-1L subresolved particle counts, since the Wallingat River had the highest in situ light attenuance yet produced the fewest subresolved counts (Figures 9 and 5).

[32] By assuming OPC-1L measurements of net zooplankton best represent actual concentrations of zooplankton, the sum of the differences over all bin sizes in total counts and biomass between OPC-1L measurements of net zooplankton and in situ OPC-2T measurements must be due to coincidence of subresolved particles. Positive correlations were found between $\log_{10}$ OPC-1L subresolved particles (determined from 100 μm filtrate of 20 L samples) and the sum of the differences between $\log_{10}$ of OPC-1L measurements of net zooplankton and in situ OPC-2T measurements for total counts (Figure 10c, d; $r = 0.46$, $p < 0.01$, $n = 65$), but was not significant for total biomass. In situ OPC-2T measurements of total zooplankton abundance could therefore be corrected for erroneous counts of subresolved particles using OPC-1L measurements of 100 μm mesh filtered water samples from the same site, but corrected values would have large error because of the low coefficient of determination of the corresponding regression ($r^2 = 0.22$), and information on the size distribution of zooplankton is lost. The use of the in situ OPC-2T is severely confounded in turbid estuarine water.

3.3. Influence of Catchment Disturbance on Estuarine NBSS

[33] The intercepts and slopes of net zooplankton NBSS obtained using the OPC-1L (Figure 11 and Table 6) were assessed using two-factor ANOVA to determine any effects of time and river on zooplankton biomass and productivity. The effect of time and river significantly affected NBSS intercepts, or total zooplankton biomass (Table 7). Tukey's pairwise multiple comparisons revealed that zooplankton biomass in December was not significantly different to all other months, but was significantly lower in March compared to January and February. The lower biomass at the beginning of March was observed two weeks after 61 mm rain fell in the Manning River catchment from 23 to 25 February, and 70 and 82 mm rain fell in the Wallamba and Wallingat River catchments, respectively, from 21 to 22 February (Bureau of Meteorology). Increased river flow associated with this rain event likely flushed much of the resident zooplankton from the estuaries, which had subsequently not recovered at the time of sampling in March. The effect of river was highly significant, and a comparison of the mean square values from the analysis indicated that river explained the greatest proportion of variance in NBSS intercepts (74%). Tukey's pairwise multiple comparisons revealed lower zooplankton biomass in the forested Wallingat River compared to the disturbed Manning and Wallamba rivers (Figure 11). This was expected due to increased nutrient inputs from the higher proportions of livestock agriculture and residential land use in Manning and Wallamba River catchments supporting a higher biomass of zooplankton.

[34] The effects of time, river and their interaction significantly affected NBSS slopes (Table 7). Steeper slopes, indicating higher productivity of small zooplankton (or predation of larger zooplankton), were generally observed in January for the Manning and Wallingat River, but in the Wallamba River, slopes were flatter in January,

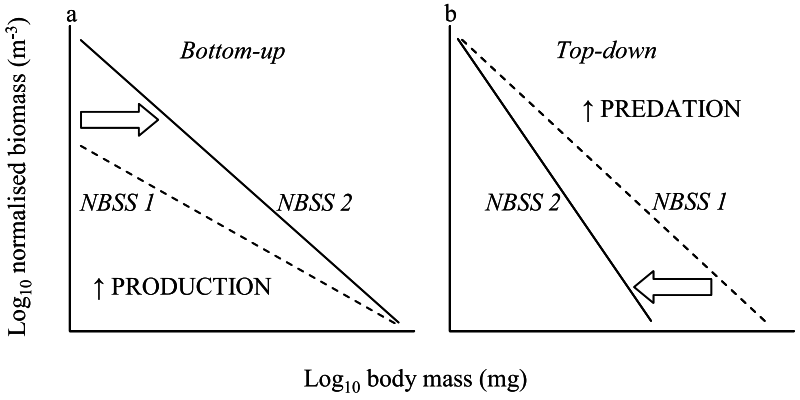

Figure 12. Schematic diagram showing the (a) bottom-up and (b) top-down factors that can influence of zooplankton normalized biomass size spectra (NBSS). Bottom-up factors include nutrient enrichment and increase the productivity of zooplankton in smaller size classes. Top-down factors such as predation by planktivorous fish selectively remove zooplankton in larger size classes. Both of these processes act to increase the slope of the NBSS, seen as the shift from NBSS 1 to NBSS 2.

producing the interaction (Figure 11). The increased productivity in January is likely a result of zooplankton in smaller size classes responding to the increased input of nutrient from a rain event that occurred one month earlier on 11 December 2002 (S. K. Moore and I. M. Suthers, manuscript in preparation, 2006). The effect of river explained the greatest proportion of variance from the analysis (71% of the total mean square) with NBSS slopes indicating comparatively higher productivity in the Wallingat River (Figure 11 and Table 7). However, it was expected that productivity would follow trends in biomass and be greater in the disturbed Manning and Wallamba rivers due to increased nutrient inputs.

[35] The slopes of NBSS can be influenced by both bottom-up and top-down processes. Bottom-up factors such as increased nutrients act to increase the productivity and abundance of zooplankton in smaller size classes producing steeper slopes (Figure 12a), whereas top-down factors such as predation by zooplanktivorous fish will selectively remove zooplankton in larger size classes and also act to steepen slopes (Figure 12b). The abundance of zooplankton in smaller size classes in the forested Wallingat River is generally less compared to the disturbed Manning and Wallamba rivers (Figure 11), so the comparatively steeper slopes of NBSS from the Wallingat River cannot be due to increased production. Therefore predation must be comparatively greater in the forested Wallingat River with the zooplankton community under top-down control. This could be due to either increased abundances or increased predation rates of zooplanktivorous fish in the Wallingat River. Zooplanktivorous fish are primarily visual predators and when light levels are adequate they will select larger organisms for prey [*Brooks and Dodson*, 1965]. In turbid waters, such as those of the disturbed Manning and Wallamba rivers as determined from increased numbers of subresolved counts in 20 L water samples, rates of predation can be greatly reduced [*Utne*, 1997; *Vinyard and O'Brien*, 1976]. However, high concentrations of dissolved organic carbon, such as in the Wallingat River, has also been found to reduce predation on larger zooplankton in brown water lakes [*Wissel et al.*, 2003]. Therefore further research is required to identify the mechanism of top-down control of larger zooplankton in the tannin-rich waters of the forested Wallingat River.

4. Conclusions

[36] This study examines the relationships between subresolved particles, light attenuance and in situ OPC-2T measurements of zooplankton in estuaries for the first time. Expected positive relationships between light attenuance and subresolved particle biomass and concentrations were confounded by tannin-rich waters with low turbidity producing highest in situ light attenuations. Only in situ OPC-2T measurements of total zooplankton concentrations could be corrected for the influence of subresolved particles using OPC-1L measurements of 100 μm mesh filtrate from the same site, but corrected values would have large error. Therefore OPC-1L measurements of net samples is preferable over in situ OPC measurements to establish zooplankton NBSS in estuaries. Net samples, using preferably 80 μm mesh, are improved by the extrusion of subresolved particles yet efficiently retain ellipsoid particles, such as copepods, that are large enough to be detected by the OPC if presented to the light beam on the longest axis [*Gallienne and Robins*, 2001; *Hopcroft*, 2001]. The NBSS of net zooplankton revealed that the disturbed Manning and Wallamba rivers had higher biomasses of zooplankton compared to the forested Wallingat River. The NBSS of zooplankton from the OPC-1L is a useful indicator of nutrient enrichment in estuaries.

[37] **Acknowledgments.** The authors acknowledge the Australian Research Council (ARC) Strategic Partnership with Industry-Research and Training (SPIRT) program (C00107004). Industry partners are Great Lakes Council, Department of Environment and Conservation and the Department of Infrastructure, Planning and Natural Resources. We thank our partner investigators G. Tuckerman, T. Pritchard, and G. Carter. Thanks to R. Piola, J. Dela-Cruz, T. Gaston, K. Wright, R. Moore, and K. Moore for assistance and support at various times during field data collection.

References

Brooks, J. L., and S. I. Dodson (1965), Predation, body size, and composition of plankton, *Science*, *150*, 26–35.

Cyr, H., and M. L. Pace (1993), Allometric theory: Extrapolations from individuals to communities, *Ecology*, *74*, 1234–1245.

Dela-Cruz, J., J. H. Middleton, and I. M. Suthers (2003), Population growth and transport of the red tide dinoflagellate, *Noctiluca scintillans*, in the coastal waters off Sydney Australia, using cell diameter as a tracer, *Limnol. Oceanogr.*, *48*, 656–674.

Edvardsen, A., M. Zhou, K. S. Tande, and Y. Zhu (2002), Zooplankton population dynamics: Measuring in situ growth and mortality rates using an optical plankton counter, *Mar. Ecol. Prog. Ser.*, *227*, 205–219.

Gallienne, C. P., and D. B. Robins (2001), Is *Oithona* the most important copepod in the world's oceans?, *J. Plankton Res.*, *23*, 1421–1432.

Heath, M. (1995), Size spectrum dynamics and the planktonic ecosystem of Loche Linnhe, *J. Mar. Sci.*, *52*, 627–642.

Herman, A. W. (1988), Simultaneous measurements of zooplankton and light attenuance with a new optical plankton counter, *Cont. Shelf Res.*, *8*, 205–221.

Herman, A. W. (1992), Design and calibration of a new optical plankton counter capable of sizing small zooplankton, *Deep Sea Res.*, *39*, 395–415.

Herman, A. W., B. Beanlands, and E. F. Phillips (2004), The next generation of optical plankton counter: The laser-OPC, *J. Plankton Res.*, *26*, 1135–1145.

Hopcroft, R. R. (2001), Comparison of plankton size spectra from net tow samples and OPC measurements in marine waters, in *Global Ocean Ecosystem Dynamics, Optical Plankton Counter Workshop (17–20 June 2001, Tromsø, Norway)*, *GLOBEC Rep. 17*, edited by M. Zhou and K. Tande, pp. 7–11, GLOBEC Int. Proj. Off., Plymouth, U.K. (Available at http://www.pml.ac.uk/globec/products/reports/globecrep.htm)

Huntley, M. E., M. Zhou, and W. Nordhausen (1995), Mesoscale distribution of zooplankton in the California Current in late spring, observed by optical plankton counter, *J. Mar. Res.*, *53*, 647–674.

Huntley, M. E., A. González, Y. Zhu, M. Zhou, and X. Irigoien (2000), Zooplankton dynamics in a mesoscale eddy-jet system off California, *Mar. Ecol. Prog. Ser.*, *201*, 165–178.

Nogueira, E., G. González-Nuevo, A. Bode, M. Varela, X. A. G. Morán, and L. Valdés (2004), Comparison of biomass and size spectra derived from optical plankton counter data and net samples: Application to the assessment of mesoplankton distribution along the Northwest and North Iberian Shelf, *J. Mar. Sci.*, *61*, 508–517.

Platt, T. R. (1985), Structure of the marine ecosystem: Its allometric basis, *Can. Bull. Fish. Aquat. Sci.*, *213*, 55–64.

Platt, T. R., and K. Denman (1978), The structure of pelagic marine ecosystems, *Rapp. Proces Verbaux Reunions Cons. Permanent Int. Explor. Mer*, *173*, 60–65.

Remsen, A., T. L. Hopkins, and S. Samson (2004), What you see is not what you catch: A comparison of concurrently collected net, optical plankton counter (OPC), and Shadowed Image Particle Profiling Evaluation Recorder (SIPPER) data from the northeast Gulf of Mexico, *Deep Sea Res., Ser. I*, *51*, 129–151.

Rissik, D., I. M. Suthers, and C. T. Taggart (1997), Enhanced zooplankton abundance in the lee of an isolated reef in the south Coral Sea: The role of flow disturbance, *J. Plankton Res.*, *19*, 1347–1368.

Rodriguez, J., and M. M. Mullin (1986), Relation between biomass and body weight of plankton in a steady state oceanic ecosystem, *Limnol. Oceanogr.*, *31*, 361–370.

Roman, M., X. Zhang, C. McGilliard, and W. Boicourt (2005), Seasonal and annual variability in the spatial patterns of plankton biomass in Chesapeake Bay, *Limnol. Oceanogr.*, *50*, 408–492.

Sprules, W. G., and M. Munawar (1986), Plankton size spectra in relation to ecosystem productivity, size and perturbation, *Can. J. Fish. Aquat. Sci.*, *43*, 1789–1794.

Sprules, W. G., J. M. Casselman, and B. J. Shuter (1983), Size distribution of pelagic particles in lakes, *Can. J. Fish. Aquat. Sci.*, *40*, 1761–1769.

Sprules, W. G., E. H. Jin, A. W. Herman, and J. D. Stockwell (1998), Calibration of an optical plankton counter for use in fresh water, *Limnol. Oceanogr.*, *43*, 726–733.

Stockwell, J. D., and W. G. Sprules (1995), Spatial and temporal patterns of zooplankton biomass in Lake Erie, *J. Mar. Sci.*, *52*, 557–564.

Suthers, I. M., C. T. Taggart, D. Kelley, J. H. Middleton, and D. Rissik (2004), Entrainment and advection in an island's wake, as revealed by light attenuance, zooplankton, and ichthyoplankton, *Limnol. Oceanogr.*, *49*, 283–296.

Utne, A. C. W. (1997), The effect of turbidity an illumination on the reaction distance and search time of the marine planktivore *Gobiusculus flavescens*, *J. Fish Biol.*, *50*, 926–938.

Vinyard, G. L., and W. J. O'Brien (1976), Effects of light and turbidity on the reactive distance of bluegill (*Lepomis macrochirus*), *J. Fish. Res. Board Can.*, *33*, 2845–2849.

Wissel, B., W. J. Boeing, and C. W. Ramcharan (2003), Effects of water color on predation regimes and zooplankton assemblages in freshwater lakes, *Limnol. Oceanogr.*, *48*, 1965–1976.

Zhang, X., M. Roman, A. Sanford, H. Adolf, C. Lascara, and R. Burgett (2000), Can an optical plankton counter produce reasonable estimates of zooplankton abundance and biovolume in water with high detritus?, *J. Plankton Res.*, *22*, 137–150.

Zhou, M., and M. E. Huntley (1997), Population dynamics theory of plankton based on biomass spectra, *Mar. Ecol. Prog. Ser.*, *159*, 61–73.

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