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Can an optical plankton counter produce reasonable estimates of zooplankton abundance and biovolume in water with high detritus?

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Abstract. The Optical Plankton Counter (OPC) has been used in oceanic and fresh waters to estimate zooplankton abundance and biovolume. However, it is not clear whether the OPC can produce accurate estimates of zooplankton abundance and biovolume in waters with high detritus. In order to test the capability of the OPC to estimate zooplankton abundance and biovolume in Chesapeake Bay, two sets of laboratory experiments were conducted using water with high detritus concentrations collected from the upper Choptank estuary of Chesapeake Bay and laboratory cultured *Artemia*. Our results suggest that the OPC is able to produce accurate estimates of zooplankton biovolume after correcting for the influence of background detritus in all the detritus concentrations used, but accurate estimates of zooplankton abundance only in water with background detritus <100 particles l^{-1}. The relationship between light attenuation and OPC background particle concentrations provides a useful way to estimate OPC background particle concentrations when direct OPC background particle measurements are not available. Light attenuation corrected OPC particle abundance and particle volume gave accurate estimates of zooplankton abundance and biovolume. However, the accuracy of the corrected OPC measurements by the estimated background particle concentrations was not as high as the corrected OPC measurements by the direct background particle measurements.

Introduction

The Optical Plankton Counter (OPC) has been used in oceanic and fresh waters to estimate zooplankton abundance and biovolume on fine temporal and spatial scales (Herman *et al*. 1993; Huntley *et al*., 1995; Stockwell and Sprules 1995; Wieland *et al*., 1997). When the size distributions of different species of zooplankton overlap, OPC measurements may not provide the same detailed zooplankton taxonomic information as traditional techniques. However, the increased temporal and spatial resolution of OPC zooplankton measurements is essential for studying the coupling between physical processes and zooplankton distributions, and for modeling zooplankton population dynamics (Huntley *et al*., 1995; Wieland *et al*., 1997). We have been using the OPC to study the temporal and spatial distribution of zooplankton in Chesapeake Bay.

Estuaries often contain high amounts of organic and inorganic detrital particles (Lenz, 1972). As the OPC cannot distinguish between zooplankton and detrital particles, detrital particles may influence OPC zooplankton abundance and biovolume estimates. Herman suggested that the largest source of bias for OPC estimates of zooplankton abundance and biovolume may be the presence of detrital particles (Herman, 1992). There has apparently been no research done to evaluate systematically the errors of OPC zooplankton abundance and biovolume estimates in waters with high detritus concentration. Understanding the effects of detritus on OPC measurements is essential to ground-truth OPC zooplankton abundance and biovolume estimates in Chesapeake Bay and other coastal waters. In order to evaluate the accuracy of OPC zooplankton abundance and biovolume estimates in Chesapeake Bay, laboratory experiments were conducted using high detritus containing water collected from the upper Choptank estuary of Chesapeake Bay and laboratory cultured *Artemia*. The objectives of this study were to: (i) describe the influence of detritus on OPC zooplankton abundance and biovolume measurements; (ii) compare the accuracy of OPC zooplankton abundance and biovolume measurements at different detritus concentrations; and (iii) explore the possibility of using light attenuation to correct for the influence of detritus on OPC measurements.

Method

Two sets of laboratory experiments to study the relationships between OPC measurements and detritus were conducted using water containing high detritus collected from the upper Choptank estuary of Chesapeake Bay and laboratory cultured *Artemia*. Like most zooplankton, the body shape of *Artemia* can be approximated by an ellipsoid. As *Artemia* are easily cultured and specific age groups are of uniform size, we used *Artemia* to evaluate the ability of the OPC to measure zooplankton abundance and biovolume in our laboratory experiments. Pre-filtered 100 μm mesh high detritus water was mixed with 0.2 μm-filtered water at various percentages to attain a dilution series of 100, 50, 25, 12.5 and 0% of the original water. We refer to the 50 and 100% of the original water as the higher detritus water and to the 0, 12.5 and 25% dilution as the lower detritus water.

Particle abundance, particle volume concentration and light attenuation of these samples, with and without *Artemia* additions, were measured with a laboratory OPC (Model OPC-1L, Focal Technologies, Inc., Dartmouth, Canada). The OPC detects and sizes particles by measuring the amount of light blocked, which is proportional to the projected areas of particles passing through the OPC sampling tunnel (Herman, 1988). To ensure that area measurements are independent of variations in light attenuation of the water (i.e. the amount of light absorbed by the water), the OPC automatically adjusts the intensity of the light source according to the light attenuation (Herman, 1988). A semi-empirical relationship is used to convert the amount of light blocked to the Equivalent Spherical Diameter (ESD) for particles that are larger than 250 μm ESD (Herman, 1992). Therefore, only particles larger than 250 μm ESD can be measured accurately by the OPC. For simplicity, particle volume was calculated using a spherical model with diameter = ESD. The cross-section of the sampling tunnel of the OPC used in our experiments was 2 × 2 cm, and the flow rate of water passing through the OPC sampling tunnel was approximately 10 l min^{-1}. Three measurements on each sample were made and the average value of these measurements was used. In most cases, the coefficient of variation of these three measurements was less than 15%.

Artemia were added at densities of 10, 20, 40 and 80 animals l^{-1}. To test whether the accuracy of OPC zooplankton abundance and biovolume estimates is affected by variations in zooplankton size, two different size groups of *Artemia* were used in the first and second sets of experiments. The average length and width of *Artemia* used in the first set of experiments were 877 and 245 μm, respectively. The average length and width of *Artemia* used in the second set of experiments were 565 and 192 μm, respectively. The amount of *Artemia* biovolume added was estimated from microscopic length and width measurements by assuming an ellipsoid shape for each animal. Since the shape of *Artemia* body is not exactly ellipsoid, there may be some errors associated with the *Artemia* biovolume estimate.

As only particles larger than 250 μm ESD can be measured accurately by the OPC (Herman, 1992), a Coulter Counter (Model Coulter Multisizer II) equipped with a 280 μm aperture orifice tube was also used to measure particle concentrations of the 100 μm mesh pre-filtered water. With this configuration, the range of particle sizes that can be measured accurately by the Coulter Counter is 5 to 168 μm ESD.

An SAS (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA) program 'Multiple Comparisons of Slopes' located in the SAS Sample Library was used to compare simultaneously multiple slopes at various experimental conditions. This program is basically an Analysis of Variance program and employs General Linear Model and Tukey's Studentized Range (HSD) Test to compare multiple slopes. The program controls the type I experiment-wise error and will be referred to as MCS in the following text.

Results

Ideally, there should be no particles counted by the OPC in the 100 μm mesh pre-filtered water because as individual particles, the ESD is below the limit of detection with the OPC. However, this was not the case. OPC particle abundance in the 100 μm mesh pre-filtered water without addition of *Artemia* decreased as ESD increased (Figure 1A). Unlike OPC particle abundance, OPC particle volume concentration was dominated by particles with ESD between 350 and 500 μm (Figure 1B). The water used in the experiments contained high amounts of detritus. When particle abundance is high, many small particles may be counted by the OPC as a single large particle due to coincidence. In addition to coincidence, the geometric shapes and orientations of detritus may also contribute to background OPC measurements. The OPC measures the projected area of a particle through the sampling tunnel. Much of the detritus is presumably flat and may have become folded and passed through the mesh during the filtration process. With certain orientations, some of this flat detritus could produce projected areas large enough to be detected by the OPC.

Zooplankton abundance

The relationships between the total OPC particle abundance and the number of *Artemia* added at the different detritus concentrations from the two sets of experiments are shown in Figure 2. The slopes of the lines from the higher detritus waters were significantly smaller than those from the lower detritus waters

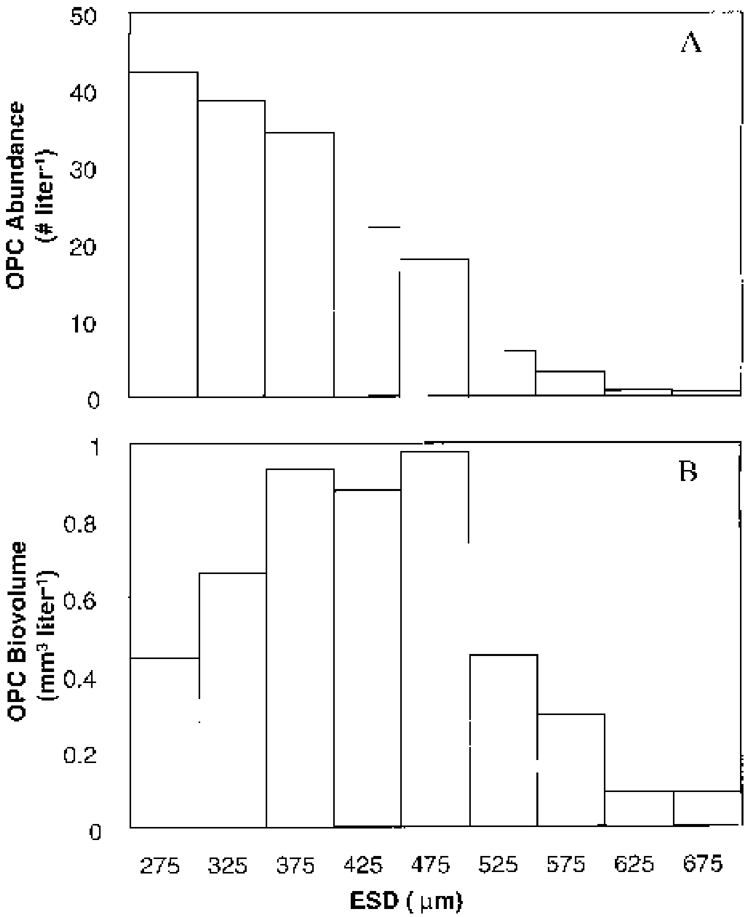

Fig. 1. Size-frequency (**A**) and size-volume (**B**) distributions of 100 µm mesh pre-filtered water measured with a laboratory Optical Plankton Counter (OPC). ESD represents Equivalent Spherical Diameter.

(MCS, $P < 0.05$). The slopes of the two lines from the higher detritus waters were not significantly different (MCS, $P > 0.05$). The slopes of the three lines from the lower detritus waters were not significantly different (MCS, $P > 0.05$). The intercepts of these lines represent the background particle abundance (i.e. particle abundance in various dilutions of the 100 µm mesh pre-filtered original water without addition of *Artemia*). These intercept values were subtracted from the OPC measurements to derive a corrected zooplankton abundance estimate (Figure 3). The intercepts of the regression lines between the corrected OPC particle abundance and the number of *Artemia* added from the lower and higher detritus waters were not significantly different from the expected value of 0 (*t*-test, $P > 0.05$). The slopes of the regression lines between corrected OPC

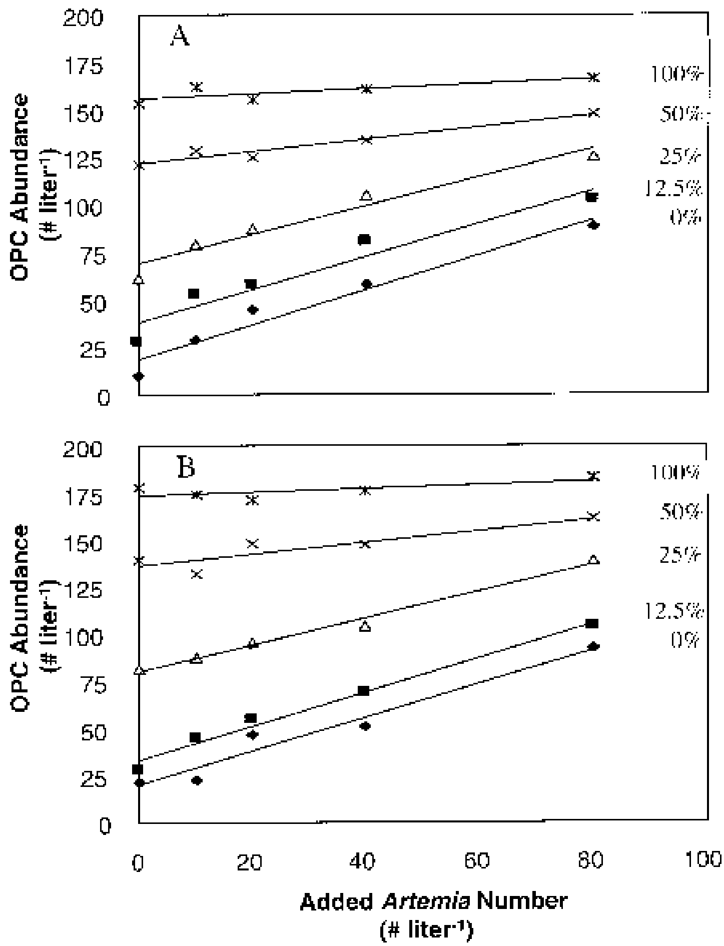

Fig. 2. Relationships between the total OPC particle abundance measurements and the number of *Artemia* added in different mixtures of sea water and filtered sea water. **A** and **B** represent data from the first and the second sets of experiments, respectively. The percent signs represent the percentage of the original sea water in the mixture.

particle abundance and the number of *Artemia* added were 0.84 and 0.21 for the lower and higher detritus waters, respectively (Figure 3). Although the slope from the lower detritus water was higher than that from higher detritus water (t-test, $P < 0.05$), both slopes were less than the expected value of 1 (t-test, $P < 0.05$) (Figure 3).

There was a significant linear relationship between the light attenuation and the OPC background particle abundance (i.e. particle abundance in various dilutions of the 100 μm mesh pre-filtered original water without addition of *Artemia*) in our laboratory studies (t-test, $P < 0.05$; Figure 4). We used this regression to estimate the background particle abundance, which was subtracted from each

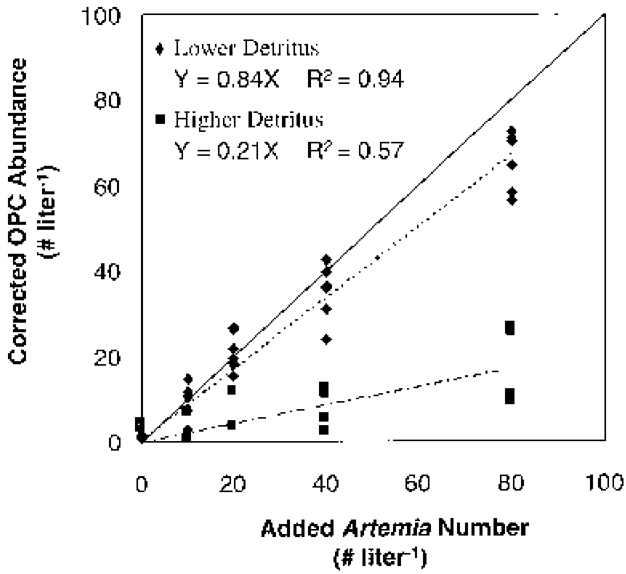

Fig. 3. Relationships between the direct background corrected particle abundance measured by the OPC (by subtracting the corresponding intercept in Figure 2) and the number of *Artemia* added for both sets of experiments. The dotted line represents the regression line between the corrected OPC particle abundance and the number of *Artemia* added in the lower detritus water (0, 12.5 and 25%; diamonds). The dot-and-dash line represents the regression line between the corrected OPC particle abundance and the number of *Artemia* added in the higher detritus water (50 and 100%; squares). The solid line represents the 1:1 relationship.

corresponding OPC measurement. There was not a significant linear relationship between the light attenuation corrected OPC abundance from the higher detritus water and the number of *Artemia* added (t-test, $P > 0.05$; Figure 5). However, there was a significant linear relationship between the light attenuation corrected OPC abundance from the lower detritus water and the number of *Artemia* added (t-test, $P < 0.05$). The intercept and the slope of the regression line are 8.29 and 0.81, respectively (Figure 5). The intercept was significantly larger than the expected value of 0 (t-test, $P < 0.05$), and the slope was significantly less than the expected value of 1 (t-test, $P < 0.05$).

Zooplankton biovolume

We added the same number of *Artemia* for both sets of experiments. However, *Artemia* used in the first set of experiments were larger than those used in the second set of experiments (Figure 6). Unlike the relationship between the total OPC particle abundance and the number of *Artemia* added (Figure 2), the slopes of the biovolume from the different detritus concentrations were not significantly different (MCS, $P > 0.05$; Figure 6). The intercepts of these lines represent the background particle volume (i.e. particle volume in various dilutions of the 100 μm mesh pre-filtered original water without addition of *Artemia*). The contribution of background counts was corrected by subtracting the background particle volume

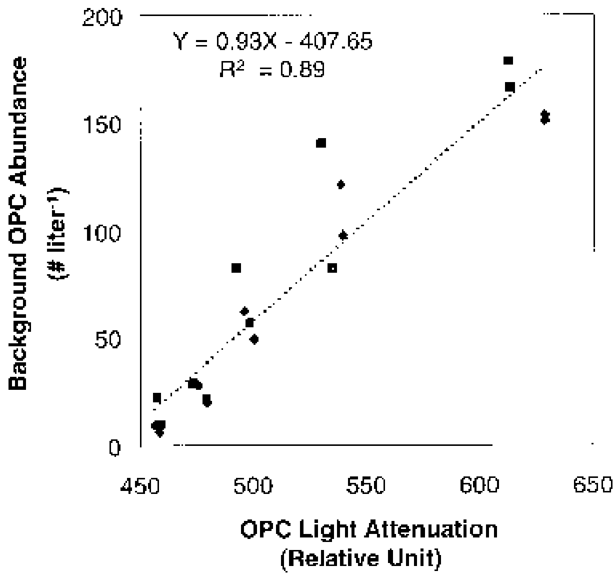

Fig. 4. Relationships between light attenuation and OPC background particle abundance in different mixtures of sea water and filtered sea water. Diamonds and squares represent data from the first and the second sets of experiments, respectively. The unit of light attenuation is relative and is typically set for a reading in air of around 500 ± 200 for the laboratory OPC (FOCAL Technologies, 1998).

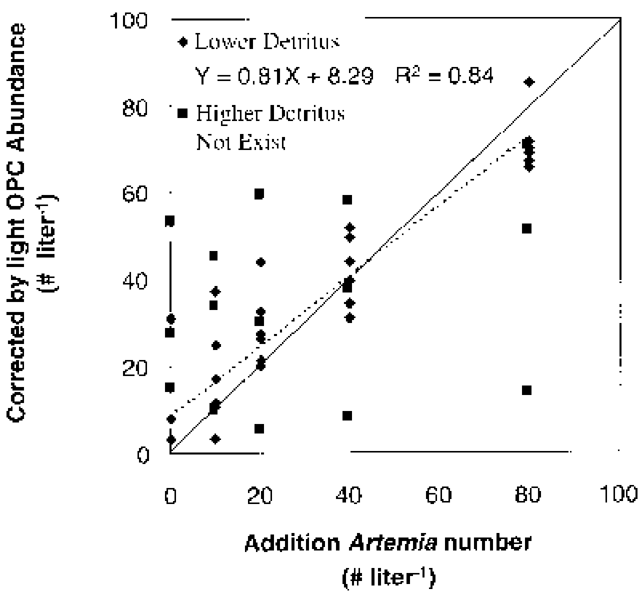

Fig. 5. Relationship between the light attenuation background corrected OPC total particle abundance and the number of *Artemia* added. The dotted line represents the regression line between the light attenuation background corrected OPC total particle abundance and the number of *Artemia* added in the lower detritus water (0, 12.5 and 25%; diamonds). The light attenuation background corrected OPC total particle abundance from the higher detritus water (50 and 100%; squares) and the number of *Artemia* added were not correlated. The solid line represents the 1:1 relationship.

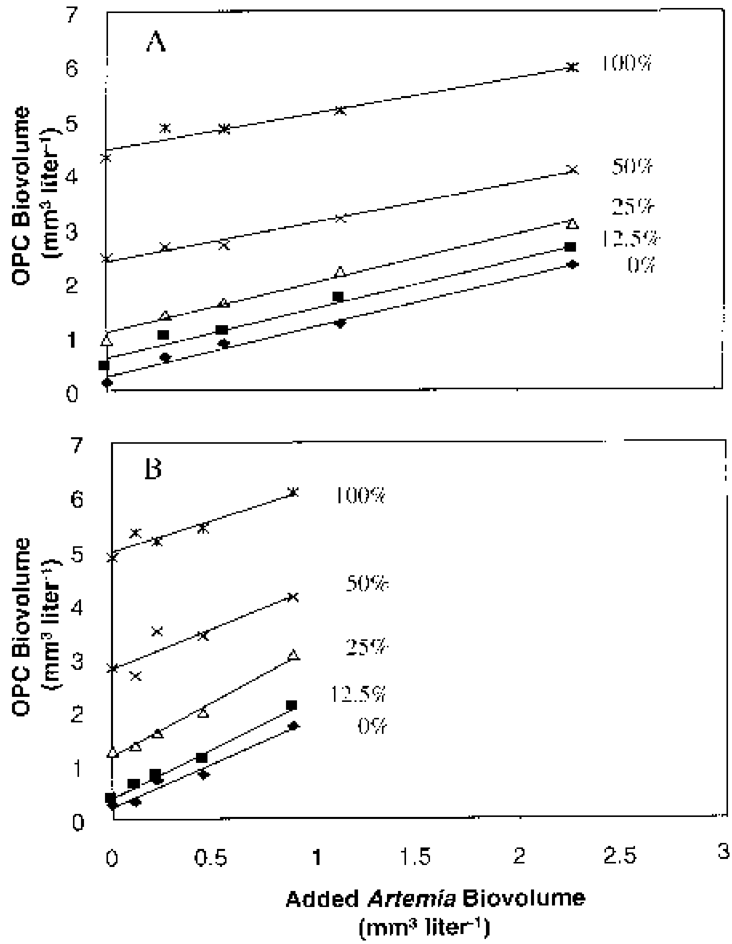

Fig. 6. Relationships between the total particle volume concentration measured by the OPC and the amount of *Artemia* biovolume added in different mixtures of sea water and filtered sea water. **A** and **B** represent data from the first and the second sets of experiments, respectively. The percent signs represent the percentage of the original sea water in the mixture.

from each corresponding OPC measurement. The intercept and the slope of the regression line between the corrected OPC particle volume and the amount of *Artemia* biovolume added were 0.14 and 0.83, respectively (Figure 7). The intercept was significantly larger than the expected value of 0 (t-test, $P < 0.05$), and the slope was significantly less than the expected value of 1 (t-test, $P < 0.05$).

There was a significant linear relationship between light attenuation and OPC background particle volume concentration (i.e. particle volume concentration in various dilutions of the 100 μm mesh pre-filtered original water without addition of *Artemia*) in our laboratory studies (t-test, $P < 0.05$; Figure 8). We used this regression to estimate the background particle volume, which was subtracted

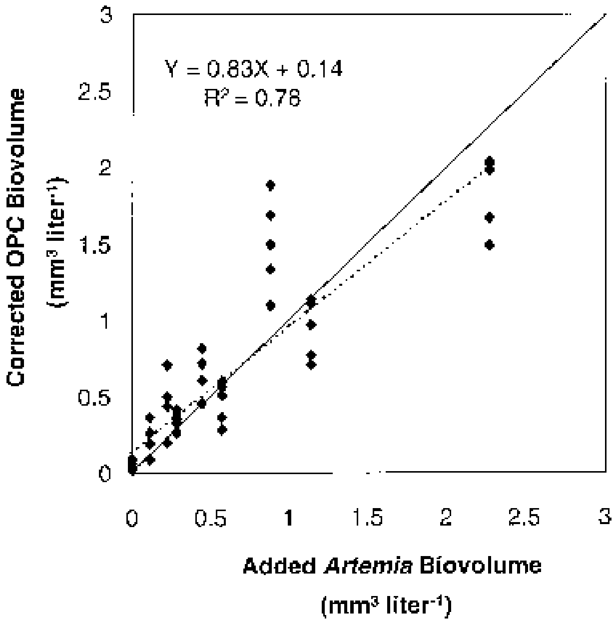

Fig. 7. Relationship between the direct background corrected total particle volume concentration measured by the OPC (by subtracting the corresponding intercept in Figure 6) and the amount of *Artemia* biovolume added for both sets of experiments. The dotted line represents the regression line between the corrected OPC particle volume from the different detritus concentrations and the amount of *Artemia* biovolume added. The solid line represents the 1:1 relationship.

from each corresponding OPC measurement. There was a significant linear relationship between the light attenuation corrected OPC particle volume from the different detritus water and the amount of *Artemia* biovolume added (*t*-test, $P < 0.05$; Figure 9). The intercept and the slope of the regression line were 0.36 and 0.69, respectively. The intercept was significantly larger than the expected value of 0 (*t*-test, $P < 0.05$), and the slope was significantly less than the expected value of 1 (*t*-test, $P < 0.05$).

Discussion

All particles other than *Artemia* with an ESD >250 μm were classified as detritus in this study. The accuracy of the OPC to estimate *Artemia* abundance and biovolume was evaluated by comparing the differences in OPC abundance and volume measurements before and after addition of *Artemia* to 100 μm mesh pre-filtered water. The particle abundance and particle volume in various dilutions of 100 μm mesh pre-filtered water without addition of *Artemia* were treated as the background detritus concentration in our laboratory experiments. However, zooplankton and detrital particles are mixed together in field samples. In order to get the background particle concentration in field samples, zooplankton and detrital

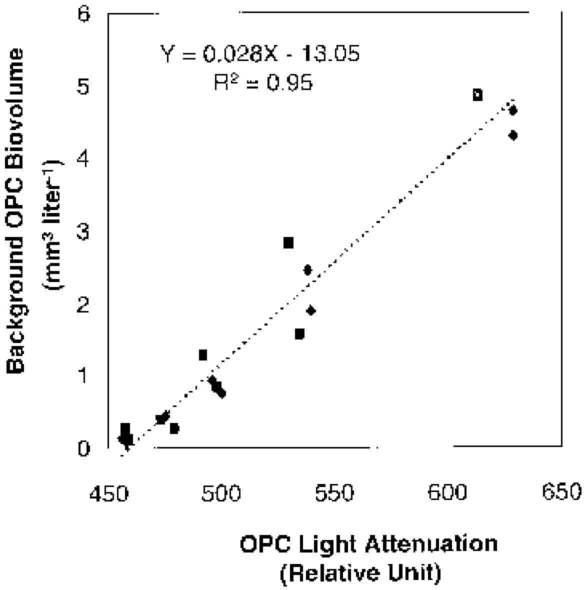

Fig. 8. Relationship between light attenuation and OPC background particle volume concentration in different mixtures of sea water and filtered sea water. Diamonds and squares represent data from the first and the second sets of experiments, respectively. The unit of light attenuation is relative and is typically set for a reading in air of around 500 ± 200 for the laboratory OPC (FOCAL Technologies, 1998).

particles have to be separated before background particle concentrations can be measured. Separation of these two components under a microscope is possible, but very time consuming. Thus, we suggest removal of zooplankton by filtering water through a 100 μm mesh and estimating the OPC background 'detritus' concentration in field studies. However, some experimental errors may be introduced by the filtration. As filtration removes zooplankton as well as large detrital particles, the estimate of OPC background detritus concentration tends to be underestimated. This underestimate should not be significant, since the abundance of large particles is usually lower than small particles in sea water (Sheldon and Parsons, 1967; Sheldon *et al.*, 1972; Lenz, 1972). Additionally, the filtration may break apart some larger detrital particles into smaller particles. Some of these broken small particles may be too small to be detected by the OPC. Therefore, the natural background particle abundance and particle volume may be altered by the filtration. Given the lower abundance of large particles in sea water (Sheldon and Parsons, 1967; Sheldon *et al.*, 1972; Lenz, 1972), we believe that the alternation of natural detrital composition by the filtration is not significant.

In order to test the capability of the OPC to estimate zooplankton abundance and biovolume in water with high detritus, different numbers of *Artemia* were added to the 100 μm mesh pre-filtered water. There were positive linear relationships between the OPC particle abundance and the number of *Artemia* added

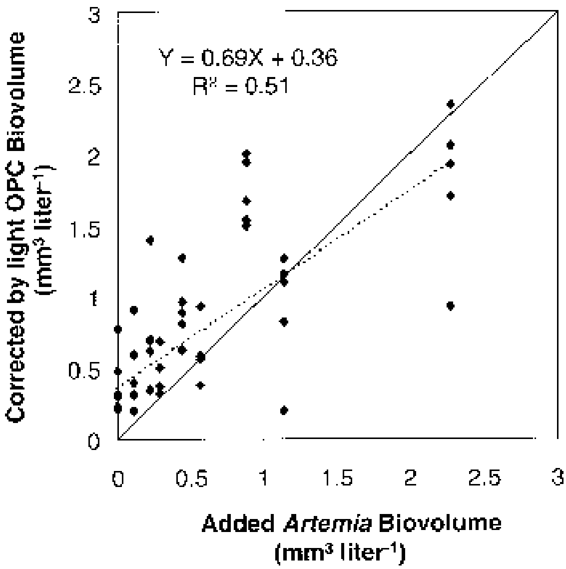

Fig. 9. Relationship between the light attenuation background corrected OPC total particle volume and the amount of *Artemia* biovolume added. The dotted line represents the regression line between the light attenuation background corrected OPC total particle volume and the amount of *Artemia* biovolume added in the different detritus waters. The solid line represents the 1:1 relationship.

(Figure 2). However, the slopes of the lines tended to be lower in the higher detritus waters. These results suggest that the relationships between the total OPC particle abundance and the number of *Artemia* added are affected differentially by the variation in detritus concentrations. Regression lines between the direct background corrected OPC particle abundance and the number of *Artemia* added from the lower and higher detritus waters passed through the origin, but the slopes were less than the expected value of 1 (1 versus 0.84 and 1 versus 0.21) (t-test, $P < 0.05$; Figure 3). Therefore, the OPC may underestimate particle abundance in both the higher and lower detritus water. This underestimate of particle abundance may be due to coincidence counts. For a laboratory OPC, the coincidence error was predicted to be less than 10% when particle abundance was lower than 121 particles l^{-1} (Sprules *et al.*, 1992). The particle abundance measured by the OPC was above 121 particles l^{-1} from the higher detritus waters in our laboratory studies (Figure 2). Our results suggest that the OPC may underestimate particle abundance by only 16% in the lower detritus water, but by as much as 79% in higher detritus water. Therefore, the coincidence counts tend to affect the OPC particle abundance estimate more seriously in the higher detritus water than in the lower detritus water. Our results suggest that the OPC is able to produce accurate estimates of zooplankton abundance in the lower (<100 particles l^{-1}) detritus water after correcting for the influence of detritus. These results are consistent with previously published results (Herman, 1988; Sprules *et al.*, 1998).

Similar to the OPC particle abundance measurements, there were positive linear relationships between the total OPC particle volume and the amount of *Artemia* biovolume added (Figure 6). However, unlike the relationships between the total OPC particle abundance and the number of *Artemia* added, the slopes of the lines from the different detritus concentrations were not significantly different (MCS, $P > 0.05$). These results suggest that the relationships between the total OPC particle volume and the amount of *Artemia* biovolume added are not affected differentially by the variation in detritus concentrations. Unlike the relationships between the direct background corrected OPC particle abundance and the number of *Artemia* added, the slopes of the regression lines between the direct background corrected OPC particle volume and the amount of *Artemia* biovolume added were not significantly different between the higher and lower detritus waters (MCS, $P > 0.05$). Both the intercept and the slope of the regression line between the direct background corrected OPC particle volume and the amount of *Artemia* biovolume added were different from the expected values (0 for the intercept and 1 for the slope; *t*-test, $P < 0.05$; Figure 7). However, the value of the intercept only is equivalent to the biovolume of less than seven *Artemia* l^{-1} and the slope is only 17% less than the expected value. Unlike the particle abundance measurements, the OPC is able to produce reasonable estimates of zooplankton biovolume after correcting for the influence of detritus from the higher and lower detritus waters. These results also suggest that OPC particle volume measurements more accurately represent zooplankton volume than OPC particle abundance measurements estimate zooplankton abundance when detritus concentrations are high (Figures 3 and 7). Note that particle biovolume was calculated using a spherical model with diameter = ESD. However, the spherical model only works well for the spherical particles in biovolume calculation. For non-spherical particles, using the spherical model may introduce some errors. The biovolume of the non-spherical particles may be over- or underestimated depending on the orientations of the particles in the OPC sampling tunnel (Herman, 1992).

In many field studies it will not be possible to measure OPC background particles directly and thus, indirect estimates of background counts are necessary. Light attenuation is mainly affected by dissolved substances and small particles (Yang, 1992; Bricaud *et al.*, 1995). Particle concentrations in the 100 μm mesh pre-filtered water were measured using both the OPC and the Coulter Counter. The range of particle sizes measured by the Coulter Counter was 5 to 168 μm ESD. The particle abundance of the 100 μm mesh pre-filtered water measured by the Coulter Counter was a few orders of magnitude higher than that by the OPC. The particle volume of the 100 μm mesh pre-filtered water measured by the Coulter Counter was a few fold higher than that by the OPC. However, the corresponding measurements between the OPC and the Coulter Counter were highly correlated (Pearson's product-moment correlation, $P < 0.05$). Given this correlation, it was natural to find a linear relationship between light attenuation and OPC background particle abundance and particle volume, respectively (Figures 4 and 8). This relationship provides a useful way of estimating OPC background particle abundance and particle volume.

There was no significant difference between the slopes of the regressions of

Artemia addition and corrected counts by direct and light attenuation background estimates in the lower detritus water (t-test, $P > 0.05$). However, unlike the regression of the direct background corrects, the Y intercept of the light attenuation background estimate regression was significantly larger than the expected value of 0 in the lower detritus water (t-test, $P < 0.05$). The large positive Y intercept resulted in the light attenuation corrected OPC particle abundance tending to overestimate particle abundance when the number of *Artemia* added was small. However, the overestimation for most cases was within a factor of two (Figure 5).

There was no significant difference between the slopes of the regressions between the added *Artemia* biovolume and the direct and light attenuation corrected OPC biovolume (t-test, $P > 0.05$). Although the Y intercepts of both lines were larger than the expected value of 0 (t-test, $P < 0.05$), the Y intercept of the light attenuation corrected regression was larger than that of the direct background corrected regression (t-test, $P < 0.05$). The larger positive Y intercept resulted in the light attenuation corrected OPC particle volume tending to overestimate particle volume when the amount of *Artemia* biovolume added was small. Similar to the light attenuation corrected OPC particle abundance, the overestimation for most cases was within a factor of two (Figure 9).

Although the light attenuation corrected OPC particle abundance and particle volume are able to produce reasonable estimates of zooplankton abundance and biovolume, the accuracy of the light attenuation corrected OPC measurements was not as high as the direct background corrected OPC measurements. Therefore, the relationship between light attenuation and OPC background particle concentrations should be used when direct background particle measurements are not available. The relationship between light attenuation and OPC background particle concentrations is complex. As well as light attenuation, other characteristics of water samples (such as composition and size distribution of <100 μm particles) may need to be analyzed to estimate OPC background particle concentrations accurately. However, given that more than 89% of the OPC background particle concentration can be explained by light attenuation in our laboratory studies (Figures 4 and 8), the relationship between light attenuation and OPC background particle concentrations is fairly robust. We derived the relationship between light attenuation and OPC background particle concentrations from water collected from the upper Choptank estuary of Chesapeake Bay. We do not know whether this relationship can be generalized to the entire Chesapeake Bay or to other coastal waters. Therefore, researchers that want to use this approach to correct for the influence of background detritus on OPC estimates of zooplankton abundance and biovolume should conduct background measurements in their areas of study.

In summary, our results suggest that the OPC is able to produce accurate estimates of zooplankton biovolume after correcting for the influence of background detritus in the different detritus waters. However, the OPC is only able to produce reasonable estimates of zooplankton abundance after correcting for the influence of background detritus in the lower (<100 particles l^{-1}) detritus waters. Direct OPC background particle measurements provide more accurate OPC adjustments than indirect background particle volume estimates based on light attenuation. If the relationship between light attenuation and OPC background particle concentrations can be characterized for each water mass, this relationship will provide a useful way of estimating OPC background particle concentrations when direct OPC background particle measurements are not available.

Acknowledgements

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