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Abstract

Two recently developed instruments, the Laser Optical Plankton Counter (LOPC) and the Zooscan, have been applied to study zooplankton biomass size spectra in tropical and subtropical marine ecosystems off Brazil. Both technologies rely on optical measurements of particles and may potentially be used in zooplankton monitoring programs. Vertical profiles of the LOPC installed in a 200 µm ring net have been obtained from diverse environmental settings ranging from turbid and nearshore waters to oligotrophic open ocean conditions. Net samples were analyzed on the Zooscan and counted under a microscope. Particle biovolume in the study area estimated with the LOPC correlated with plankton displacement volume from the net samples, but there was no significant relationship between total areal zooplankton biomass determined with LOPC and the Zooscan. Apparently, normalized biomass size spectra (NBSS) of LOPC and Zooscan overlapped for particles in the size range of 500 to 1500 µm in equivalent spherical diameter (ESD), especially at open ocean stations. However, the distribution of particles into five size classes was statistically different between both instruments at 24 of 28 stations. The disparities arise from unequal flow estimates, from different sampling efficiencies of LOPC tunnel and net for large and small particles, and possibly from the interference of non-zooplankton material in the LOPC signal. Ecosystem properties and technical differences therefore limit the direct comparability of the NBSS slopes obtained with both instruments during this study, and their results should be regarded as complementary.

Introduction

Research in zooplankton ecology is undergoing rapid change in recent years due to the development of new technologies for in situ measurements and sample analysis. Optical instruments, such as particle counters or imaging systems, now automatically sense and measure plankton distribution in the water column. These methods achieve higher resolution than net sampling, and they considerably increase processing speed of collected plankton samples (Benfield et al. 2007). The Laser Optical Plankton Counter (LOPC) provides real-time information on the size and abundance of particles by measuring the cross-sectional area of particles passing through its laser beam (Herman et al. 2004). It minimizes the problem of coincidence counting associated with the former Optical Plankton Counter (OPC) at high concentrations of small particles (Herman 1988; Herman 1992; Sprules et al. 1998). Compared with sophisticated in situ imaging systems (Gorsky et al. 2000; Davis et al. 2004; Remsen et al. 2004), which allow visual discrimination of plankton and marine snow, the LOPC may represent a robust alternative for zooplankton ecologists. Yet, it needs to be calibrated for the presence of fragile particles. In combination with conventional zooplankton net tows, the predecessor OPC was successfully applied to investigate zooplankton biomass distribution and size composition in a variety of coastal and oceanic ecosystems such as the Chesapeake Bay (Roman et al. 2005; Zhang et al. 2006), California current

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(Huntley et al. 1995), Southern Ocean (Pollard et al. 2002), and along latitudinal transects across the Atlantic (Woodd-Walker et al. 2001). Developed as recently as the LOPC, the Zooscan allows rapid estimation of biomass, size distribution and taxonomic classification of the zooplankton community (Grosjean et al. 2004). A scan of the collected plankton sample and subsequent image analysis provide a permanent archive of the sample, considerably reduces the time of sample processing, and avoids sample destruction (Grosjean et al. 2004; Hernández-León and Montero 2006).

Both the LOPC and the Zooscan generate the so-called biomass size spectra of zooplankton communities. These metrics have emerged as valuable indices for the analysis of population dynamics and ecosystem production in aquatic environments (Zhou and Huntley 1997; Vidondo et al. 1997; Kerr and Dickie 2001). They have also been successfully applied to the calibration of optical in situ determination of zooplankton distribution in the field (Herman and Harvey 2006), especially when the presence of non-zooplankton particles, marine snow, or other detritus interfere with the optical signal in turbid waters (González-Quirós and Checkley 2006).

This study, as part of the Brazilian PROABROLHOS project and the international oceanographic monitoring program ANTARES (www.antares.ws), provides an intercomparison of the LOPC and the Zooscan as emerging tools to evaluate zooplankton distribution and biomass dynamics in relation to the physical and biological environment. Tropical and subtropical mesozooplankton populations off Brazil are highly diverse (Valentin and Monteiro-Ribas 1993; Lopes et al. 2006) and usually dominated by small-sized (<1 mm) copepods and meroplankton. The additional presence of gelatinous zooplankton, as well as large microcrustaceans and ichthyoplankton in the upper size range detected by the LOPC, make the study sites ideal settings for a combined evaluation of LOPC measurements and Zooscan sample analyses. We evaluated how reliably one can estimate zooplankton biomass and size spectra in varying environmental settings using these optical instruments and whether Zooscan and LOPC yielded comparable results.

Materials and procedures

LOPC set-up and deployment—The LOPC is commercially available through ODIM Brooke Ocean (http://www.brooke-ocean.com/lopc.html) and is supplied with a deck unit and Windows-based software. As suggested by Herman et al. (2004), the LOPC was installed inside a conic-cylindrical ring net (diameter 60 cm) fitted with either 80 µm or 200 µm mesh to collect the zooplankton susceptible to have passed in the LOPC tunnel during the tow. The instrument was mounted on a T-frame, as described in the LOPC user manual, with an interoperable Micro-CTD (AML Microsystems) and a flowmeter (General Oceanics; Mechanical Digital Flowmeter Model 2030). The flowmeter was calibrated with vertical tows over a known distance (3 m) in the pool of the university sports club. The mean distance traveled per number of revolutions was calculated from six replicate tows. A pre-cruise calibration check of the LOPC under dry conditions in the laboratory with three sizes of beads provided by the manufacturer indicated optimal functioning of the system.

The LOPC was deployed at 56 stations to a maximum depth of 200 m on the Eastern coast of Brazil during the PROABROLHOS winter expedition (Fig. 1). At the first 13 stations (75 to 66), an 80 µm net was used. The mesh size was changed to 200 µm from station 65 onwards because of high accumulation of phytoplankton aggregates in the zooplankton sample collected with the finer mesh size. Average tow speed was 0.77 m s^{-1} (std 0.13). In addition, the LOPC has been deployed at monthly intervals on the ANTARES station off Ubatuba, on the southern coast of Brazil (23°44′S; 45°00′W). Particle abundance was recorded from a depth of 38 m (2 m above the ground) to the surface at an average tow speed of 0.41 m s^{-1} (std 0.08). In all cases, the net samples were recovered immediately and fixed in buffered formaldehyde (4% final concentration) for subsequent scanning and microscopic analysis.

LOPC data recording and processing—LOPC and CTD provide real-time records of data acquisition during the deployment, which are graphically displayed for immediate interpretation. The raw data files written by the LOPC software yield information on the abundance and size of particles passing through the LOPC tunnel, and an estimate of flow speed. Temperature, conductivity, and depth information recorded by the AML Micro-CTD are also included. A playback mode allows further visualization of LOPC data as time or depth charts, for example to check the upper cut-off (see below) and whether the tow showed major singularities.

Raw files were inspected on a text editor to determine the exact range of sample counts for which the raw data were extracted. The range was chosen in order to obtain the complete vertical profile starting from the sample count at greatest depth up to the sample count when the instrument first reaches 1.5 m depth. This upper cut-off minimizes integration of false LOPC counts due to wave action and bubble formation near the surface. Only data from the upcast was integrated into the profile calculation since the net impedes a constant flow of water through the LOPC tunnel on the downcast.

Based on the exact sample counts, a postprocessing routine (*LOPC_PostPro*, available at www.alexherman.com) was used to extract data with a vertical resolution of 1 m. *LOPC_PostPro* configurations were generally set to "sample-time-count" processing, which takes into account CTD data recorded in parallel, to an ellipsoid axis ratio of 3:1, assuming that most zooplankton passing the tunnel have the shape of an prolate spheroid. Both the ellipsoid axis ratio and the assumed biomass density (see below) are oversimplifications considering the diversity of the sampled zooplankton communities. This simplification is imposed by the limited data processing options available at the present time, in combination with large amounts of data needing to be assimilated.

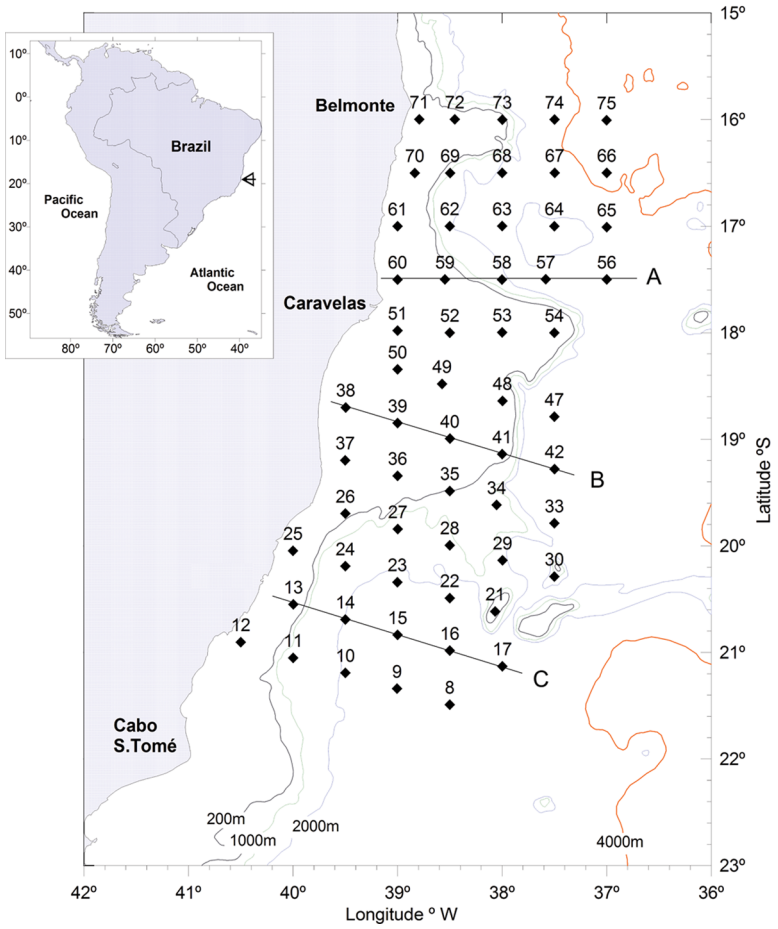

Fig. 1. Map of the sampling grid in the Abrolhos ecosystem.

Biomass estimates of mesozooplankton given by *LOPC_PostPro* are expressed in wet weight and are derived from particle volume assuming a density of 1 mg mm^{-3} (Herman and Harvey 2006). Areal biomass estimates (LOPC-AB) provided by the LOPC post processing program are calculated as

$$A = LOPC - BB/V * z \quad (1)$$

where V is the water flow (m^{-3}) through the 7 × 7 cm LOPC tunnel, and z is the tow depth (m) recorded by the pressure sensor of the AML Micro-CTD.

We also employed a data filter to generate individual count and biomass information for ESD size classes 100-250, 251-350, 351-500, 501-1000, 1001-2000, and larger than 2000 µm.

Zooscan set-up—The Zooscan system employed in this study is a Biotom model controlled by its associated software *Zooprocess*, a plug-in for the image analysis software *ImageJ* (http://rsb.info.nih.gov/ij/). Continuous functionality of the plankton scanner and inter-comparability of data with other users requires substantial technical maintenance. The system was first calibrated for a scanning resolution of 2400 dpi, and software upgrading performed at nearly monthly intervals via download from the Zooscan web site (www.zooscan.com).

Sample scanning and image analysis—Half of the zooplankton samples collected during the LOPC tows of the PROABROLHOS winter cruise were scanned with the Zooscan system. Only samples from the 200 µm net were used. A fraction of the plankton sample, usually between 1/32 and 1/64, was obtained with a Motoda splitter, and the aliquot was poured onto the scanner previously filled with de-ionized water. Then, approximately 15 min per sample were invested to separate individual zooplankton on the scanning cell. Our experience has shown that manual separation of organisms directly on the scanning cell is more practical and permanently retained in the archived image than when using the particle separation tool provided by *Zooprocess*. Finally, the sample was scanned into a 2400 dpi, 16 bit digital image. At frequent intervals, an image containing only de-ionized water was obtained to correct for the gray level contained in the background. For processing, the image was converted from a 16-bit to an 8-bit image, and cut into two separate images, which were subsequently processed and analyzed independently. Both the conversion and split were imposed by software limitations at the time of our study, but the initial high quality images are archived for future analyses with improved

tools. Details on the image analysis with *Zooprocess* are presented by Grosjean et al. (2004).

The size distribution and biomass of the digitalized plankton sample were estimated from the "AREA" measurement provided for each recognized object in the so-called "PID-file." Upon conversion from pixel size to micrometers (ratio of 1.0 pixel : 10.56 µm), the particle area was equated to the area of a circle, which permits calculation of the ESD for each object. Biovolume, however, was derived by equating the particle area to the area of an ellipse with a 3:1 axis ratio, deducting the semimajor axis A, and calculating the particle volume assuming the shape of a prolate spheroid with an axis ratio of 3:1. This approach was chosen to assure comparability with LOPC data.

Conventional net sample analysis—Displacement volume. For the determination of total plankton displacement volume (TPV) each of the 56 net samples (80 µm and 200 µm net) was concentrated on a 65 µm mesh, washed, and suspended in a known amount of distilled water (V1). In a second step, the zooplankton was again collected on the 65 µm sieve, and the remaining amount of distilled water (V2) measured to 0.5 mL precision in a graduated cylinder. TPV was calculated as the difference between V1 and V2.

Microscopic analysis—For the microscopic counting, 15 samples were chosen, covering three coastal-ocean transects for which LOPC and Zooscan estimates were available: transect A along the northern edge of Abrolhos Bank (Stations 60 to 56), transect B over Abrolhos Bank (Stations 38 to 42), and transect C in oligotrophic waters south of the bank (Stations 13 to 17; Fig. 1). Each sample was split to a fraction of approximately 300 organisms, most often 1/64 to 1/128 of the total, and usually one split higher than the fraction scanned on the Zooscan. On average, 366 ± 71 organisms were analyzed in each of the 15 samples. For every organism, two length measurements were made on a digitizing table using the Zoopbiom software (Roff and Hopcroft 1986): one measurement along the major axis (L1) following the method of Uye (1982), and a second measurement along the minor axis (L2) approximately at the center of the animal. Volume was calculated assuming the form of a prolate spheroid with axis ratio of 3:1, considering L1 and L2 as major and minor axes. For the simplicity of comparison among methods, no attempt was made to convert volume to biomass with standard factors based on taxonomy, since the LOPC estimate does not provide taxonomic detail.

Flow calculations—The LOPC measures flow through the tunnel from the average time a particle takes to pass through the laser beam (Herman et al. 2004). A volume estimate (V_{LOPC}) is provided in the processed data file.

Flow through the 60 cm ring net was estimated in two different ways: the surface area of the net (0.28 m^2) was multiplied (i) by the tow depth as recorded by the Micro-CTD, and (ii) by the distance estimated from the flowmeter installed in the net mouth. These estimates are denoted V_{calc} and V_{flow}, respectively.

Calculation of normalized biomass spectra—Normalized biomass spectra (NBSS, Kerr and Dickie 2001) have been calculated for the results of 28 stations at which LOPC and Zooscan data were available, in addition to 15 spectra derived from microscopic measurements. Calculations closely followed the method of Herman and Harvey (2006). For the LOPC, log normalized biomass (µg m^{-3} $\Delta\mu g^{-1}$) estimated in 55 size bins (90–5678 µm ESD) are provided in the output of the postprocessing software. In the calculations based on Zooscan and microscopy results, the ESD of each organism was used to sum biomass into the size bins used by the LOPC postprocessing software. Finally, log normalized biomass concentrations in each bin were adjusted for the different fractions analyzed by the LOPC, Zooscan, and microscope.

Assessment

In situ LOPC profiling—Between July 2007 and September 2008, the LOPC has been deployed for vertical profiling in various environmental settings. During the PROABROLHOS winter cruise, 56 stations of 58 planned stations could be sampled both over the continental shelf and in deep oceanic waters. Only two samplings were lost due to rough weather conditions and minor technical problems with the LOPC, respectively. Four raw data files, all recorded in turbid nearshore areas (Station 61, 51, 37, 12) could not be processed. At the ANTARES monitoring station, the LOPC has been deployed for 15 consecutive months, with successful data recording and processing for 11 months. On one occasion, difficult weather conditions made sampling impossible, and on three occasions raw data files again could not be processed.

For the OPC, the predecessor of the LOPC, coincidence counting was a major problem. It resulted in the misinterpretation of many small particles as large zooplankton counts (e.g., Sprules et al. 1998). With the new LOPC technology, the segmented detector improves discrimination between larger particles and high concentrations of small particles. Depending on whether one or several neighboring subunits of the detector are activated, particles are recognized as Single-Element-Plankton (SEP) or Multi-Element-Plankton (MEP). A coherent MEP sequence will display the letter M followed by the number of the active detector element (e.g., M 5) for several elements in decreasing order. Two examples are given below, both particles activating three consecutive detectors, M5 to M3 and M21 to M19:

M 5 36089 3 32869
M 4 36091 14 178
M 3 36089 17 177
M 21 36442 38 32957
M 20 36474 46 324
M 19 36512 1 102

On several occasions, we observed incoherent MEP sequences, which display the element numbers out of order, exemplified in the following two data streams:

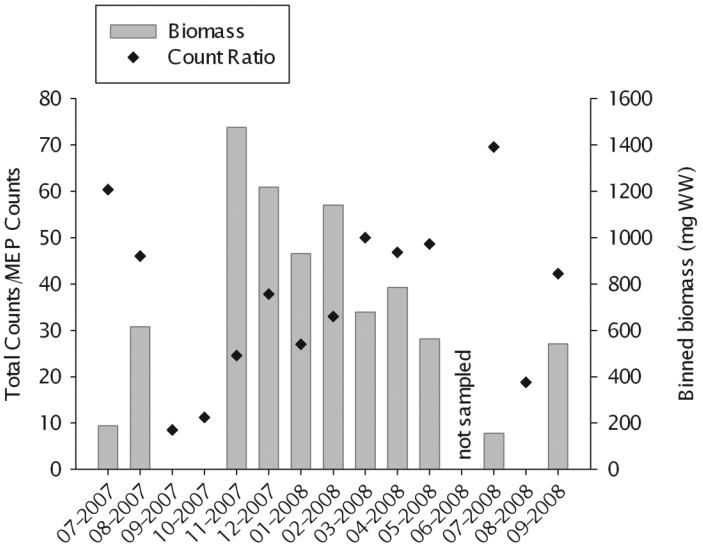

Fig. 2. Biomass (mg wet weight) binned by the LOPC during vertical tows at the ANTARES monitoring station. The ratio of total counts (TC) to multi-element plankton counts (MEP) registered by the LOPC during the tow is indicated (◆).

M 2 43398 1878 3366	M 24 2059 1153 4016
M 2 11055 855 32870	M 1 56 1961 4012
M 0 63749 1110 1381	M 33 14389 1296 4012
M 1 60699 1878 33892	M 9 12290 296 4015
M 4 54278 86 33635	M 51 10301 520 4007
M 2 99999 342 34402	M 40 99999 520 4007

Such data streams indicate that the MEP signal has been overloaded (Herman pers. comm.) with excessively high MEP counts. Data files with faulty MEP data cannot be processed reliably, and will probably result in an overestimation of MEP counts. Fig. 2 presents biomass estimates from the ANTARES monitoring station in comparison to the ratio of total counts to MEP counts (TC/MEP). Biomass could not be estimated when the TC/MEP ratio dropped below 20. During the PROABROLHOS winter expedition, the average TC/MEP ratio was 110 (std 28) for deep stations, and 69 (std 15) at shallow stations. The difference is statistically significant ($t = 4.449$, $P < 0.0001$, $n = 28$). Similar to observations made at ANTARES station, difficulties in processing files were encountered when the TC/MEP was close to 20 or lower (data not shown). The TC/MEP ratio may be a useful indicator for potential technical limitations of the instrument, similar to coincidence counting in the OPC. Stations with a low TC/MEP ratio will have to be viewed with caution in terms of MEP biomass, since part of the MEP biomass may in fact be caused by coincident activation of several subunits by high concentrations of small particles.

Comparison of biovolume estimates—Plankton biovolume in the PROABROLHOS study area was determined with four independent methods: from the LOPC profiles (Fig. 3a), with a simple volumetric method (Fig. 3b), from the Zooscan, and from microscopic counts (both shown in Fig. 3c). The ease of the method and the time allotted for sample analysis impose a decreasing amount of data being available for each of the four. Biovolume results will be compared sequentially, beginning

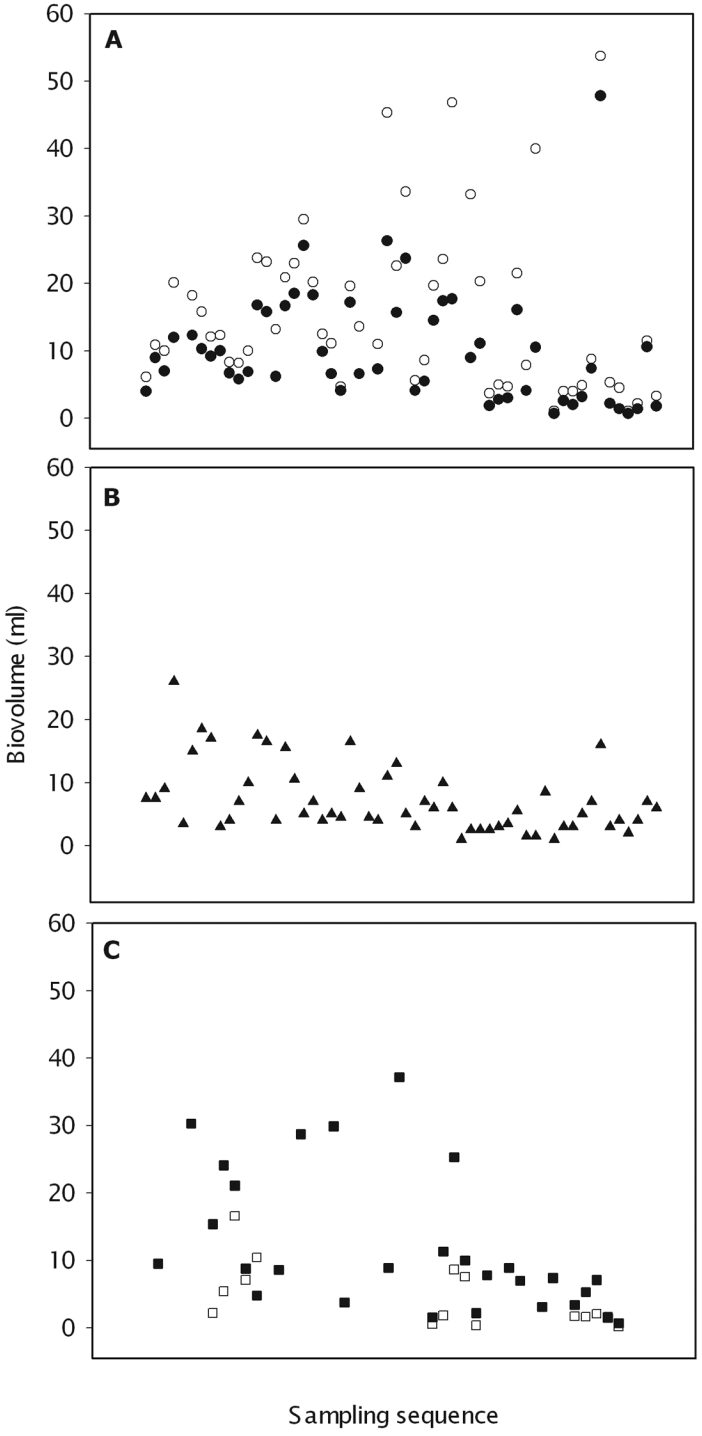

Fig. 3. Biovolume (ml) estimated from LOPC (A), with the volumetric method (B), the Zooscan and the microscope (both in panel C). LOPC-BB (○); LOPC-BB_{500} (●); TPV (▲); Zooscan (■); Microscope (□). Data are aligned along the x axis following the sampling sequence. See text for further explanation.

Table 1. Results of a linear correlation analysis between plankton displacement volume (TPV) and LOPC-binned biomass from the PROABROLHOS winter cruise.

	LOPC binned biomass		
Correlation of TPV with/for	**Total size spectrum**	**<500 µm ESD**	**>500 µm ESD**
All stations (n = 52)	0.347*	0.035‡	0.479*
Shallow stations (n = 24)	0.282‡	–0.059‡	0.607*
Deep stations (n = 28)	0.509*	0.751†	0.413*

*$P < 0.05$; †$P < 0.001$; ‡nonsignificant

with results from the LOPC and the volume displacement method, for which the largest data set is available.

Two methods (n = 52)—TPV (mL) estimated with the displacement method showed a lot less variability than biomass binned by the LOPC during the upward tow (LOPC-BB). TPV ranged from 1 to 26 mL, whereas the LOPC determined biovolume up to 53.7 mL. LOPC-BB was weakly correlated with TPV for the entire size class of particles when all stations are considered in the analysis (Table 1). The correlation improved for all stations when LOPC-BB estimated only for particles >500 µm ESD was used in the calculation (full symbols in Fig. 3a). At deep stations, TPV was correlated with LOPC-BB for the total particle size spectrum, for particles >500 µm ESD, and most significantly with particles <500 µm ESD. At shallow stations, LOPC results correlated with TPV only for the size class >500 µm ESD. The 200 µm LOPC net apparently retained particles >500 µm ESD most efficiently as has been shown previously by Herman (2005) in a comparative study of optical plankton counters with vertically towed nets. The consistent correlation between TPV and LOPC biomass >500 µm ESD should be viewed from this perspective.

Three methods (n = 28)—For 50% of the stations sampled during PROABROLHOS, net samples were analyzed on the Zooscan, allowing us to compare results of plankton volume based on the image analysis (ZSCN-PV), on LOPC-BB, and on TPV. Based on the findings from the previous correlation analysis, LOPC-BB was only considered for the particle size class > 500 µm ESD (LOPC-BB_{500}). A highly significant correlation exists between TPV and ZSCN-PV ($r = 0.859$; $P < 0.001$), whereas LOPC-BB_{500} correlated to a lesser degree with TPV ($r = 0.335$; $P < 0.05$) and ZSCN-PV ($r = 0.420$; $P < 0.05$). The good comparability of the results obtained with the displacement method and with the image analysis is confirmed by a linear regression model [$ZSCN\text{-}PV = 1.46 * TPV + 0.55$ ($r^2 = 0.71$, F = 51.7; $P < 0.0001$)] that can be fitted to the data. The slope value of 1.46, however, indicates a consistently higher biovolume estimate obtained with the Zooscan. This apparent overestimation by the Zooscan may be due to the fact that biovolume is calculated from the two dimensional area of an organism on the image, assuming a perfect spheroid shape. In reality, most organisms are not geometrically homogenous bodies. In addition, surface area varies depending on the orientation of the zooplankton on the scanning cell and on the precise detection of appendages.

Table 2. Results of a linear correlation analysis for plankton volume estimated with the displacement method (TPV), Zooscan (ZSCN-PV), microscopy, and based on LOPC-binned biomass for particles >500 µm (LOPC-BB_{500}). n = 15 if not indicated differently; n_A, excluding stations 14, 15, 40; n_B, excluding stations 39, 41, 58.

	TPV	**ZSCN-PV**	**Microscopy**
TPV	1.00		
ZSCN-PV	0.92†	1.00	
Microscopy	0.47*	0.60*	1.00
LOPC-BB_{500}	0.34‡	0.38‡	0.13‡
LOPC-BB_{500} n_A = 12	0.54*	0.64*	0.13‡
LOPC-BB_{500} n_B = 12	0.62*	0.71*	0.13‡

*$P < 0.05$; †$P < 0.001$; ‡nonsignificant

Four methods (n = 15)—Microscopic estimates from 15 stations were added to the previous comparison showing that biovolume estimated with microscopy, Zooscan, and displacement method are correlated with each other (Table 2). Results from the LOPC, however, correlate with none of the three other methods. High environmental variability along the three coastal ocean transects in a comparatively small data set could be the reason for this lack of correlation. These results demonstrate higher robustness of plankton volume estimates from the Zooscan than from the LOPC or from microscopy, when compared with the displacement method. LOPC-BB_{500} is related again to ZSCN-PV and TPV when the three stations with highest biovolume estimates, either based on LOPC (St. 39, 41, 58) or Zooscan (St. 14, 15, 40), are withdrawn from the analysis. Difficulties to bring LOPC and Zooscan estimates into agreement seem to be associated with stations of high zooplankton biomass.

Comparison of areal biomass estimates—Inherent to the calculation of areal biomass is the sensitivity of results to tow depth and the surface area of the sampling device, both influencing the volume of water that has been sampled. Volume estimates based on the flowmeter reading (V_{flow}) and LOPC estimates (V_{LOPC}) appear to be in good agreement for shallow and deep tows (Fig. 4). Volume estimates based on tow depth (V_{calc}) converge with V_{LOPC} only for shallow tows and are on average by a factor of 2.3 lower than V_{flow}. The substantially reduced and stable estimate obtained for V_{calc} at the deep stations

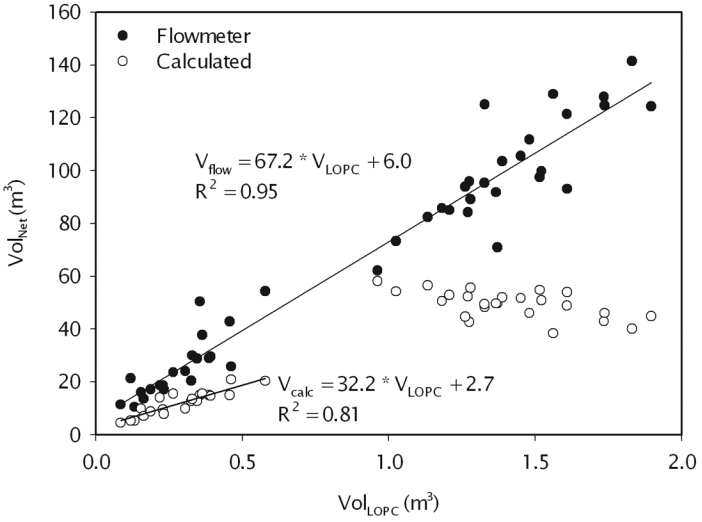

Fig. 4. Comparison of volume filtered by the LOPC with volume filtered by the net. Calculation for the net is indicated based on the flowmeter reading (●) and tow depth (○).

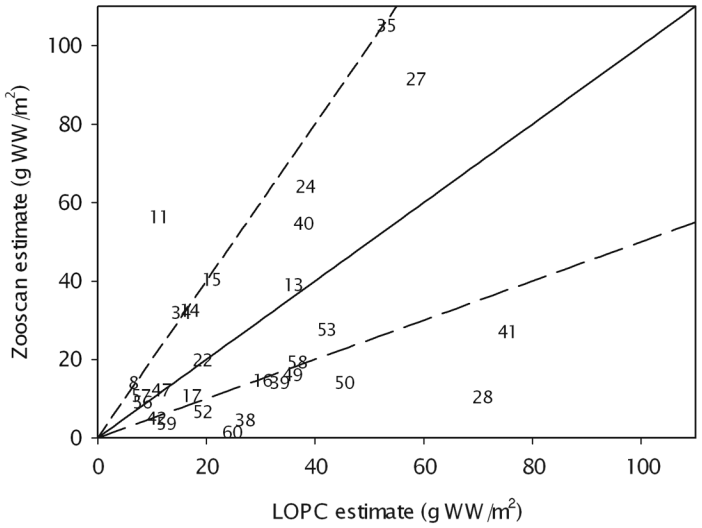

Fig. 5. Comparison of areal biomass (g wet weight/m^2) estimated by the Zooscan and the LOPC (>500 µm ESD). Numbers on graph indicate the station number. The 1:1 line and the range within the LOPC and the Zooscan agree within a factor of two (dashed lines).

most certainly underestimates the true volume, because the varying inclination of the cable is not taken into account in the calculation. Based on the flowmeter reading, volume estimates generally appear to be too high for the sampled depths. Assuming a maximum depth of 200 m, an angle of 30° and a net surface of 0.28 m^2, the filtered volume should not exceed 64 m^3 but V_{flow} reaches values of up to 140 m^3 (Fig. 4). Therefore the flowmeter, and potentially the LOPC, overestimate the truly filtered volume.

In theory, the volume filtered by the net should be 57.7 times the volume filtered by the LOPC, i.e., the ratio between the surface area of the net and that of the LOPC tunnel. However, neither the slope of the regression between V_{LOPC} and V_{calc} for the shallow tows, nor the one between V_{LOPC} and V_{flow} for all tows fall onto this theoretical value, although the latter only deviated by 15% of the theoretical estimate (Fig. 4). For the sake of comparability, the volume of water sampled by the net has been set to $57.7 * V_{LOPC}$, henceforward called the corrected volume (V_{corr}) and used in all biomass calculations.

Areal standing stock of zooplankton biomass estimated with LOPC and Zooscan ranged from 6.4 to 75.4 g WW m^{-2} and from 1.4 to 105.1 g WW m^{-2}, respectively (Fig. 5). A substantial number of the 28 stations fall outside the range for which LOPC and Zooscan estimates agree within a factor of two (dashed lines in Fig. 5). Clearly, both estimates cannot be brought into agreement and a case-by-case analysis for the origin of this variability is necessary. Seventy-eight percent of stations with a more than 2-fold higher biomass estimate by LOPC compared with Zooscan are characterized by low TC/MEP ratios, indicating limiting environmental conditions for use of LOPC. The extent to which the high variability observed in the areal biomass data are due to differences in particle detection will be further explored in the following section.

Normalized biomass spectra—The influence of technical difference and the environmental setting are illustrated within the biomass spectra obtained by the LOPC and the Zooscan for 5 stations on transect A (Fig. 6). First, the Zooscan NBSS generally showed a maximum in the log biomass bin of 1.54, equivalent to 487 µm ESD. This is interpreted as the lower limit for efficient plankton retention by the 200 µm net, and in close agreement with previous observations in tropical ecosystems (Hopcroft et al. 2001). Below this size, LOPC and Zooscan NBSS diverge in all of the 28 compared spectra. Second, going from coastal (St. 60) to offshore (St. 56), biomass spectra from both methods gradually converge for log biomass of 1.5 and higher. All spectra from deep stations (n = 15) show reasonable agreement comparable to the examples from stations 56 and 57, indicating that particles detected by the LOPC were predominantly zooplankton.

LOPC spectra from stations closer inshore and over Abrolhos Bank generally show offset from the Zooscan spectra along the y-axis, or binned-biomass-axis, with station 60 depicting the most extreme case. Most certainly, this offset is introduced by superfluous counts of suspended matter of non-zooplankton origin, since waters at station 60 and 59 were visibly turbid. The complete offset between spectra measured with in situ vertical tows and spectra from net samples resembles the results obtained by Finlay et al. (2007; their Fig. 5), who attribute the offset as inefficient retention of zooplankton in the net. In our study, LOPC data from stations with an offset are, with one exception, among the 33% of stations with the lowest TC/MEP ratio, indicating a potential overesti-

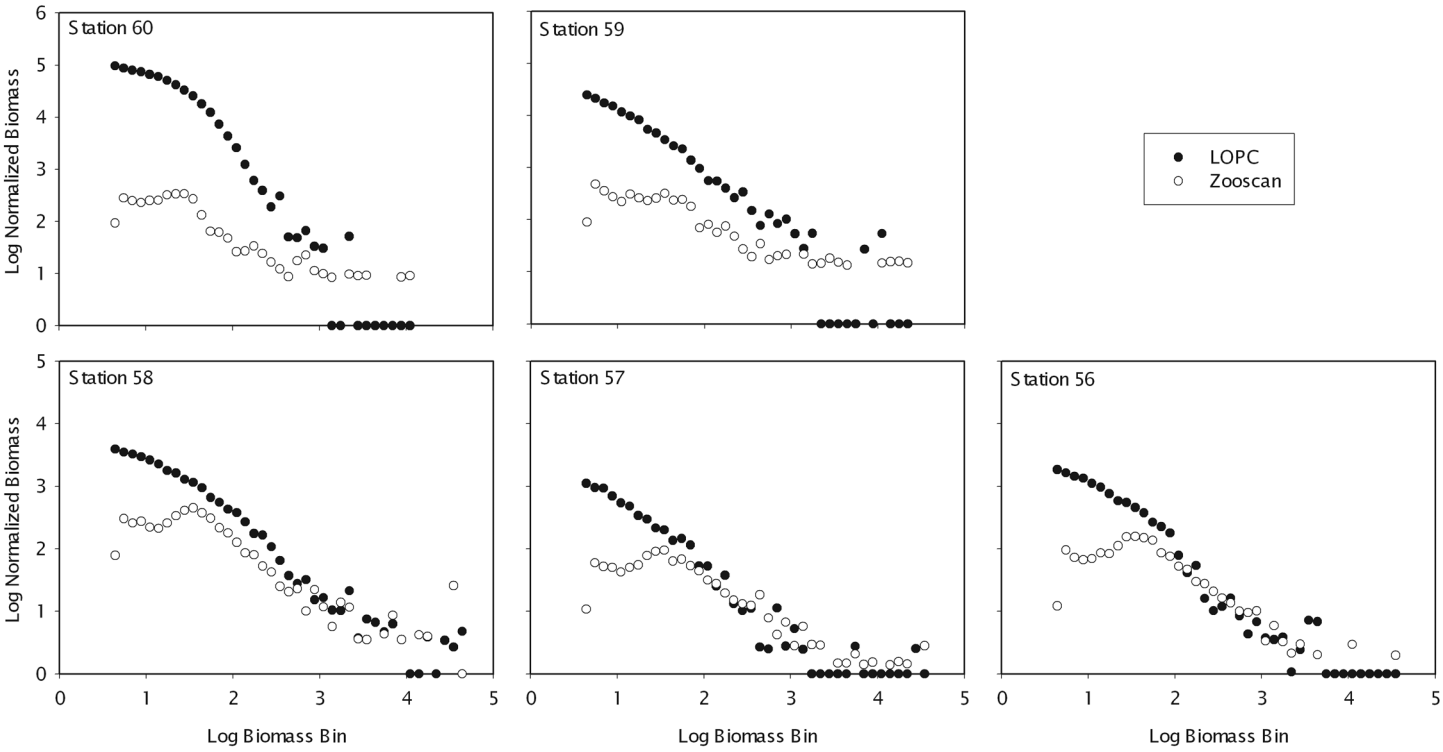

Fig. 6. Comparison of normalized biomass spectra calculated from LOPC and Zooscan results for a coastal ocean transect (A) during PROABROLHOS winter expedition. Station 60 is the coastal-most station, station 56 furthest offshore.

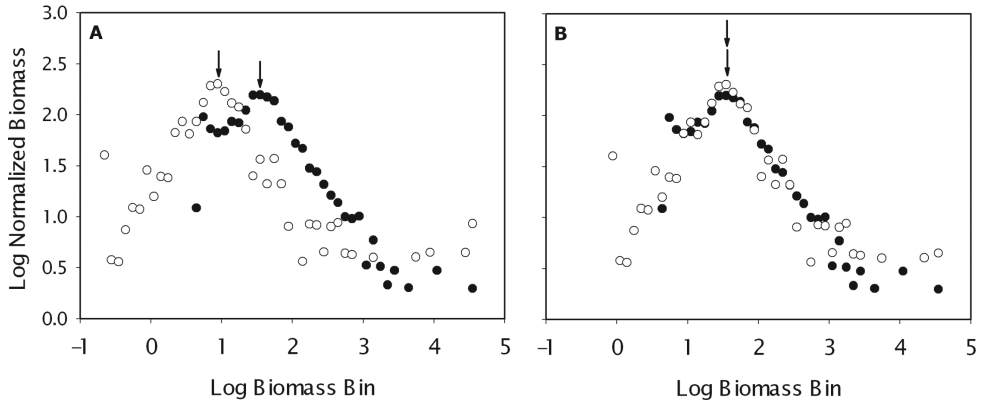

Fig. 7. Comparison of normalized biomass spectra calculated from Zooscan (●) and microscopy (○) results at Station 56. Original spectra (a) and aligned spectra (b) are presented. Arrows indicate the fullest bin in each spectrum.

mation of MEP biomass due to false MEP counts. Further tests need to confirm the utility of this ratio for the calibration of LOPC data, but it has proven useful since results on total counts or MEP counts alone do not permit us to isolate and identify problematic stations (data not shown).

Spectra determined from microscopic counts have a shape similar to the Zooscan spectra, but are offset along the x-axis, or size-bin-axis (Fig. 7a). In a standardized calibration test using (uncooked) rice grains, it was noticed that for a similar count (Fig. 8a) the Zooscan provides a 13% lower major:minor axis ratio than determined from the measurement with the microscope (Fig. 8b). This would lead in theory to a 32% higher biovolume estimate and a 10% increase in ESD, potentially guiding the offset of the Zooscan spectrum to the right along the size-bin-axis in comparison to the microscope spectrum. In reality, the increase in biovolume per rice grain is only 5% and statistically not significant. For calanoid copepods, a similar effect can be observed, causing an effective increase in biovolume per individual of 67% (Fig. 8c). Whereas the slight difference noticed for rice grains is probably due to

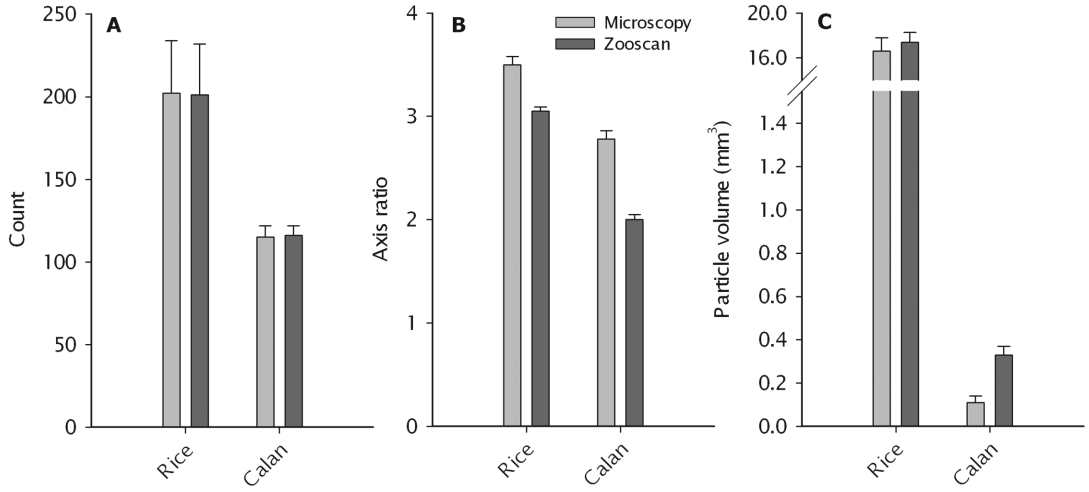

Fig. 8. Results for rice grains (Rice) and calanoid copepods (Calan) counted (panel A) and measured on the microscope and the Zooscan. For measurements, ratio of the major to minor axis (panel B) and the average volume per particle (panel C) are shown. Bars represent the average of three replicates with the standard deviation.

a minor optical deformation detected with spherical calibration beads (data not shown), the disparity noted for calanoid copepods is rather an effect of different measurement techniques. On the microscope, major and minor axes are digitized from the prosome (Uye 1982), whereas on the Zooscan, they are estimated from the best-fit ellipse having the same area as the region of interest, including caudal ramii and antennae. Hence, the measurement based on the Zooscan appears to be more realistic. Considering that appendices probably have a lower biomass density than the prosome, results from the microscope and the Zooscan indicate a lower and upper limit for the shift of biomass spectra along the size-bin-axis, respectively. To correct for the apparent artifact and compare with the LOPC results, Zooscan and microscope spectra were realigned. The microscope spectrum was shifted to the right on the size-bin-axis in order to superpose the value of its fullest bin with the maximum value observed in the Zooscan spectrum (Fig. 7b).

Most frequently, NBSS data are interpreted in terms of the slope of a linear regression fitted to the data, and the slope value introduced into modeling approaches (e.g., Zhou and Huntley 1997). Comparability of NBSS data from different methods is therefore only given when slope values show agreement. For the linear fit to the data, results were considered only for log biomass bin 1.54 (midpoint 487 µm ESD) and higher, since below this limit net retention leads to an underestimation of biomass in Zooscan and microscope spectra. To the right of the size-bin-axis, data are included until the first empty bin occurs in either Zooscan or LOPC spectrum. Beyond this point, reliability of normalized biomass calculations is reduced because of few binned particles, and data points have a potentially large influence on the slope value (Sourrisseau 2002). An example of fitted regressions to the Zooscan and LOPC data are shown for transect A (Fig. 9). NBSS slopes for all transects (A, B, and C) and methods (LOPC, Zooscan, and microscopy) are summarized in Fig. 10. Variation of the slopes introduced by different methods is in the same range as environmental variation of slopes determined with the same method on the three transects. Zooscan and microscope measurements are in reasonable agreement, especially along transect C. In comparison, slopes determined with the LOPC are steeper at 12 of 15 stations.

During the time of sampling, ostracods were an important part of the zooplankton community in the Abrolhos ecosystem. At station 35 to the south of the bank, the high abundance of ostracods created a distinct peak in the NBSS from both instruments (Fig. 11). The different environmental setting over Abrolhos bank (stations 40, 49, 50, 52) leads to a generally higher LOPC biomass estimate, shifting the intercept of the spectral line upwards along the biomass-axis. Only at station 35, the intercepts of the LOPC and Zooscan spectra yield similar values of 3.15 and 3.12 µg m^{-3} $\Delta\mu g^{-1}$, respectively. The erosion of the ostracod peak can be traced in the Zooscan NBSS of all five stations, but vanishes from the LOPC NBSS due to higher non-zooplankton particle load in shallow waters over Abrolhos bank. These results demonstrate well the environmental limitations encountered for comparability of data obtained with Zooscan and LOPC.

Discussion

In the framework of our study, the LOPC has been deployed on more than 70 occasions in very diverse environmental settings. Only in 9% of these deployments have problems been encountered preventing data acquisition. During the PROABROLHOS cruise, this occurred in turbid nearshore waters where problems for the use of particle counters are to be expected. The exact reasons for the corrupted files generated during the ANTARES monitoring need to be investigated in

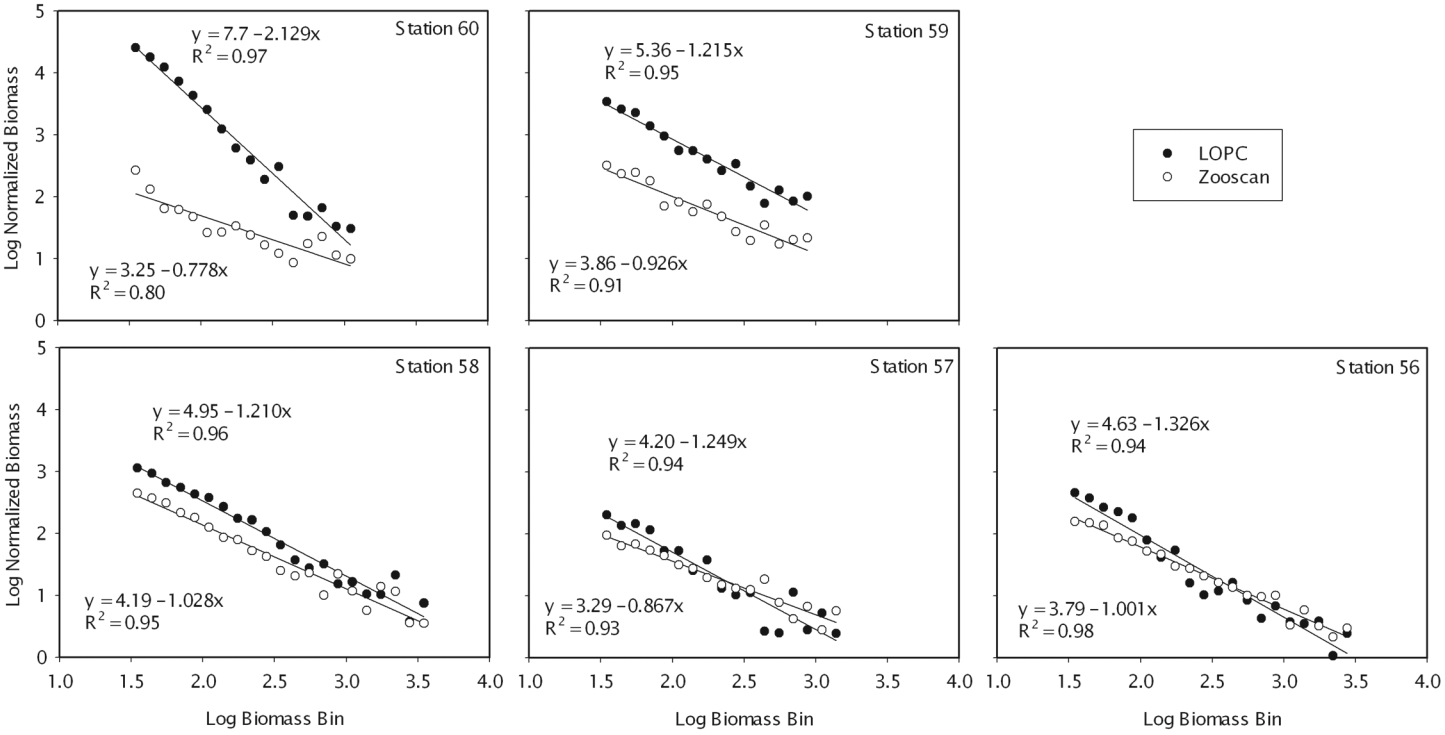

Fig. 9. Linear regressions fitted to the NBSS spectra from LOPC and Zooscan on transect A during PROABROLHOS winter expedition. The equation to the linear fit and its regression coefficient are indicated for each plot.

greater depth, for example, the possible interference of colored dissolved organic matter (cDOM) with the laser. Particle abundance was far below 10^6 m^{-3}, stated by Herman et al. (2004) to be limiting for the operation of the LOPC. To trace potential coincidence counting, we propose to rapidly identify stations with abnormally high MEP biomass based on the ratio TC/MEP ratio of the profile. Generally though, the problem of coincidence counting seemed to be of minor importance in our study, as is confirmed by other recent assessments of zooplankton abundance using the LOPC (e.g., Herman et al. 2004; Finlay et al. 2007).

Major uncertainty was associated with the estimation of flow through the net. This complicated the comparison of LOPC and Zooscan at stations with high zooplankton biomass (Table 2) and during deep tows. Trapping of superfluous zooplankton in the net during the descent of the instrument and regurgitation of sample during resurge is of concern and cannot be corrected for. Depth profiles of several tows indicate that the net frequently stagnated in the water column. It also cannot be precluded that some volume was recorded by the flowmeter on the down-cast. Clogging of the net, however, as experienced by Nogueira et al. (2004), had no apparent influence on our results since it would have reduced the flowmeter estimate in comparison to the volume calculated based on tow depth. Alternatively, flow through the net can be estimated from LOPC data. Based on average tow speed and the duration of the tow, the distance the net travels through the water can be estimated. A second possibility is to extrapolate the volume estimate provided in the processed data files of the LOPC to the net surface, i.e., our V_{corr}. Both approaches were compared to V_{flow} and V_{calc} for one coastal-ocean transect (data not shown) and indicate that they provide a robust alternative.

The general downside of particle counters is that they sense more than just zooplankton. Net casts in turn will lose a substantial part of gelatinous zooplankton and other fragile particles detected by particle counters or imaging systems (Remsen et al. 2004). At shallow stations situated over the shelf and Abrolhos Bank, the LOPC counted up to 33% of biovolume in the particle size class between 100 and 250 µm ESD, compared with not more than 14% contribution of this size class at deep stations (data not shown). It is likely that LOPC biomass estimates for the total size spectrum overestimate true zooplankton biomass due to accidental counting of terrigenous particulate matter at shallow stations. At most oceanic stations, a strong peak at the bottom of the mixed layer was detected and associated with counts in the small size classes (data not shown). Microscopic analysis of samples from oceanic stations 57, 56, 42, 17, and 16 indicated a predominance of appendicularians and cyclopoid copepods in the size class < 500 µm ESD. A possible contribution of phytoplankton aggregates to the LOPC counts is indicated by high in situ fluorescence measurements from a probe on the hydrographic cast (Nonnato pers. comm.). It is probable that the high correlation observed at deep stations between plankton volume caught in the net—presumably retaining zooplankton larger than 500 µm ESD—

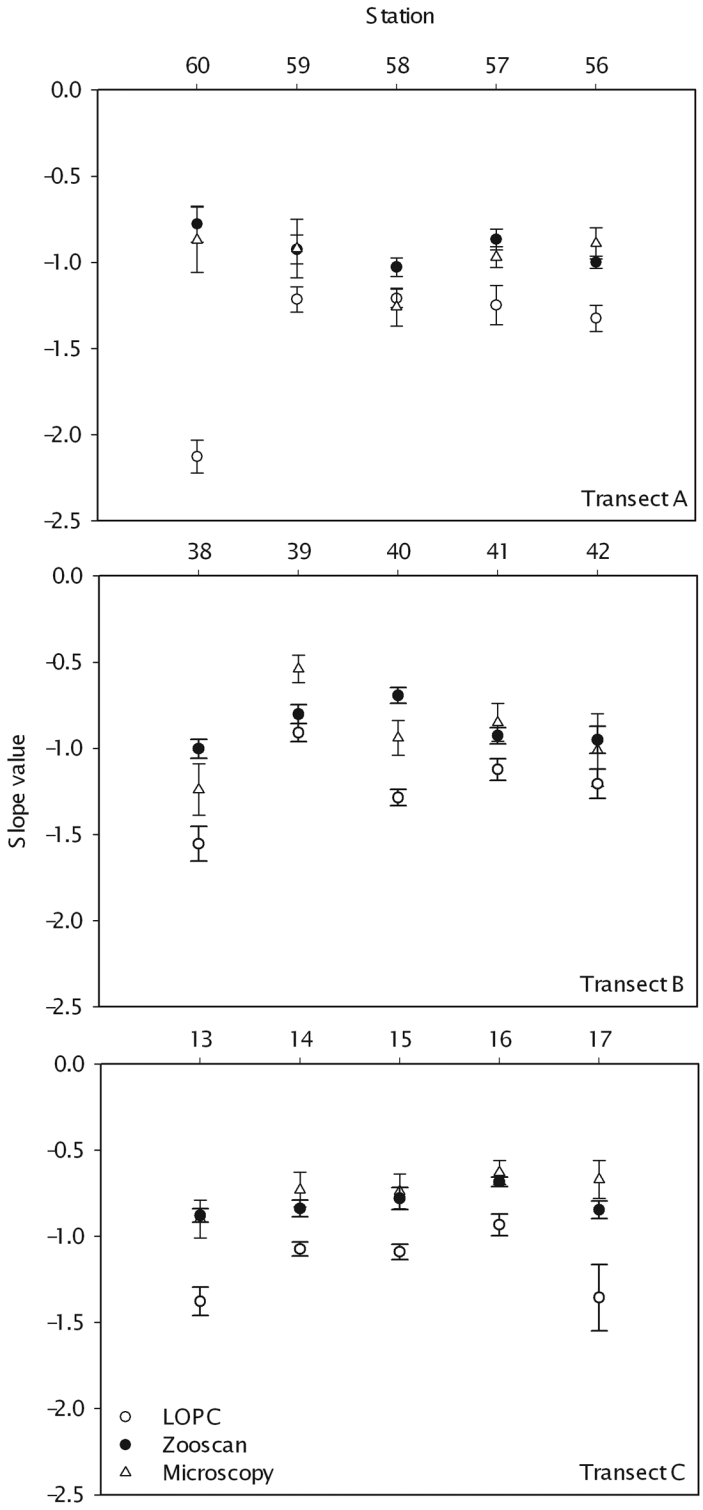

Fig. 10. Slopes of the linear regression fitted to the NBSS spectra from LOPC, Zooscan, and microscopy for transects A, B, and C. Values represent the slope with the standard error of the estimate.

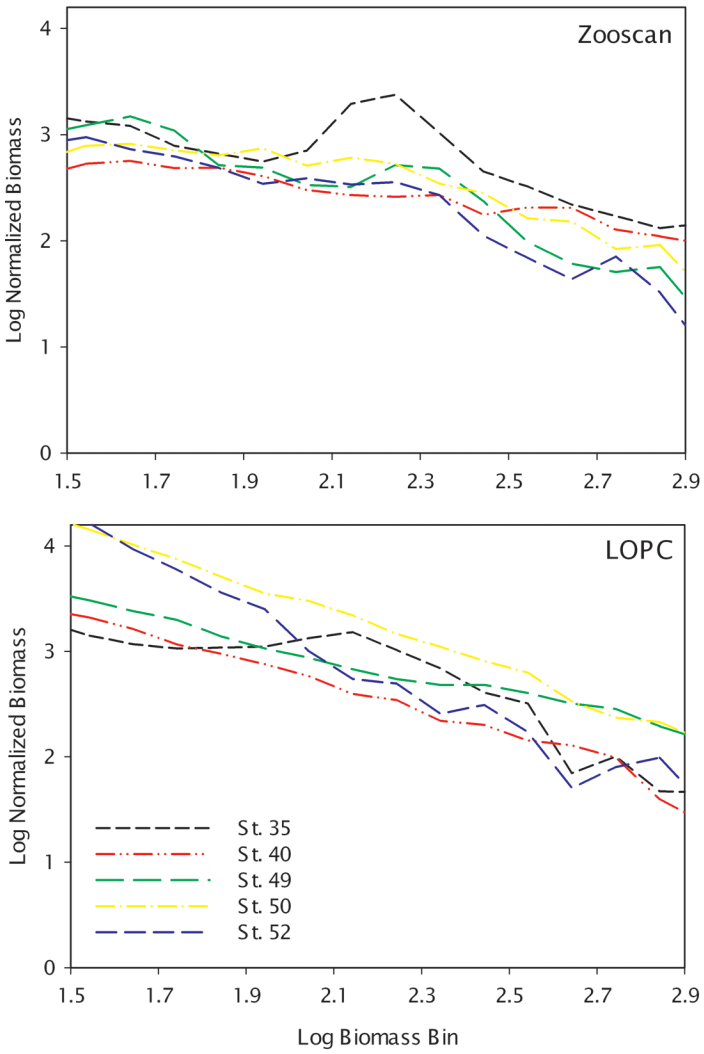

Fig. 11. Biomass spectra determined with LOPC and Zooscan at several stations over Abrolhos-Bank during PROABROLHOS winter expedition.

and LOPC biomass < 500 µm ESD is spurious, and that the instrument counted larvacean houses or aggregates, which have been destroyed by the net. Although a recent comparison of LOPC data with in situ imaging data from California waters (Herman et al. unpubl. data) indicates a reduced sensitivity of the LOPC to fragile transparent particles, excluding 90% of counts compared with the imaging system, the reliability of zooplankton biomass estimated with the LOPC continues to require knowledge on the environmental setting.

The Zooscan permitted rapid determination of zooplankton biomass and particle size spectra from the collected net samples. Compared with the measurements on the microscope, the Zooscan analysis yields slightly larger size and biovolume estimates, probably due to variable orientation of the organisms in the automated method. This introduces a shift of the biomass spectrum along the size-axis, analogous to results obtained by Sprules et al. (1998) and Finlay et al. (2007) for OPC and LOPC calibrations with a microscope. The shift is

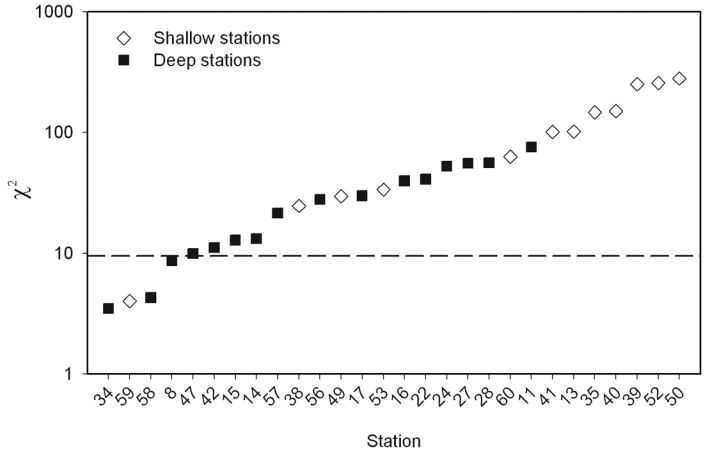

Fig. 12. Results of the chi-square statistics comparing the particle size distribution estimated in five size classes (I: 468-588 µm ESD, II: 589-740 µm ESD, III: 741-932 µm ESD, IV: 933-1174 µm ESD, V: 1174-1479 µm ESD) with LOPC and Zooscan at 28 stations. Dashed line indicates the level of the critical chi-square (see text).

not observed for spectra emanating from LOPC and Zooscan intercomparison in this study. Compared with the number of particles passing the LOPC, and to the fraction of the sample analyzed on the microscope, the Zooscan analysis uses the largest sample sizes. This increases both robustness and precision of the obtained results.

The LOPC and the Zooscan cover different size ranges of the biomass spectrum, and effectively overlap in this study from 500 to 1500 µm ESD only. The lower limit is set by the retention efficiency of the net. The upper limit is indicated by empty biomass bins occurring in the LOPC data for bin 37 and higher (equivalent 1426 µm ESD). The distribution of particles into five size classes, each cumulating three consecutive size bins, has been compared between the LOPC and the Zooscan for 28 stations based on chi-square (χ^2) statistics (Scherrer 1984). Only at four stations (34, 59, 58, 8), the calculated χ^2 is lower than the critical value ($\chi^2 = 9.46$, DF = 4, $\alpha = 0.05$), indicating that particle distribution is the same for both methods with a probability of 95% (Fig. 12). The four stations are situated in different environmental settings, which make it difficult to determine the reason for such result. An influence of taxonomy, for example the contribution of gelatinous zooplankton or appendicularians, or of overall biomass is not indicated. χ^2, however, is generally lower at deep oceanic stations and increases to very high values for shallow stations over the bank (Fig. 12), suggesting again an effect of terrigenous or resuspended matter.

Steeper NBSS slopes estimated with the LOPC compared with the Zooscan occur all the way from coastal to offshore stations. Overestimation of small-sized zooplankton biomass due to accidental counting of non-zooplankton material, for example at station 60, cannot entirely explain this observation. The smaller sampling surface of the LOPC compared with the net is less susceptible to catch and measure larger zooplankton at the upper end of the spectrum. A significant linear relationship ($y = 0.012* x - 1.024$; $R^2 = 55.4$, $P < 0.01$, $n = 15$) can be established between the slope determined with the Zooscan (y) and the contribution of zooplankton with a major axis > 1000 µm (x) to the net sample. This regression is not significant when the LOPC slope is considered instead. Potential underestimation of larger zooplankton in LOPC counts therefore appears to be a second factor leading to steeper NBSS slopes.

Comments and recommendations

The comparison of results from in situ LOPC vertical profiles and analysis of net samples with the Zooscan system in this study revealed advantages and limitations of both approaches to determine particle size distribution and biomass of zooplankton communities. Straightforward comparability of particle distribution into size classes and normalized biomass spectra is hampered by three major differences: (i) the LOPC detects particles, independently whether they are zooplankton or not; (ii) the 200 µm net sample analyzed on the Zooscan underestimates small-sized zooplankton; and (iii) the net and the LOPC tunnel catch larger and less abundant zooplankton with different efficiencies. Variability introduced by these factors is of the same range as environmental variability of slopes observed during the PROABROLHOS cruise and in other studies (Zhou and Huntley 1997; Sourrisseau and Carlotti 2001; Herman and Harvey 2006). Therefore, interpretation of slopes from different ecosystems should only rely on data obtained with the same method. Comparison of normalized biomass spectra and their slope determined with the LOPC and the Zooscan on coastal-ocean transects (Figs. 6 and 10) suggests that oligo- to mesotrophic conditions remain the primary field of deployment for the LOPC to obtain highly resolved spatial data on the distribution of particles in the zooplankton size range.

Potentially, the 7 × 7 cm LOPC tunnel is not entirely appropriate for quantitative sampling of larger (>1 mm ESD) zooplankton, and the large tunnel version may represent a better choice, even though escape reactions should not be neglected for both LOPC models. On the contrary, the LOPC covers an important and frequently under-sampled size fraction of mesozooplankton, notably from 100 to 500 µm ESD, representing naupliar and young copepodite stages of most copepod species, as well as appendicularians, foraminiferans, and radiolarians. This size range dominates biomass spectra in a range of ecosystems (Hopcroft et al. 2001). Future analysis of the 80 µm net samples for comparison with LOPC spectra will allow us to explore the possible advantage of LOPC measurements in areas dominated by very small zooplankton.

The Zooscan analysis provides robust estimates of zooplankton biovolume and the image archive contains impor-

tant taxonomic information, which can help to interpret LOPC data. Size spectra of both instruments resolved singularities such as the peak created by the ostracod "bloom" in the Abrolhos ecosystem. Comparison of the results from a morphometric filter applied to the LOPC signal, and from automated recognition of the Zooscan image may help to detect the influence of taxonomy and non-zooplankton material on the particle size spectra provided by the LOPC. For a higher precision of biomass estimates from digital images appropriate geometric models (e.g., Patoine et al. 2006) and biomass conversion factors will have to be introduced and tested for the level of confidence in comparison to a microscopic analysis.

In the timeframe of this study, many software features and optical or morphometric parameters provided in raw data files from both instruments could not be explored in further detail due to the lack of enhanced processing tools. Clearly, the broad use of the new optical instrumentation would greatly benefit from more user-friendly open-source software, allowing laboratories engaged in zooplankton ecology and observation to tackle and value the large amounts of data emanating from their applications.

The combined use of the LOPC and the Zooscan in the PROABROLHOS and ANTARES projects has the potential to enhance our understanding on how physical forcing induced by seasonality of current systems and topography along the Brazilian coast (Silveira et al. 2008) relates to changes in size spectra, diversity, and energy flow through small-sized zooplankton communities. Such process-oriented approach is crucial to understand the role of planktonic food webs in marine ecosystems of the Southwest Atlantic (Lopes 2007). On a larger scale, the acquisition of similar data sets from oceanic areas and to greater sampling depths can provide information on the variability of particle size spectra (zooplankton and non-zooplankton), much needed in global biogeochemical models.

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