Zooplankton biomass estimated from digitalized images in Antarctic waters: A calibration exercise

Santiago Hernández-León[1] and Irene Montero[1]

Received 18 January 2005; revised 9 January 2006; accepted 30 March 2006; published 27 May 2006.

[1] The direct measurement of zooplankton biomass following the different analytical procedures normally requires the destruction of the samples. The use of conversion factors to estimate biomass from nondestructive methods is still a challenge. The widespread use of image analyzers and optical counters in biological oceanography provides a useful tool to measure the abundance and size spectrum of zooplanktonic organisms in real or quasi-real time. Both methodologies measure the equivalent spherical diameter and/or the body area of organisms. In order to estimate biomass from the highly valuable information generated by the size spectrum of the sample, we measured the relationship between individual body area and individual biomass of the most common species and groups of zooplankton in Antarctic waters. The slope of the regression for each different species and groups of taxa was not significantly different from that obtained by pooling all taxa, thus providing a general relationship for the entire size spectrum of zooplankton. The biomass estimated from the body area spectrum of samples obtained around the Antarctic Peninsula agreed with other measurements of biomass in the region. The proposed conversion factor could provide for rapid estimates of biomass of net-collected zooplankton from imaging devices or optical plankton counters.

Citation: Hernández-León, S., and I. Montero (2006), Zooplankton biomass estimated from digitalized images in Antarctic waters: A calibration exercise, *J. Geophys. Res., 111,* C05S03, doi:10.1029/2005JC002887.

1. Introduction

[2] Measuring zooplankton biomass and preserving the sample for taxonomic, size spectrum analysis and collection is still a problem in biological oceanography. Biomass determination following the different analytical procedures available [see *Postel et al.*, 2000], require destruction of the sample for weight measurements. However, zooplankton samples and, in general, samples in biological oceanography are an important source of information for future studies. The time and cost of acquiring such samples argues against the destruction of the highly valuable information they contain. Because of this problem, the use of conversion factors to estimate carbon or nitrogen biomass from nondestructive methods such as the use of displacement volumes or wet weight is a common solution [*Wiebe et al.*, 1975; *Le Borgne*, 1975; *Corral et al.*, 1981; *Postel et al.*, 2000].

[3] The advent of the image analysis techniques allowed the estimation of the biovolume of organisms, assuming a geometric volume approximation, to convert the generated volume to biomass using different conversion factors from the literature [see *Billones et al.*, 1999]. Another procedure is to assess the biomass by using a previously obtained relationship between total biovolume in the sample and biomass in a unit-volume basis [see *Alcaraz et al.*, 2003]. Although the inherent problems of classical procedures such as the different interstitial water content of samples of different taxonomic groups are avoided using image analysis, the taxonomic composition of the sample as well as the size spectrum of organisms will affect the choice of conversion factor. The geometric volume approximation gives a rather artificial volume and this erratic volume of different groups of animals is then converted to biomass using general assumptions. The conversion from the biovolume to wet weight, dry weight and carbon also promotes increased error. The procedure given by *Alcaraz et al.* [2003] partly avoids the latter problems but still relies on a general conversion factor that cannot account for the large heterogeneity and size spectrum of zooplankton. Whether the general relationship obtained by these authors works in constantly changing communities of zooplankton remain to be tested. In any case, its use for biomass estimates is restricted to entire samples, precluding the use of the method for assessing biomass of different portions of the size spectrum of zooplankton generated by image analysis or optical counters, which may contain highly useful information.

[4] The common use of image digitalization and automatic counting and sizing of zooplankton by optical procedures (e.g., optical plankton counter, OPC), which produces measurements of size and area of individual organisms, has encouraged biological oceanographers to obtain estimates of biomass from the information generated by those devices. Particularly, the use of the OPC in oceanographic studies provides a potential standard procedure to rapidly count and

[1]Biological Oceanography Laboratory, Facultad de Ciencias del Mar, Campus Universitario de Tafira, Las Palmas de Gran Canaria, Canary Islands, Spain.

0148-0227/06/2005JC002887

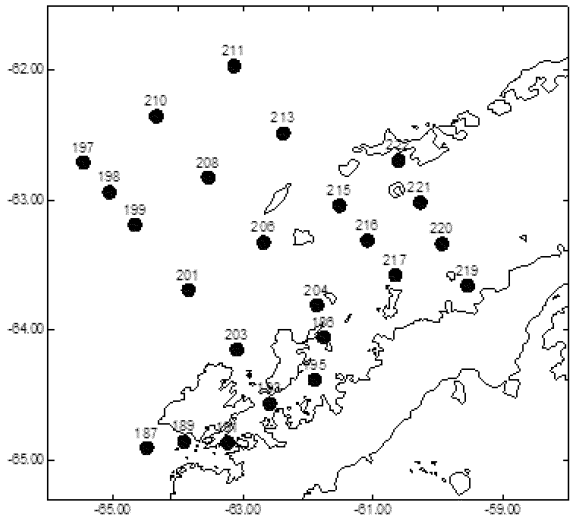

Figure 1. Location of the stations sampled north of the Antarctic Peninsula during January-February 1996 during the Fruela 96 cruise.

size zooplankton. However, the use of these data to provide reliable estimates of biomass remains in question, mainly because of the lack of conversion factors relating the equivalent spherical diameter (ESD) measured by the OPC, or the area generated by image analyzers, to the biomass of individuals producing such signals.

[5] Image analyzers and OPCs produce similar and interconvertible measures. The latter generates the ESD by converting the organism's volume into a spherical form [*Herman*, 1992]. However, the dimension measured by the OPC should be considered as the equivalent circular diameter (ECD) [*Sprules et al.*, 1998; *Beaulieu et al.*, 1999], the diameter of a circle with the same area as the silhouette of the organisms passing the OPC beam [*Sprules et al.*, 1998]. Image analyzers measure the area of the animal by summing up the pixels generated by its shadowed silhouette. Both measurements are directly interconvertible, since the area of the organism can also be obtained from the ECD generated by the OPC. In the present work we attempted to directly calibrate the area of the most common zooplanktonic organisms of Antarctic waters with the individual biomass of each organism. The relationship we obtained can be used directly to estimate the biomass of a sample by converting each individual ECD, or area of the size spectrum generated by the optical devices, into individual biomass. The application of this relationship produced biomass estimations that agree with the values expected in Antarctic waters during summer.

2. Material and Methods

[6] In order to compare individual body mass and the area of the zooplanktonic organisms, we produced the silhouette of each different organism using a previously published procedure [*Edgerton*, 1981] and also directly measured its individual biomass. Silhouette photography was done on shipboard in a darkroom using orthocromatic film (AGFA Litex 0711p). The film was positioned under a transparent tray containing the sample in a thin layer of water and a stroboscopic lamp (EG&G FX-6A) was placed at 50 cm from the tray. The film was later processed following the instructions of the manufacturer. Digital image analysis was made on the resulting silhouette photograph using a CCD camera placed in a Wild M8 stereoscope microscope for the 200–500 μm size fraction; we used the camera's macro lens for larger size classes. Images were digitized using a personal computer, a frame grabber (Data Translation) and image analysis software (Global Lab Image). After correcting for grey level threshold of the images, the area obtained by the computer-generated perimeter of all organisms in the image was recorded and stored. The system was calibrated for both the microscope and the macro lens.

[7] In order to estimate biomass from the area of each organism, the body area of specimens of different species (*Calanus propinquus*, *Metridia gerlachei*, *Rhicncalanus gigas*, unidentified small copepods, ostracods and euphausiids) was always measured in a similar position respect to the stroboscopic lamp, and individual dry weight was then measured using standard procedures [*Lovegrove*, 1966]. Specimens were dried at 60°C for 24 hours and later weighed, first allowing the sample to reach room temperature and avoiding humidity. For small copepods, organisms of similar size were grouped (2 to 6 per sample), photographed and processed for body area, and later dried in order to obtain a reliable measure of weight using the ultra microbalance (Sartorius supermicro, ±0.2 μg).

[8] The field samples used to estimate biomass from the body area spectrum of zooplankton samples were obtained from 17 January to 5 February 1996 on board the R/V *Hespérides* [see *Anadón and Estrada*, 2002]. A grid of 22 stations (Figure 1) was sampled in the shelf waters of the Bellinghaussen Sea and the Bransfield and Gerlache straits (Antarctica). Zooplankton samples were obtained in vertical hauls from 200 m (or 10 m from the bottom at shallower stations) to the surface, using a WP-2 net [*UNESCO*, 1968] equipped with 200 μm mesh. Samples from the net were size fractionated into 200–500, 500–1000 and >1000 μm classes and processed for silhouette photography and digital image collection in order to estimate biomass from the body area generated by the organisms. Finally, the sample was preserved in formalin (4%) for taxonomic analysis.

3. Results

3.1. Individual Biomass-Body Area Relationship

[9] The relationship between individual biomass and body area measured with the image analyzer was best fit by a power function. We observed better correlation coefficients for the relationship of individual weight to area than to length of organisms (not shown), denoting that the former parameter is best for calibration. The use of organism volume is likely to promote additional errors because of the need to assume cylindrical or ellipsoidal shapes, sometimes quite different from the real morphology of organisms.

[10] Different species of copepods, ostracods and euphausiids were measured for individual weight and area, obtaining the regression values shown in Table 1. Similar slopes were found in copepods except for the large copepod *Rhincalanus gigas*, which showed the largest difference in

Table 1. Regression and Correlation Parameters a, the Intercept, and b, the Slope, Between Body Area and Individual Dry Mass for Antarctic Zooplankton Species

Organism	a	b ± SE	R, μg	P	n	Body Area, mm^2
Mesozooplankton	45.72	1.19 ± 0.14	0.886	<0.001	23	0.528–8.644
Calanus propinquus	56.43	1.44 ± 0.26	0.777	<0.001	22	3.201–6.244
Metridia gerlachei	22.44	1.78 ± 0.26	0.797	<0.001	29	1.061–3.009
Rhincalanus gigas	76.71	0.63 ± 0.28	0.518	<0.05	16	5.912–17.402
Ostracods	99.46	1.28 ± 0.19	0.885	<0.001	15	1.104–4.338
Euphausia superba	87.45	1.34 ± 0.04	0.967	<0.001	71	17.248–369.1
All data	36.61	1.52 ± 0.03	0.964	<0.001	176	0.528–369.1

the slope of the regression. Pooling all the data, a highly significant ($p < 0.001$) relationship ($r^2 = 0.935$) was obtained between body area and their respective weight (Figure 2). The different slope obtained for *R. gigas* was the result of the rather small spectrum of sizes used for the calibration; in general, the weight of this species was lower than the weight expected using the general relationship. The largest zooplankton, ostracods and euphausiids showed similar slopes, highly comparable with the ones observed for copepods. Both groups showed better correlation coefficients because of the large range of sizes and weights of these organisms.

3.2. Body Area Spectrum

[11] The body area spectrum of samples from the Gerlache and Bransfield Straits was obtained using the digital analyzer. As an example, day and nighttime samples (Figure 3) show the typical bimodal spectrum in Antarctic waters with small copepods on one side and different species of large copepods, chaetognaths, pteropods and euphausiids on the other side. To compare with classical procedures of size fractionating zooplankton samples (e.g., the JGOFS protocol) and in order to have an idea of the body area of the different species, we built a schematic representation of the range covered by each organism (Figure 4). Small copepods dominated the <1 mm size fraction (<3 mm^2) while the large copepods, chaetognaths, pteropods and euphausiids were most representative in the large size fraction.

[12] Using the body area spectra and the area-weight relationship shown in Figure 2, we directly estimated the biomass for each sample. As an example, the body area spectrum shown in Figure 3 was converted into biomass (Figure 5). As expected, the highest biomass corresponded to the largest organisms, while the small copepods, although abundant, contributed only a very small percentage to total biomass. The average total biomass and the average biomass of each size fraction in the study area is shown in Table 2. Total abundance averaged 22.6 □ 10^3 individuals m^{-2}, while total biomass averaged 697.6 ± 950.9 (SD) mg dry weight m^{-2}. The greatest abundances (85% of total) appeared in the small size fraction, while the greatest average biomass (94%) was observed in the large size fraction. The medium size fraction (500–1000 μm) showed the minimum values of abundance, body area and biomass.

4. Discussion

[13] The relationship between the individual biomass of different species or groups of Antarctic zooplankton and the area generated in a picture of zooplankton or the area obtained from the OPC can easily converted into biomass values. However, the application of a single relationship could be erroneous since different species can have different area-weight relationships. This seems to be the case for *Rhincalanus gigas*, which had lower individual biomass per unit area than other species. Nevertheless, this source of error could be methodological as this very large and delicate copepod can be damaged during the vertical haul, and therefore it could lose weight due to flattening and shrinkage. In any case, as *R. gigas* was not abundant in our samples, the error involved probably did not have a significant effect on the general estimate of biomass. A similar problem can occur with gelatinous zooplankton. During the cruise, those organisms were not abundant. However, this zooplankton can be important in the Southern Ocean. Large blooms of salps (mainly *Salpa thompsoni*) are observed during certain years in Antarctic waters. However, their size is too large to be recorded by the optical plankton counter, including the laser OPC (maximum resolution of 35 mm). Thus the use of image analysis would be the method of choice for those large organisms. Further work should be done in order to calibrate for these zooplankton.

[14] The advantage of the procedure we describe in this paper is that it avoids destruction of the sample to obtain biomass estimates. At least from the stand point of methodology, the present method is more dependable than displacement volume or wet weight procedures. In our

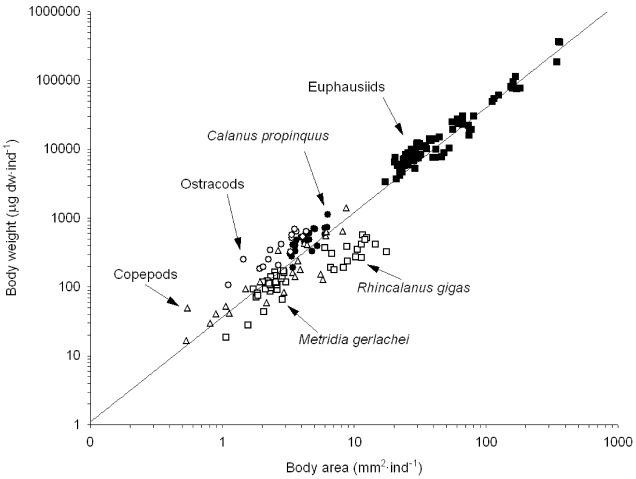

Figure 2. Relationship between individual body area and individual biomass (as dry weight) of the main zooplankton representative of Antarctic waters.

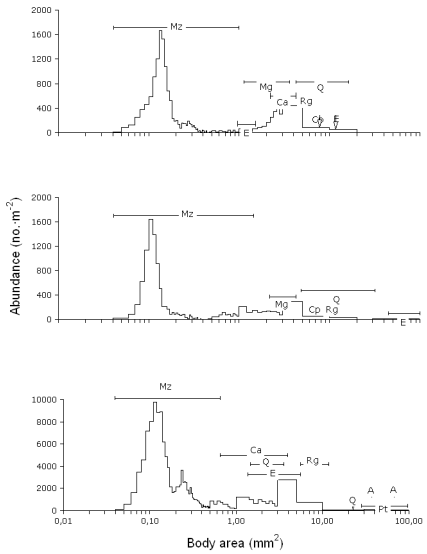

Figure 3. Examples of the body area spectrum of samples in terms of abundance. The different taxa found in the samples are indicated in relation to their body area range (mesozooplankton, Mz, *Metridia gerlachei*, Mg, *Calanoides acutus*, Ca, *Calanus propinquus*, Cp, *Rhincalanus gigas*, Rg, chaetognaths, Q, euphausiids, E, pteropods, Pt). (top) and (middle) Samples obtained during the short boreal night. (bottom) A sample obtained during daylight. Note the presence of the diel vertical migrant *Metridia gerlachei* during the night.

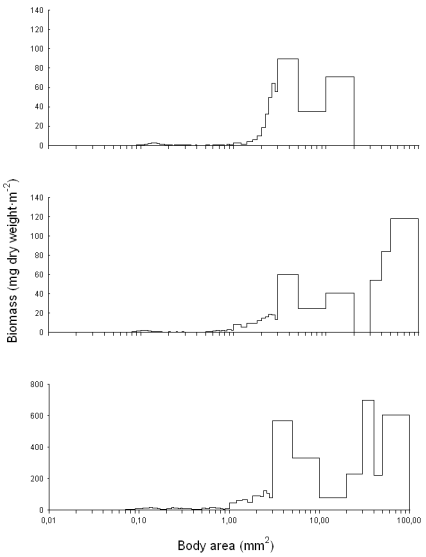

Figure 5. Examples of the body area spectrum of samples in terms of biomass. Note the inverse relationship between the peaks of abundance in Figure 3 and the peaks in biomass in this figure. (top), (middle), and (bottom) As in Figure 3.

view, our approach is also more convenient, simple and universally usable than methods based in the digitalization of the entire sample [*Alcaraz et al.*, 2003]. The latter procedure assumes no changes in the taxonomic composition of samples and although it was tested in a large range of different ecosystems, taxonomic changes can occur at the small scale or mesoscale or during temporal succession in a given water mass. Another advantage of our approach is that it avoids the use of the model II regression [*Ricker*, 1973] and allows the use of model I regression for predictive purposes. Body area of organisms can be considered as an independent variable because the organisms were recorded at the same position. The error in measuring the individual body area here is much less than the measurement of the individual body mass, allowing the use of model I regression [*Legendre and Legendre*, 1998]. In contrast, the different position of the organisms in a sample with respect to the optical device will create an error that is difficult to avoid [see *Mustard and Anderson*, 2005]. This problem is less pronounced in silhouette photography, where organisms adopt a similar position at the bottom of the tray, than in optical plankton counters where organisms pass through the optical beam in different positions.

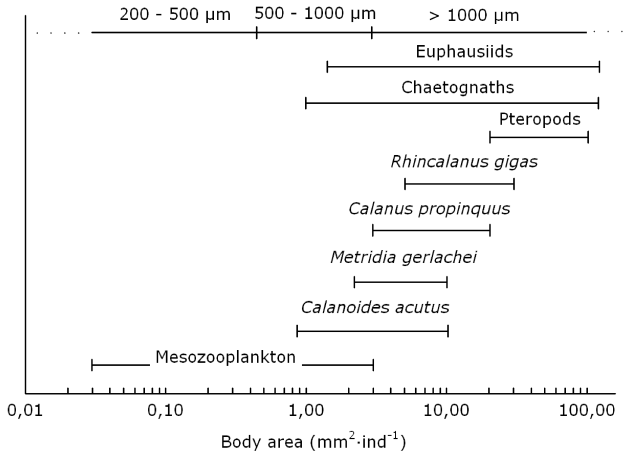

Figure 4. Body area range covered by the main species and groups of zooplankton in Antarctic waters. The relationship with the classical size fractionation of zooplankton samples is also shown.

[15] Converting our values of body area to ECD (µm) and dry weight to carbon (µgC) assuming that the latter is 40% of dry weight [*Omori and Ikeda*, 1984], we found the relationship of body carbon to ECD to be: log B = 3.04 log ECD □ 18.7, being the slope in the theoretical relationship between the linearity and volume. *Rodríguez and Mullin* [1986], however, found that log B = 2.23 log ESD □ 5.58. The difference could be due to the way to measure the diameter of the organisms. In the present work we used an analytical procedure in which we actually measured the weight and body area of every organism. By contrast, *Rodríguez and Mullin* [1986] assumed the spherical shape of organism and estimated their diameter by passing the entire sample through different mesh sizes and simply assumed the ESD to be equivalent to the nominal mesh size. The use of the equation of *Wiebe et al.* [1975] to calculate carbon from dry weight instead of the above mentioned percentage did not introduce a significant difference in our calculations.

Table 2. Abundance and Biomass for the Different Size Fractions and Their Respective Percentages in Relation to Total Abundance and Biomass

Size Class, μm	Abundance, ind 10^3	Percent	Biomass, mg dry weight m^{-2}	Percent	n
200–500	19.2 ± 16.9	85	26.0 ± 23.8	3.7	22
500–1000	1.2 ± 2.1	5.2	18.4 ± 27.9	2.7	22
>1000	2.2 ± 8.2	9.8	653.2 ± 914.3	93.6	22
Total	22.6 ± 25.7		697.6 ± 950.9		22

[16] The main point of the approach presented here is that it takes advantage of the valuable information generated by image analyzers or OPCs, namely, the size spectrum information in the sample. Using the relationship presented in Figure 2 or the ones given for each zooplanktonic group or species (Table 1), we can estimate the biomass spectrum for each sample as illustrated in Figure 5. This advantage allows the identification of the biomass contribution of key organisms or at least of a determined size or body area fraction. This is especially interesting with the use of the image analyzers as the visual selection of organisms over a digitally stored sample or the selection of a determined band of the spectrum is almost automatic and straightforward.

[17] The results of biomass obtained in the area we sampled near the Antarctic Peninsula are comparable with other estimates of biomass in the same area. In the Bransfield Strait, values of biomass of the 200–500 μm size fraction in the upper 200 m layer is a rather small percentage of total biomass [*Robins et al.*, 1995] and average values ranged from 32.3 to 162.5 mg dry weight m^{-2} [*Hernández-León et al.*, 1999, 2000; *Cabal et al.*, 2002]. Similarly, the 500–1000 μm size fraction also shows low biomass (range of average values 8.6–52.5 mg dry weight m^{-2}). The >1000μm size fraction is the best represented in terms of biomass in these waters [see *Robins et al.*, 1995] and average values were in the range of 30.5–395.0 mg dry weight m^{-2} [*Hernández-León et al.*, 1999, 2000; *Cabal et al.*, 2002]. Our estimation of biomass agreed with the ranges obtained for the smaller size fractions, while for the larger we found a higher value (Table 2). However, in terms of biomass there are two main peaks in Antarctic waters, one in the 1 to 5 mm which is dominated by large copepods (mainly *Calanoides acutus*, *Calanus propinquus* and *Metridia gerlachei*), and a second one in the 14 to 40 mm range, mainly dominated by large euphausiids [*Boysen-Ennen et al.*, 1991]. Since the size fractionation data available were obtained using standard nets in vertical hauls (mainly the WP-2 net), the large variability in the large size fraction should be related to the underestimation of the large organisms in vertical hauls. The measurement of biomass in this large size fraction is better accounted by using large nets in oblique hauls. In fact, *Boysen-Ennen et al.* [1991] used the Rectangular Mid-water Trawl (RMT 1+8) for their evaluation of zooplankton in Antarctic waters. Their results ranged from 0.8 to 3.6 g dry weight m^{-2} for the <14.5 mm fraction, showing that our biomass for the Antarctic waters are in the lower bound of this range. Our results for the biomass spectrum of different samples (Figure 5) showed that the greatest biomass was in the larger size fraction (>3 mm^2) but inside this class the very large organisms have an important influence on the total biomass of the sample. A few organisms are responsible for the important variability in the average biomass observed (Table 2). This variability is even larger when gelatinous zooplankton dominates the large size fraction (e.g., salps). Therefore the use of vertical hauls in Antarctic waters and the collection of large animals such as euphausiids at random using this sampling procedure imply an important source of variability in the final biomass estimate. The use of oblique hauls in order to account for the patchiness of large zooplankton seems of a paramount importance.

[18] Finally, the use of the individual body area-biomass relationship presented here also provide a useful tool to convert the OPC equivalent circular diameter of net-collected zooplankton into area and then into biomass using the size spectrum of samples provided by this apparatus. However, the use of this relationship to estimate individual biomass from optical devices in situ should be taken with caution. Particles other than zooplankton (e.g., aggregates) can be counted and sized, and assigned erroneously to the body area spectrum. This source of error could only be solved with the use of pattern recognition from imaging devices. This technological step would provide an important amount of data in real or quasi-real time, a prerequisite to match the temporal and spatial scales of data provided by physical and chemical oceanographers, or even by fisheries scientists using acoustics.

[19] **Acknowledgments.** The authors are indebted to two anonymous reviewers for their valuable comments and criticisms. We also acknowledge R. Anadón, cruise leader of Fruela 96 and to the crew and technical staff of the BIO *Hespérides*. This work was supported in part by projects Fruela (ANT94-1010), Breddies (REN2001-2650), and Icepos (REN2002-04165) from the Spanish Ministry of Science and Technology.

References

Alcaraz, M., E. Saiz, A. Calbet, I. Trepat, and E. Broglio (2003), Estimating zooplankton biomass through image analysis, *Mar. Biol.*, *143*, 307–315.

Anadón, R., and M. Estrada (2002), A carbon flux study in productive areas of the Antarctic Peninsula (Fruela cruises), *Deep Sea Res., Part II*, *49*, 567–583.

Beaulieu, S., M. Mullin, V. Tang, S. Pyne, L. Andrews, and B. Twining (1999), Using an optical plankton counter to determine the size distributions of preserved zooplankton samples, *J. Plankton Res.*, *21*, 1939–1956.

Billones, R. G., M. L. M. Tackx, A. T. Flachier, L. Zhu, and M. H. Daro (1999), Image analysis as a tool for measuring particulate matter concentrations and gut content, body size, and clearance rates of estuarine copepods: Validation and application, *J. Mar. Syst.*, *22*, 179–194.

Boysen-Ennen, E., W. Hagen, G. Hubold, and U. Piatkowski (1991), Zooplankton biomass in the ice-covered Weddell Sea, Antarctica, *Mar. Biol.*, *111*, 227–235.

Cabal, J. A., F. Alvarez-Marqués, J. L. Acuña, M. Quevedo, R. Gonzalez-Quirós, I. Huskin, D. Fernández, C. R. del Valle, and R. Anadón (2002), Mesozooplankton distribution and grazing during the productive season in the northwest Antarctic Peninsula (FRUELA cruises), *Deep Sea Res., Part II*, *49*, 869–882.

Corral, J., J. Masso, and M. T. Alvarez-Ossorio (1981), Un estudio comparado preliminar de la biomasa seca, materia orgánica, carbono, nitrógeno, relación C/N y del contenido energético del zooplancton de la Rías de Arosa y Muros, *Bol. Inst. Esp. Oceanogr.*, *6*, 221–240.

Edgerton, H. E. (1981), Electronic flash sources and films for plankton photography, *J. Biol. Photogr.*, *49*, 25–26.

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

Hernández-León, S., S. Torres, M. Gómez, I. Montero, and C. Almeida (1999), Biomass and metabolism of zooplankton in the Bransfield Strait (Antarctic Peninsula) during austral spring, *Polar Biol.*, *21*, 214–219.

Hernández-León, S., C. Almeida, A. Portillo-Hahnefeld, and I. Montero (2000), Biomass and potential feeding, respiration and growth of zooplankton in the Bransfield Strait (Antarctic Peninsula) during austral summer, *Polar Biol.*, *23*, 679–690.

Le Borgne, R. (1975), Equivalences entre les mesures de biovolumes, poids secs, poids secs sans cendres, carbone, azote et phosphore du mésozooplancton de l'Atlantique tropical, *Cah. ORSTOM Ser. Oceanogr.*, *13*, 179–196.

Legendre, P., and L. Legendre (1998), *Numerical Ecology*, 853 pp., Elsevier, New York.

Lovegrove, T. (1966), The determination of the dry weight of plankton and the effect of various factors on the values obtained, in *Some Contemporary Studies In Marine Science*, edited by H. Barnes, pp. 429–467, Allen and Unwin, St. Leonards, N.S.W., Australia.

Mustard, A. T., and T. R. Anderson (2005), Use of spherical and spheroidal models to calculate zooplankton biovolume from particle equivalent spherical diameter as measured by an optical plankton counter, *Limnol Oceanogr.*, *3*, 183–189.

Omori, M., and T. Ikeda (1984), *Methods in Marine Zooplankton Ecology*, 332 pp., John Wiley, Hoboken, N. J.

Postel, L., H. Fock, and W. Hagen (2000), Biomass and abundance, in *ICES Zooplankton Methodology Manual*, edited by R. P. Harris et al., pp. 83–192, Elsevier, New York.

Ricker, W. E. (1973), Linear regression in fishery research, *J. Fish. Res. Board Can.*, *30*, 409–434.

Robins, D. B., R. P. Harris, A. W. Bedo, E. Fernández, T. W. Fileman, D. S. Harbour, and R. N. Head (1995), The relationship between suspended particulate material, phytoplankton and zooplankton during the retreat of the marginal ice zone in the Bellinghausen Sea, *Deep Sea Res., Part II*, *42*, 1137–1158.

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

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

UNESCO (1968), *Zooplankton Sampling*, *Monogr. Oceanogr. Methods*, vol. 2, 174 pp., Paris.

Wiebe, P. H., S. Boyd, and J. L. Cox (1975), Relationships between zooplankton displacement volume, wet weight, dry weight and carbon, *Fish. Bull.*, *73*, 777–786.

S. Hernández-León and I. Montero, Biological Oceanography Laboratory, Facultad de Ciencias del Mar, Campus Universitario de Tafira, 35017 Las Palmas de GC, Canary Islands, Spain. (shernandez@dbio.ulpgc.es)