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Zooplankton biomass estimation from digitized images: a comparison between subtropical and Antarctic organisms

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Abstract

The measurement of mesozooplankton biomass in the ocean requires the use of analytical procedures that destroy the samples. Alternatively, the development of methods to estimate biomass from optical systems and appropriate conversion factors could be a compromise between the accuracy of analytical methods and the need to preserve the samples for further taxonomic studies. The conversion of the body area recorded by an optical counter or a camera, by converting the digitized area of an organism into individual biomass, was suggested as a suitable method to estimate total biomass. In this study, crustacean mesozooplankton from subtropical waters were analyzed, and individual dry weight and body area were compared. The obtained relationships agreed with other measurements of biomass obtained from a previous study in Antarctic waters. Gelatinous mesozooplankton from subtropical and Antarctic waters were also sampled and processed for body area and biomass. As expected, differences between crustacean and gelatinous plankton were highly significant. Transparent gelatinous organisms have a lower dry weight per unit area. Therefore, to estimate biomass from digitized images, pattern recognition discerning, at least, between crustaceans and gelatinous forms is required.

Introduction

Zooplankton plays a central role in structuring pelagic food webs and mediating biogeochemical cycles. Understanding their biomass and distribution within the world ocean is a requisite to predict their contribution to the global organic matter and energy fluxes (Banse 1995). Analytical measurement of biomass using standard methods (see Postel et al. 2000) requires the destruction of the sample (dry weight, ash-free dry weight, elemental analysis of carbon, nitrogen, etc.). To avoid these procedures and allow further taxonomical and ecological studies, nondestructive methods should be used. However, adequate methods for suitable estimation of mesozooplankton biomass are still not standard.

Several optical imaging techniques have been developed over the past decades to examine zooplankton organisms. The use of an electronic flash source was the first tool to capture instantaneously the silhouette of living plankton (Ortner et al. 1979; Edgerton 1981). An in situ silhouette camera system was also designed for zooplankton genera identification and abundance estimation (Ortner et al. 1981). Other systems based on video cameras were developed to classify zooplankton. For instance, a video camera was interfaced to extract the silhouette of preserved organisms and further classify them into different taxonomic groups (Jeffries et al. 1984). Microscope image processing systems have been used to classify and identify different stages of copepods (Dietrich and Uhlig 1984) and to study the size distribution of zooplankton samples (Rolke and Lenz 1984). A modified method was used to extract body area and size of copepods, but only preliminary results concerning analysis and treatment of samples were presented (Gorsky et al. 1989). A submersible 35-mm camera system was also used to compare plankton density from in situ silhouette photographs with concurrent preserved net collections (Olney and Houde 1993). A recent method based on the digitalization of a net sample by a scanner (Zooscan) was also developed to identify and automatically detect zooplankton organisms (Grosjean et al. 2004). The enumeration and measurement of a thousand specimens of zooplankters can be done in a short time, and various morphological parameters such as body length, shape, and area can be extracted. One of the major attributes of the Zooscan is rapid sample processing (Gorsky and Grosjean 2003).

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Because of the evolution of image acquisition techniques of zooplankton, it is now possible to indirectly estimate zooplankton biomass using digital image processing. A high-performance CCD camera mounted on a tripod was used to study *Daphnia magna* population growth using ellipsoid conversion factors to estimate its body volume (Færøvig et al. 2002). Similarly, a CCD video camera installed in a stereomicroscope and connected to a computer was used to measure the length of various zooplankton organisms and calculate the biovolume of the whole sample (Alcaraz et al. 2003). The relationship between the biovolume and biomass was extracted from the integrated samples, although changes in the taxonomical composition of the sample can modify the relationship obtained.

To estimate biomass of the most common species and groups of zooplankton in Antarctic waters, Hernández-León and Montero (2006) used a CCD camera connected to a stereoscope microscope to compare individual biomass with body area. The conversion of the body area spectrum into the biomass spectrum allowed the estimation of total and size-fractionated biomass. This method gave results comparable to previous measures carried out in the same region (see Hernández-León and Montero 2006). Gelatinous organisms like salps and chaetognaths were not considered in this study, however, owing to poor representation in the samples. Biomass of chaetognaths is estimated to be 10% to 30% of that of copepods in the world oceans (Bone et al. 1991), and the tunicate *Salpa thompsoni* is among the most important filter-feeding metazoans of the Southern Ocean, ranking only after copepods in terms of total biomass (see Pakhomov et al. 2002). Thus, the gelatinous forms play a significant role in the transfer of energy to higher trophic levels (Bone et al. 1991), and their biomass estimation is of paramount importance.

The objective of the present study was first to extract the relationship between biomass and digitized body area of the most common taxa of mesozooplankton in subtropical waters around the Canary Islands and then to compare our results with those obtained in the Southern Ocean by Hernández-León and Montero (2006). Second, we tested the suitability of the method to extract biomass for gelatinous organisms. Results indicate that the body area and biomass relationship for subtropical crustaceans fits well with measurements obtained in Antarctic waters for those organisms. As expected, a different relationship for gelatinous plankton was obtained.

Materials and methods

To compare body area with individual biomass, different specimens of copepods, chaetognaths, siphonophores, and euphausiids were sampled around the Canary Islands in vertical hauls from 200 m to the surface during April–May 2006 using a WP-2 net (UNESCO 1968) with a 200-µm mesh. Samples from the net were size fractionated into 200–500, 500–1000, and >1000 µm size classes. The organisms of the different size fractions were gently washed to remove particles and immediately digitized for image processing and analysis.

Salps (*Salpa thompsoni*) were captured in the shelf waters of the Bransfield Strait (Antarctic Peninsula) during January–February 2005. A BIONESS (Bedford Institute of Oceanography Net and Environmental Sensing System) net was deployed in oblique hauls from 400 m depth to the surface. We were unable to digitize them on board; therefore, organisms were then frozen at –20°C for further processing and image analysis.

To obtain the relationship between individual body area and dry weight, the body shape of each organism was photographed and body area was measured on a computer. Salps were gently defrosted from –20°C, and body area was measured by image analysis. The nucleus of the organism was also measured in addition to body area because of its sharper and more visible form for optical devices. Therefore, we also tested the relationship between the nucleus and the biomass of the whole organism.

Organisms were dried using standard procedures (Lovegrove 1966). Except for salps, all specimens were first digitized and then stored at –20°C before drying at 60°C for 24 h, allowing the sample to reach room temperature, avoiding humidity, and then weighed using an ultra microbalance (Sartorius supermicro, ± 0.2 µg).

To generate a digital image of the silhouette of organisms, a standard digital camera with a CCD sensor was used (Nikon D100 equipped with a 55-mm Nikkor macro lens). In this study, each organism was individually photographed whatever its natural position, and the specific dry weight was measured. The camera was positioned at the smallest vertical distance to the organism to have exactly the right focus and maximum resolution. Three neon lamps (8W each) were placed at 5 cm below the transparent tray (Nunc) containing the organisms in a thin layer of distilled water. A frosted glass of 5 mm was used to attenuate and diffract the beam homogeneously. Diaphragm opening diameter, shutter speed, and film sensitivity were controlled to obtain the best contrast to distinguish the silhouette from the background. Image files were stored as Tagged Image File Format (tiff) and processed with a personal computer using the image analysis software Global Lab Image/2. After correcting for all band-level thresholds of the image of each organism, the silhouette was extracted by the program-generated area and stored. The system was calibrated with a micrometer-graduated ruler (Leica) to a resolution of 7.8 µm pixel size for copepods, chaetognaths, siphonophores, and euphausiids and 33.3 µm for salps. Adopted resolutions were suitable for morphometric measurements and recognition to taxonomic group.

Finally, body area of organisms can be considered as an independent variable, because 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 for predictive purposes (Legendre and Legendre 1998), avoiding the use of the model II regression (Ricker 1973).

Results

Our results showed that individual dry mass and body area were best fitted by a power function (see Fig. 1) for subtropical copepods and euphausiids, showing a high correlation coefficient ($r = 0.967$ and $r = 0.955$, respectively). Similar slope coefficients were found in these taxa (1.59 ± 0.027 versus 1.47 ± 0.078; see Table 1). As differences between the regression curves were not significant, we pooled all the data for a general subtropical crustacean relationship between body area and respective dry weight. A high correlation coefficient was again obtained ($r = 0.968$). Subtropical euphausiids showed similar slope coefficients of the area–biomass relationships to those found in Antarctic waters (Hernández-León and Montero 2006), but the intercept values of the regression curve (see Table 1) were significantly different ($P < 0.01$). Similar intercept values of the area–biomass relationships were also observed for the Antarctic and subtropical mesozooplankton, but the slope coefficients were significantly different ($P < 0.01$). A general area–biomass relationship extracted from our results and data from Hernández-León and Montero (2006) for mesozooplankton, euphausiids, and both were also obtained, showing good correlation coefficients ($r = 0.947$, $r = 0.987$, $r = 0.972$, respectively).

As expected, however, gelatinous zooplankton showed quite different regression parameters (Fig. 1). Relationships between individual biomass and area for salps, siphonophores and chaetognaths showed slightly lower correlation coefficients than for crustaceans ($r = 0.902$, $r = 0.926$, and $r = 0.840$, respectively). Significant differences for slope coefficients and intercepts were found within these gelatinous organisms. Regression results for the different zooplankton are given in Table 1.

To compare the methodology for assessment of individual biomass in salps, we obtained a better relationship between the area of the nucleus and their total dry weight (see Table 1) than measurement of the entire body area for the same organisms. The same conclusion was drawn in a previous study (Alcaraz et al. 2003). This relationship will allow establishing a more precise estimation of the dry weight of those transparent organisms.

Discussion

Our results show the usefulness of extracting precise information on individual biomass from direct body area measurements. The question of whether to pool crustaceans into a general relationship from the two different regions, however, is open to debate. In this sense, when all data for subtropical crustaceans (mesozooplankton and euphausiids) were

Table 1. Regression and correlation parameters obtained between body area and individual dry mass for subtropical and Antarctic organisms.

Organism	*a*	*b* ± SE	*r*	*P*	*n*	Body area, mm²	Source
Mesozooplankton	45.72	1.19 ± 0.14	0.886	<0.001	23	0.528–8.644	Hernández-León and Montero 2006
Calanus propinquus	56.43	1.44 ± 0.26	0.777	<0.001	22	3.201–6.244	Hernández-León and Montero 2006
Metridia gerlachei	22.44	1.78 ± 0.26	0.797	<0.001	29	1.061–3.009	Hernández-León and Montero 2006
Rhincalanus gigas	76.71	0.63 ± 0.28	0.518	<0.001	16	5.912–17.402	Hernández-León and Montero 2006
Ostracods	99.46	1.28 ± 0.19	0.885	<0.001	15	1.104–4.338	Hernández-León and Montero 2006
Euphausia superba	87.45	1.34 ± 0.04	0.967	<0.001	71	17.248–369.1	Hernández-León and Montero 2006
Antarctic mesozooplankton[a]	42.38	1.47 ± 0.08	0.828	<0.001	89	0.528–8.644	Hernández-León and Montero 2006
Antarctic crustaceans[a]	41.35	1.52 ± 0.04	0.981	<0.001	160	0.528–369.1	Hernández-León and Montero 2006
All data	36.61	1.52 ± 0.05	0.961	<0.001	176	0.528–369.1	Hernández-León and Montero 2006
Chaetognaths	23.45	1.19 ± 0.13	0.840	<0.001	33	24.9–187.5	This study
Salps sp.	4.03	1.24 ± 0.08	0.902	<0.001	21	170.19–997.49	This study
Salps nucleus sp.	67.66	0.78 ± 0.07	0.940	<0.001	21	10.70–95.57	This study
Siphonophores	43.17	1.02 ± 0.38	0.916	<0.001	9	2.64–59.51	This study
Subtropical euphausiids	43.81	1.47 ± 0.08	0.955	<0.001	17	1.67–9.99	This study
Subtropical copepods	45.25	1.59 ± 0.03	0.967	<0.001	138	0.10–8.31	This study
Subtropical crustaceans	44.78	1.56 ± 0.02	0.968	<0.001	155	0.10–9.99	This study
General mesozooplankton	43.38	1.54 ± 0.03	0.947	<0.001	227	0.10–8.644	This study and Hernández-León and Montero 2006
General euphausiids	49.58	1.48 ± 0.05	0.987	<0.001	88	1.67–369.1	This study and Hernández-León and Montero 2006
General crustaceans[a]	43.97	1.52 ± 0.02	0.972	<0.001	315	0.10–369.1	This study and Hernández-León and Montero 2006

Regression was determined from DW (µg) = $a\ S^b$, where a is the intercept, S is body area in mm², and b is slope. SE, standard error of the regression coefficient r. Body area shows the range observed.
[a]Without *Rhincalanus gigas* (see text).

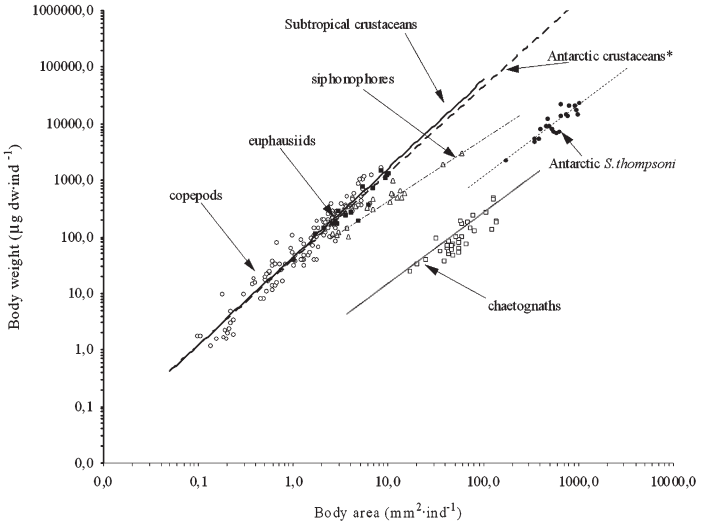

Fig. 1. Relationship between individual body area and individual biomass (as dry weight) of zooplankton in subtropical and Antarctic waters. Dashed line represents the relationship obtained by Hernández-León and Montero (2006) excluding *Rhincalanus gigas* (see text).

combined, the intercept of the potential regression curve for crustaceans from subtropical waters was significantly different ($P < 0.01$) from those from the Southern Ocean, but the slope coefficient was highly similar. Excluding the large, soft-bodied copepod *Rhincalanus gigas* from the general relationship resulted in subtropical and Antarctic crustacean regression parameters being highly similar. Therefore, the problem of extracting a general relationship between biomass and body area depends on the taxonomical composition of the sample. Thus, a general area–biomass relationship for crustaceans ($r = 0.972$) can be obtained for these two quite different ecosystems (excluding *R. gigas*; see Table 1) whatever its dorsal or lateral position. The two regressions obtained for mesozooplankton and euphausiids or even the one observed for the entire range size of crustaceans can be applied in both areas of the ocean.

Gelatinous organisms showed a lower individual biomass per unit area than crustacean zooplankters (Fig. 1). Moreover, the regression parameters of gelatinous organisms showed a higher standard deviation than crustaceans. The main reasons for this are the high amount of water and high variability in organic content of their tissues, as well as the difficulty of the software to accurately detect the edges of these transparent planktonic organisms.

Differences between the regression parameters of salps, siphonophores, and chaetognaths were significant ($P < 0.01$), so a general body area–biomass relationship cannot be applied for the gelatinous groups. Therefore, to estimate biomass from body area–biomass relationships, the crustaceans and gelatinous forms should be discerned. Although a universal relationship is envisaged for crustaceans, this will not be the case for all mesozooplankton, as observed for salps, siphonophores, or chaetognaths. Even crustaceans show important differences among species (see Hernández-León and Montero 2006), and the soft-bodied *Rhincalanus gigas* is a clear example. Thus, the procedure to follow should be the exercise started in Antarctic copepods and euphausiids to obtain single relationships for every taxon. Unfortunately, pattern recognition methods are not a standard at present, but important advances to discern between copepods, euphausiids, and some gelatinous organisms at a genus level can be used for routine work (Grosjean et al. 2004).

These body area and dry mass relationships will allow a reassessment of zooplankton biomass in the world ocean. An important problem in biological oceanography is the very high percentage of zooplankton biomass data estimated using wet weight analysis (Postel et al. 2000). The dry weight to wet weight relationships should be avoided (Le Borgne 1975; Wiebe 1988) because of the poor relationships between the two measurements. Gelatinous forms with their high water content introduce important errors in the estimation of biomass using wet weight. This problem is solved using optical devices and appropriate software.

Since the advent of high-resolution 2D digital image acquisition system (scanners, video, and digital photographic cameras) and the development of simple pattern recognition software (WHOI Silhouette digitizer, Little and Copley 2003; Plankton Visual Analyser, PVA 2005; Zooimage, Zooimage 2006), it is possible to use these optical devices to extract body area of crustacean and gelatinous forms and to estimate biomass without destruction of the organisms. Thus, the use of the body area spectrum generated by simple, low-cost digital cameras or scanners is suggested as a standard method to first estimate biomass and then preserve samples for collection and further taxonomic analysis.

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