Journal of Sea Research 66 (2011) 69–75

Contents lists available at ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Using ZooImage automated system for the estimation of biovolume of copepods from the northern Argentine Sea

R. Di Mauro [a,b,*], G. Cepeda [a,c,d], F. Capitanio [b,c], M.D. Viñas [a,c,e]

[a] Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo No. 1, Escollera Norte CP 7600 Mar del Plata, Argentina
[b] Universidad de Buenos Aires (UBA), Facultad de Ciencias Exactas y Naturales, Intendente Güiraldes 2160, Ciudad Universitaria CP 1428EGA, Buenos Aires, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917 - CP C1033AAJ, Buenos Aires, Argentina
[d] Centro de Estudios de Biodiversidad y Biotecnología-Fundación para Investigaciones Biológicas Aplicadas (CEBB-MdP-FIBA), Vieytes 3103, Mar del Plata, Argentina
[e] Universidad Nacional de Mar del Plata (UNMDP), Facultad de Ciencias Exactas y Naturales, Funes 3250, CP 7600, Mar del Plata, Argentina

ARTICLE INFO

Article history:
Received 10 September 2010
Received in revised form 4 April 2011
Accepted 24 April 2011
Available online 4 May 2011

Keywords:
Zooplankton
Image analysis
Southwestern Atlantic
Biomass

ABSTRACT

A total of 46 samples from coastal and shelf sectors from the northern Argentine Sea (34°–41° S) were digitized to compare the performance of the new ZooImage analysis method for copepod abundance and biovolume estimations. A training set of 1437 objects were used for automatic discrimination using a Random Forest algorithm with a general accuracy of 83.92%. A total of 11 taxa were automatically classified. Copepods were divided in three categories: Large calanoids, small calanoids and cyclopoids and identified with an accuracy of 83.15%, 79.5% and 85.7% respectively.

The discriminant analysis revealed both the equivalent circular diameter (ECD) and the area were the best variables to differentiate the three copepod categories. Samples were previously quantified by optical methods in order to compare with automated results. Automated copepod biovolume measurements were estimated from individual calculations applying new ZooImage allometric parameters, and were compared with manual calculations using specific size/biovolume equations. It was demonstrated that ZooImage can potentially be used as a tool for abundance and biovolume estimations of calanoid and cyclopoid copepods and allow us to obtain results more rapidly by reducing the time lag involved using traditional measuring methods.

1. Introduction

Copepods are among the most numerous multi-celled organisms on Earth (Mauchline, 1998). They are considered keystones in aquatic ecosystems because of their important role in the transfer of matter and energy from primary producers to higher trophic levels (e.g. fish larvae) (Irigoien et al., 2009). Biomass measurements are necessary steps towards quantifying that energy flow across planktonic food webs (Alcaraz et al., 2003), specially in fisheries science, in which most approaches require the incorporation of accurate production estimations of food web components (Christensen and Pauly, 1992). This taxon has been a focus of major international programmes such as GLOBEC (Irigoien et al., 2009).

Most standard biomass indicators may be expressed in terms of wet mass, dry mass, ash-free dry mass, carbon mass or nitrogen mass, but direct methods require destructive analytical procedures, precluding the use of samples for other studies (Alcaraz et al., 2003 and references therein). On the other hand, there are non-destructive estimates of biomass based on a combination of independent factors including counts, biovolume determined from measures of individual body size (length and width), conversion factors determined from body shape and chemical measurements. Biovolume estimates and conversion factors required by indirect methods increase opportunities for error, because error associated with multiple independent factors can be propagated at each stage of calculation (Baguley et al., 2004). However, these procedures preserve the samples and allow further taxonomical and ecological studies.

While traditional methods provide invaluable information about zooplankton species composition and biomass, they are labor-intensive and time-consuming, given that counting and obtaining manual measurements of organisms are necessary for abundance and biomass estimates. In light of these constraints, several optical imaging techniques have been developed over the past decades to examine zooplankton using permanent electronic records, from silhouette photography (Ortner et al., 1979) to a variety of digital imaging technologies that combine with algorithms for machine learning. Consequently, it is now possible to enumerate and measure thousands of zooplankters in a short time, and extract various morphological parameters such as body length, shape, and area. Hence, image acquisition techniques have evolved that indirectly estimate zooplankton biovolume using digital image processing (Benfield et al., 2007; Sieracki et al., 2010).

* Corresponding author at: Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo No. 1, Escollera Norte CP 7600 Mar del Plata, Argentina. Tel.: +54 2234862586; fax: +542234861830.
E-mail address: rdimauro@inidep.edu.ar (R. Di Mauro).

1385-1101/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.seares.2011.04.013

ZooImage is an open software tool that utilizes, a semi-automated method based on image analysis and automated recognition. It was developed to offer a fast, simple, and non-destructive mechanism for broad taxonomic identification of zooplankton, including the estimation of abundance and biovolume with minimum sample manipulation (Benfield et al., 2007; Culverhouse et al., 2006). Despite the growing worldwide interest in the application of this software (Benfield et al., 2007; Gislason and Silva, 2009), few studies to date have used it to estimate copepod biomass (Zarautz et al., 2008). Image analysis though, has long been used for biovolume estimation of copepods and zooplankton (Alcaraz et al., 2003; Baguley et al., 2004; Billones et al., 1999; Clark et al., 2001).

In the Argentine Sea, copepods are one of the richest and taxonomically best known zooplankton groups (Bradford-Grieve et al., 1999). Even though they have been the subject of many studies focused in their diversity and spatial distribution (Berasategui et al., 2006; Cepeda, 2006; Di Mauro et al., 2009; Fernández Aráoz et al., 1994; Marrari et al., 2004; Ramírez, 1969, 1970; Ramírez and Santos, 1994; Santos and Ramírez, 1991; Viñas et al., 2002), biomass estimates remain scarce (Fernández Aráoz et al., 1991; Viñas et al., 2010).

In the present study we undertake a comparison between traditional and ZooImage automated methods to estimate copepod biovolume for the first time in the northern Argentine Sea region. We suggest new allometric parameters to obtain direct estimates of calanoid and cyclopoid biovolume with ZooImage.

2. Materials and methods

2.1. Samples collection and laboratory analysis

A total of 46 samples were collected with a 220-μm meshed small bongo net (0.20 m diameter), on three cruises carried out in coastal and shelf sectors off the Buenos Aires province (34°S–41°S) during spring 2002 to 2004, as part of the *Engraulis anchoita* assessment project of INIDEP (Instituto Nacional de Investigación y Desarrollo Pesquero) (Fig. 1). Oblique tows were performed from a depth near the bottom to the surface without clogging of the net. Filtered water was estimated from a Hydrobios flowmeter placed at the mouth of the net. The ship moved at 2.5 knots and tows were short in duration (towing time: 2 minutes; towing rate: 20 m/minute). Samples were preserved in 4% formaldehyde for further analysis.

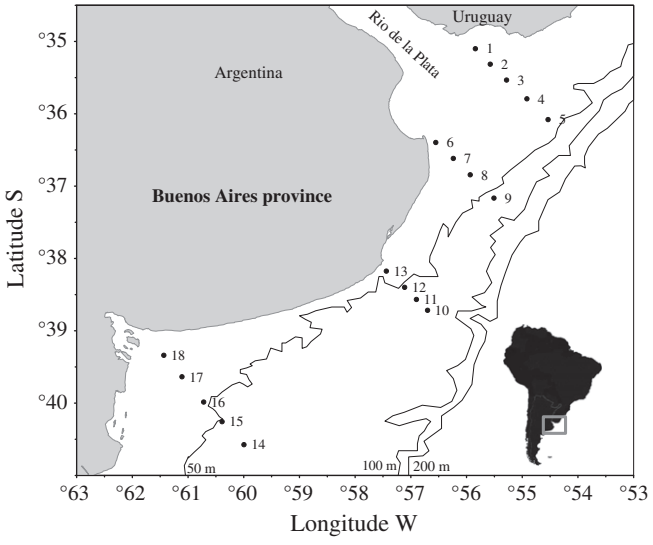

Fig. 1. Distribution of zooplankton sampling stations (1 – 18) of the study area.

Before image analysis, all samples were quantified by traditional optical methods in order to compare with automated results. Copepod species present in the samples were identified and counted under a compound microscope. For this purpose, a subsample was obtained from each sample and its volume determined in order to include at least 100 adults of the dominant copepod species. Taxonomic identification of copepods was based on the current literature for the region (e.g., Björnberg, 1981; Bradford-Grieve et al., 1999; Ramírez, 1970, 1971, 1981; Ramírez and Sabatini, 2000).

In order to compare manual and automaedc counts of copepods, the species were arranged in 3 categories consistent with those of the training set (Section 2.3).

2.2. Image acquisition

Samples were first mixed thoroughly in a beaker, in a total volume that varied between 200 mL and 600 mL depending on the zooplankton density in each sample. Then, each sample was sieved into two fractions using a 500 μm mesh to separate small and large mesozooplankton. From each fraction A and B (<500 μm and >500 μm respectively) sufficient aliquots (approximately 30 mL approximately) were taken and stained with Bengal Rose for 24 hours to ensure good contrast at the time of scanning, and avoid counting the detrital material at the time of processing the images. Then, aliquots were distributed in 3 polystyrene cells (127×85 mm) to be scanned. This procedure must allow the best representation of the zooplankton diversity of each sample with minimum overlap of the animals. Nevertheless, some manipulation may be necessary, because specimens near the borders and those floating in the surface must be manually positioned.

Following the procedure mentioned above a total of 46 zooplankton samples were digitized using a commercial scanner (Epson Perfection Photo 4490, Epson Scan software).

From the scanning process at a resolution of 1200 dpi, 276 raw images were obtained, including 6 images per sample (3 from each fraction A and B). At the end of the process, the 6 images were integrated, brightness and contrast (+41 and +15 points respectively) were adjusted for their processing using ZooImage software, version 1.2-1 (http://www.sciviews.org/zooimage). The number of individual vignettes (individual images automatically extracted from raw images) obtained from each fraction, fluctuated between 200 and 600. Once the process of automatic vignette extraction and classification is complete, raw images from fraction A and B are integrated and only one result per sample is delivered. Fig. 2 shows a summary of sample treatment.

2.3. Creation of the training set

From the sampling stations, a series of 18 zooplankton samples corresponding to different locations and years were chosen in order to represent the diversity of the study area. The training set was established by selecting and sorting individual images (vignettes) of the organisms following the procedure of Grosjean and Denis (2007). Thus, the training set was built from a pool of 1437 vignettes from those 18 samples. It was possible to set a total of 13 zooplankton categories in which copepods were manually sorted into 3 subcategories: Large calanoids (LC), small calanoids (SC) and cyclopoids (CYC), relying on morphological keys and sizes. LC and SC were also classified as dorsal or lateral, given the variety of postures in which they may be found in the scanning cells.

Once the training set was created, the Random Forest algorithm was selected to build the classifier (training set + learning algorithm) according to Grosjean et al. (2004). Other algorithms provided by ZooImage, such as Linear Discriminant Analysis and Neural Networks were tested during our preliminary work, but the former offered better results. The classifier performance was then assessed by pooling the automatic identifications and comparing them to the manual identification of same individuals. This was done using a confusion matrix,

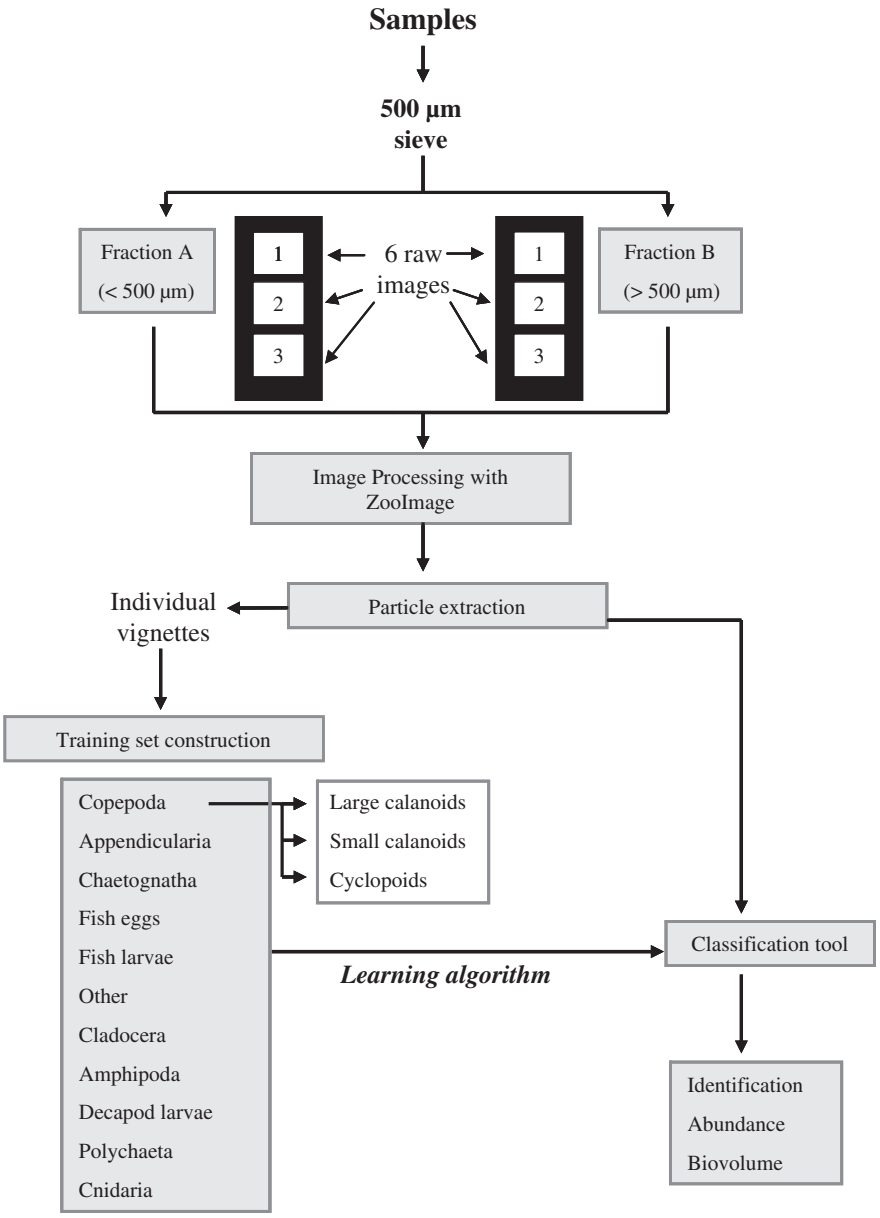

Fig. 2. Summary of samples treatment using the ZooImage automated method. Each sample is fractioned with a 500 µm sieved and 6 raw images are obtained for each of the two fractions after the scanning process. Each image is analysed using ZooImage with particle extraction. Some individual vignettes (simple images of each organism) are used to build the training set. After applying a learning algorithm, the classification tool is created and all particles can be then identified to produce abundance and biovolume data.

which is the diagnostic tool of the classifier efficiency. A confusion matrix is a square contingency table that compares all groups of the manual classification with all groups of the automatic recognition (see Table 1). The number of items in each cell corresponds to the counting of objects. The diagonal (from top-left to bottom-right) corresponds to the cells where both identifications are the same. In other words, this diagonal represents the correct counting of predicted items. All cells outside the diagonal depict disagreement in both classifications. They are usually errors in the automated classifier, assuming that there is no error in the manual training set (Grosjean and Denis, 2007).

2.4. Biovolume calculation using new allometric parameters

ZooImage calculates the surface area of the organisms from the number of pixels contained in its two-dimensional images. The individual area is defined by the silhouette of the organism after changes in its grey-level threshold, and is then automatically transformed into an ellipse of equivalent area with its major and minor axes scaled to the general shape of the organism (Alcaraz et al., 2003 and references therein). Consequently, the equivalent circular diameter (ECD) is created, which represents the estimated size of each organism and the most accurate measurement that can be obtained automatically.

To calculate biomass (B) ZooImage uses the ECD (mm) in the following equation:

$$B = (P_1 * ECD + P_2)^{P3} \quad (1)$$

where, P_1, P_2 and P_3 are allometric parameters. These parameters describe the relationship between (ECD) and other dimensions generally used in size/biomass equations taken from the literature (prosome length, prosome width, etc). As default values, ZooImage provides $P_1 = 1$, $P_2 = 0$ and $P_3 = 1$, in which case, the general Eq. (1) is thus limited to the ECD (mm).

Table 1
Confusion matrix obtained for the training set classed by the random forest algorithm. Each row of the matrix represents the groups in the training set labeled by the user, whereas columns (1–15, same categories as rows) show the classification by ZI. There are 15 categories because of the additional lateral and dorsal positions of SC and LC. The numbers in the diagonal line (in grey, from upper left to bottom right) represents the correct classification of vignettes, while those outside are misclassified individuals (false positives). Gen. Acc: General accuracy in classification performance, Acc. %: accuracy of each group.

		ZooImage classification prediction														
	Gen. Acc.: 83.92%															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
User classification in training set	Polychaetes (1)	2	0	4	0	1	0	0	1	2	1	6	0	0	0	0
	Appendicularians (2)	2	62	0	0	1	0	6	0	0	1	2	5	0	0	2
	Large calanoids dorsal (3)	0	0	111	13	0	0	0	0	2	0	2	0	0	0	0
	Large calanoids lateral (4)	0	0	8	90	0	1	0	0	0	1	4	0	0	0	0
	Chaetognaths (5)	0	3	0	0	53	0	0	0	0	0	0	0	0	0	3
	Amphipods (6)	2	1	0	6	0	22	2	0	0	2	8	0	0	0	0
	Cladocera (7)	0	1	0	0	0	0	192	0	1	1	3	0	0	0	0
	Cnidarians (8)	1	2	0	0	0	1	1	29	0	0	3	0	0	0	0
	Small calanoids dorsal (9)	1	0	0	0	0	0	1	0	132	22	0	6	0	0	0
	Small calanoids lateral (10)	0	0	0	1	0	0	5	0	16	123	2	10	0	0	0
	Other crustacea (11)	0	1	4	3	0	2	4	0	0	3	100	1	1	0	0
	Cyclopoids (12)	0	3	0	0	0	0	0	0	5	8	0	132	0	0	0
	Decapods (13)	0	0	0	0	0	4	2	0	0	1	3	0	3	0	0
	Fish eggs (14)	0	0	0	0	0	0	0	0	0	0	0	0	0	124	0
	Fish larvae (15)	0	3	1	0	6	1	0	0	0	0	6	0	1	0	31
	Total	8	76	128	113	61	31	213	30	158	163	139	154	5	124	36
	Acc. (%)	25	81.6	86.7	79.6	86.9	71	90.1	96.7	83.5	75.5	71.9	85.7	60	100	86.1

In this study, individual biomass was calculated by estimating biovolume. By changing the value of the allometric parameters and replacing them with those taken from the volume equation of a sphere, the volume of each organism (V) is then estimated by the calculation of the corresponding volume of revolution ellipsoids. To do that we consider first:

$$V = 4/3\ \Pi r^3 \quad (2)$$

where the radius (r) of the equivalent circle can be estimated using:

$$ECD = 2r \quad (3)$$

and then the ECD can be replaced in the volume Eq. (1) as follows:

$$V = 4/3\ \Pi\ (ECD/2)^3. \quad (4)$$

Now, in Eq. (4), the volume is referred to the object with a particular ECD value, and when solving this equation, the allometric parameters can be replaced in Eq. (1) by: $P_1 = \Pi/6$, $P_2 = 0$ and $P_3 = 3$.

During the ZooImage procedure with the above modifications of the allometric parameters, individual biovolume estimates of the organisms are added. Hence, sample biomass can be expressed in mm^3m^{-3} or any other biomass indicator by applying the corresponding conversion factors. Body wet weight can be derived from measurements of body biovolume by applying a factor of 1.025 for specific gravity (Chojnacki, 1983). Then, dry weight is generally obtained by multiplying the wet weight by 0.20 (Cushing et al., 1958) and the carbon content can be considered to be 40% of the dry weight (Postel et al., 2000).

To validate ZooImage calculations of individual biovolume, a series of 19 vignettes of each copepod category were selected from the training set to manually measure their prosome width (PW) using Image J (an application from the ZooImage package). PW was chosen in order to apply the following specific size/biovolume equations obtained by Viñas et al. (2010) from the geometric method:

Oithona nana: LogV: 2.751 log PW + 0.502 ($r^2 = 0.99$, $p < 0.0001$)
Ctenocalanus vanus and *Paracalanus parvus*: LogV: 3.120 log PW - 0.155 ($r^2 = 0.99$, $p < 0.0001$)
Calanoides carinatus: LogV: 3.213 log PW - 0.421 ($r^2 = 0.99$, $p < 0.0001$)

where V: volume (mm^3). These equations were chosen as representatives of the three copepod categories (CYC, SC and LC, respectively), because those species dominated in the copepod community.

The whole set of manual biovolume results were compared with ZooImage biovolume calculations on the same vignettes using the parameters estimated above ($P_1 = 0.523$, $P_2 = 0$ and $P_3 = 3$).

2.5. Data analysis

Discriminant analysis was carried out using Infostat v. 1.1 (UNC, 2002), to evaluate which of the morphometric variables used by ZooImage separated the three copepod categoriesmost effectively. A Student's *t*-test was applied both to the abundance and to the biovolume data, (log transformed), to analyze differences between estimates of automatic and manual calculations (Sokal and Rohlf, 1995). Statistica package 7.0 (StatSoft, Inc., 2004) was used for abundance and biovolume data analyses.

3. Results

The general estimated accuracy for the ZooImage classification of 13 taxonomic categories was 83.92%. The confusion matrix is provided in Table 1.

ZooImage was accurate in classifying the vignettes of the majority of the groups, particularly the fish egg category that achieved 100% accuracy. For polychaetes it was less accurate, probably because there were insufficient vignettes in the training set for this category. Nevertheless, this study focused on the classification of the three copepod categories which are the dominant members of the community. Cyclopoids were classified with 85.7% accuracy. In the case of large and small calanoids the classifier achieved accuracy between 79.5% and 85.7%, considering both dorsal and lateral views of copepods.

In order to evaluate the degree of size overlap among copepod categories, it was necessary to examine ZooImage automated classification of copepod vignettes. When the copepod size distribution was represented in a scatter plot graph showing their minor and major axis, it was apparent that smaller copepods (cyclopoids and small calanoids) caused more confusion to the program, (Fig. 3). In addition, from the actual review of classified vignettes, it was

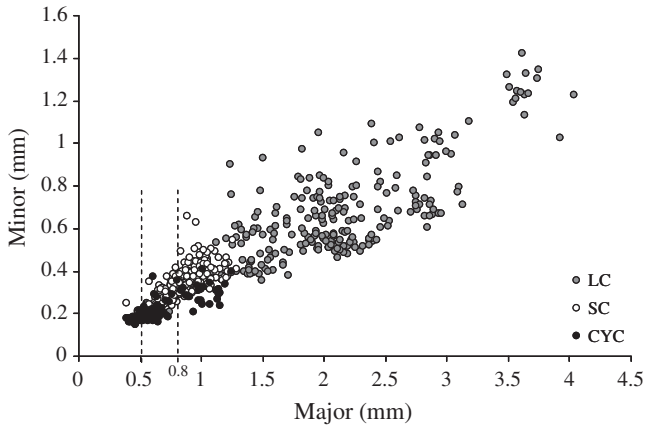

Fig. 3. Distribution of copepod categories in the training set according to major (=length) and minor (=width) axis. Data were log transformed. Copepod size spectra with major superposition in the scatter plot are indicated between dash lines. LC: large calanoids, SC: small calanoids, CYC: cyclopoids.

apparent that some copepods from small calanoids category were classified as cyclopoids and also some cyclopoid copepods were misclassified as small calanoids.

The discriminant analysis revealed that 94.84% of the total variance could be explained by the first canonical function and both the ECD and the Area were the best variables to differentiate the three copepod categories (Table 2). According to the cross validation results for the copepod classification, a total error of 12.54% was detected in reporting minimum and maximum errors (6.14% and 16.89%) for large calanoid and cyclopoid categories respectively (Table 3).

No significant differences (p value>0.05) for the total copepod counts were detected between both methods either for the large or for the small calanoid categories, in spite of the error in the automated classification performance. Cyclopoids showed significant but borderline significance (p value: 0.044), demonstrating that the software is less accurate while treating very small organisms (Table 4). Nevertheless, these results allowed comparison of total biovolume estimates by both methods and there were no significant differences when new allometric parameters were used (*t* test, p = 0.572).

4. Discussion and conclusions

The ability of ZooImage to correctly identify particles from scanned images is directly related to how well the training set represents the zooplankton composition of the samples to be analyzed (Culverhouse et al., 1994; Embleton et al., 2003). Significant knowledge of the diversity of the area is needed for a good performance in the selection of the training set categories and the interpretation of the scanned images. In this study all the samples were previously analyzed under a microscope, so the probability of finding to find an unknown organism was clearly reduced at the time of building the training set.

From the confusion matrix, we observed that ZooImage was less accurate in classifying some of the groups. Polychaetes, amphipods, decapods, and fish larvae were present at very low density compared to more abundant small animals, such as copepods. Consequently, there were poorly represented in the training set, which probably reduced accuracy below 70%. The possibility of increasing the performance in the classification of these categories might lie in the increment of the number of vignettes in the training set or in the modification of the aliquot for fraction B (see Section 2.2). With a greater aliquot for fraction B, more vignettes corresponding to these misclassified organisms could be obtained, which would produce a better representation of the zooplankton diversity.

Within copepods, the automated recognition algorithm was mostly confused when trying to differentiate the small calanoids from the cyclopoid categories. Although the general classification accuracy was higher than 80%, the actual review of vignettes demonstrated that within a certain range of body dimensions both categories were misclassified and mixed even if adults were compared. This error was probably due to the size threshold, below which the scanner did not capture sufficient morphological details from the images, at a resolution of 1200 dpi, to differentiate both copepod orders. Misclassification of copepods of the smaller size classes with ZooImage method was also reported by Gislason and Silva (2009). These authors concluded that shape and size were important features during the machine learning process, but simultaneously other image properties are taken into account for the analysis. As pointed out by Fernandes et al. (2009), it may be difficult to establish how different features are used by classification algorithms. Here, even with misclassification between smaller size copepod categories, ZooImage was considered to be successful in the quantification of copepods in a broad sense. We believe, higher resolution of the images may improve results for small particles (<0.8 mm total length) that may confuse cyclopoid and small calanoid categories.

In this work, conventional microscope abundance estimates were taken as an absolute standard. Although we know this is not completely true, and that manual estimates carry their own error depending on the experience of the researcher, it was necessary to have a starting point to compare automated estimates. In this sense, there are a few comparisons of automated and traditional counting of mesozooplankton. In

Table 2
Eigenvalues and canonical functions (Can. Func.) from discriminant analysis for copepod category classification. Var. (%): percentage of variance explained by each canonical function. Acum. (%): Accumulated Variance. The box shows the variables from the first canonical function that best explain the separation of copepod categories.

Eigenvalues	Var. (%)	Acum. (%)
5,01	94,84	94,84
0,27	5,16	100

Can. Func.	1	2
Constant	−7,10	−3,06
ECD	14,60	21,26
Area	−5,46	−3,72
Perim.	0,23	−0,75
Width	−0,56	−0,12
Height	−0,62	−0,19
Major	0,69	−5,71
Minor	−2,51	−0,23

Table 3
Cross validation table for the classification of the three copepod categories. LC: large calanoids, SC: small calanoids, CYC: cyclopoids.

Group	LC	SC	CYC	Total	Error (%)
LC	**241**	13	1	228	6.14
SC	0	**256**	46	302	15.26
CYC	0	25	**123**	148	16.89
Total	214	294	170	**678**	**12.54**

Table 4
Automated and manual mean abundances (log [ind.m^{-3} + 1]) for the three categories of copepods and for the total counts. CYC: cyclopoids, SC: small calanoids, LC: large calanoids. p: *P*-value.

	CYC	SC	LC	Total
Manual mean ± SD	2.35 ± 0.46	2.98 ± 0.36	1.67 ± 0.57	2.37 ± 0.71
Automatic mean ± SD	2.67 ± 0.39	3.10 ± 0.43	1.82 ± 0.66	2.34 ± 0.87
p	0.044	0.418	0.494	0.8

coastal waters off Concepcion, Chile, Manriquez et al. (2009) reported differences up to an order of magnitude for some mesozooplankton groups, but the quantitative dominance within the mesozooplankton community did not show major differences with both analyses. Also, copepods were classified in small and large categories, but no further details were mentioned as this comparison was not the main focus of their study. In addition, Bell and Hopcroft (2008), and Gislason and Silva (2009), reported that ZooImage was able to capture trends in mesozooplankton abundance and identify major taxa, but small copepods (<1 mm) were poorly classified.

In the present work, ZooImage was set up to calculate individual biovolume automatically, which is feasible to convert into other biomass units using published conversion factors. Individual biovolume estimations of copepods have been used extensively mesozooplankton studies. Even though all of these studies were based on the principles of geometric volume, they used approaches different than ours. Some used body dimensions (Alcaraz et al., 2003; Halliday, 2001; Svetlichny, 1983; Viñas et al., 2010), whereas others used drawings of the organisms and further geometric or morphometric approximations (Gilabert, 2001; Rodriguez et al., 1987). Others used several image techniques obtain semi-automatic length measures (Billones et al., 1999; Jeffries et al., 1984; Roff and Hopcroft, 1986; Rolke and Lenz, 1984).

It is known that the size of adult copepods depends on environmental factors acting during larval development (Gaudy and Verriopoulos, 2004), with temperature and food availability as the most important ones (Riccardi and Mariotto, 2000 and references therein). In the study area, seasonal variation in size has been described for some small species such as *Acartia tonsa* (Hoffmeyer and Torres, 2001), *Euterpina acutifrons* (Viñas and Gaudy, 1996) and *Oithona nana* (Temperoni et al., 2011).

The estimation of biovolume using a parameter like ECD, involves intrinsic measures of total length and width of the animals. This strategy allows the introduction of seasonal and latitudinal variability in copepods sizes from temperate waters (Conover and Huntley, 1991; Uye and Sano, 1998; Viñas and Gaudy, 1996) in the biovolume calculations as part of present ZooImage application.

The application of a non-destructive method such as ZooImage for the estimation of zooplankton abundance and biovolume proved to be an appropriate suitable and rapid way to obtain reliable results that otherwise would take months to achieve. In addition, our primary concern regarding ZooImage biovolume was simply to ascertain the feasibility of the automated results without major changes to the software settings. In addition however, our new ZooImage allometric parameters for individual copepod biovolume estimation could be extended to other components of the zooplankton community, given that it can be converted into other biomass indicators. Although we do not dismiss the method of Grosjean and Denis (2007), in which digital measurements of individual vignettes for each taxa have to be made in order to set the appropriate allometric parameters and relate them with a carbon content equations, it is easy to take advantage of the ECD measurements provided by the software and relate them to the volume of corresponding spheres.

We consider that this automated method encourages application in zooplankton time series within the frameworks of international programmes such as IGBP, IOC or GLOBEC, which relate to global warming and ocean observation (Valdés et al., 2007). In this sense, at the beginning of this decade, time series analysis was encouraged within several projects of the Pelagic Fisheries Programme at INIDEP. In the Northern Argentine Sea, the *Engraulis anchoita* assessment project recently incorporated ZooImage as a tool to process samples collected at a mesoscale resolution. In addition, the Marine Plankton Dynamics and Climate Change program (DiPlaMCC) of INIDEP started automated analysis of monthly samples collected at the permanent environmental studies station (EPEA) (Di Mauro and Viñas, 2009).

The fact that copepods constitute the main source of food for many fish species of economic importance in the study area (Angelescu, 1982; Pájaro, 2002; Viñas and Ramírez, 1996), and the potential to use a rapid non-destructive method to assess their abundance and biomass, will provide an improved strategy for zooplankton studies by reducing the time lag associated with traditional methods.

Acknowledgments

This study was partially supported by funds from the Agencia Nacional de Promoción Científica y Técnológica (PICT No. 15227/03) and Universidad de Buenos Aires (UBACyT X413). We are grateful to Jorge Hansen, former head of the Anchovy Project of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), and to the crew of the "Capitán Oca Balda" and "Dr. Eduardo Holmberg" RVs for the collection of plankton samples. We thank To Dr Xavier Irigoien who kindly assisted us with ZooImage use and protocols. We also thank Dr Phil Culverhouse and the SCOR Working Group 130, for the constructive comments and suggestions of an earlier version of the manuscript. We are especially grateful to anonymous reviewers for their helpful comments that greatly improved the first version of our work. This is INIDEP contribution No. 1660.

References

Alcaraz, M., Saiz, E., Calbet, A., Trepat, I., Broglio, E., 2003. Estimating zooplankton biomass through image analysis. Mar. Biol. 143, 307–315.
Angelescu, V., 1982. Ecología trófica de la Anchoíta del Mar Argentino (*Engraulidae, Engraulis anchoita*). Parte II. Alimentación, Comportamiento y Relaciones Tróficas en el Ecosistema: Ser. Contr. Inst. Nac. Inv. Des. Pesq. 409, INIDEP.
Baguley, J.G., Hyde, L.J., Montagna, P.A., 2004. A semi-automated digital microphotographic approach to measure meiofaunal biomass. Limnol. Oceanogr. Methods 2, 181–190.
Bell, J.L., Hopcroft, R.R., 2008. Assessment of ZooImage as a tool for the classification of zooplankton. J. Plankton Res. 30, 1351–1367.
Berasategui, A.D., Menu-Marque, S., Gómez- Erache, M., Ramírez, F.C., Mianzan, H.W., Acha, E.M., 2006. Copepod assemblages in a highly complex hydrographic region. Est. Coast. Shelf Sci. 66, 483–492.
Benfield, M.C., Grosjean, P., Culverhouse, P., Irigoien, X., Siracki, M.E., Lopez-Urrutia, A., Dam, H., Hu, Q., Davis, C.S., Hansen, A., Pilskaln, C.H., Riseman, E., Schultz, H., Utgoff, P.E., Gorsky, G., 2007. Research on Automated Plankton Identification. Oceanography 20, 172–187.
Billones, R.G., Tackx, M.L.M., Flachier, A.T., Zhu, L., Daro, M.N., 1999. Image analysis as a tool for measuring particulate matter concentration and gut content, body size, and clearance rates of estuarine copepods: validation and application. J. Mar. Syst. 22, 179–194.
Björnberg, T.K.S., 1981. Copepoda. In: Boltovskoy, D. (Ed.), Atlas del Zooplancton del Atlántico Sudoccidental y Métodos de Trabajo con el Zooplancton Marino. : Publicación especial INIDEP. Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, Argentina, pp. 587–679.
Bradford-Grieve, J.M., Marrkoseva, E.L., Rocha, C.E.F., Abichi, B., 1999. Copepoda. In: Boltovskoy, D. (Ed.), South Atlantic Zooplankton. Backhuys Publishers, Leiden, The Netherlands, pp. 869–1098.
Cepeda, G., 2006. Variación espacial de la biodiversidad mesozooplanctónica en un sector de la plataforma costera bonaerense (34°–41°S). Tesis de grado, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina, 44 pp.
Chojnacki, J., 1983. Standard weights of the Pomeranian Bay copepods. Internationale Revue der gesamten Hydrobiologie 68, 435–441.
Christensen, V., Pauly, D., 1992. ECOPATH II- A software for balancing steady-state ecosystem. Ecol. Model. 61, 169–185.
Clark, D.R., Khalid, V.A., Graeme, C.H., 2001. Zooplankton abundance and community structure over a 4000 km transect in the north-east Atlantic. J. Plankton Res. 23, 365–372.
Conover, R.J., Huntley, M., 1991. Copepods on ice-covered seas – distribution, adaptations to seasonally limited food, metabolism, growth patterns and life cycle strategies in polar seas. J. Mar. Syst. 2, 1–41.
Culverhouse, P., Ellis, R., Simpson, R., Williams, R., Pierce, R.W., Turner, J.T., 1994. Automatic categorization of five species of *Cymatocylis* (Protozoa, Tintinnida) by artificial neural network. Mar. Ecol. Prog. Ser. 107, 273–280.
Culverhouse, P., Williams, R., Benfield, M., Flood, P.R., Sell, A.F., Mazzocchi, M.G., Buttino, I., Sieracki, M., 2006. Automatic image analysis of plankton: Future perspectives. Mar. Ecol. Prog. Ser. 312, 297–309.
Cushing, D.H., Hunphrey, G.H., Banse, K., Laevastu, T., 1958. Report of the committee on terms and equivalents. Rapp. P.-V. Réun. Cons. Int. Explor. Mer. 144, 15–16.
Di Mauro, R., Capitanio, F., Viñas, M.D., 2009. Capture efficiency for small dominant mesozooplankters (Copepoda, Appendicularia) off Buenos Aires Province (34°S-41°S), Argentine Sea, using two plankton mesh sizes. Braz. J. Oceanogr. 57, 205–214.
Di Mauro, R., Viñas, M.D., 2009. Primeros resultados sobre la aplicación de métodos automáticos (ZooImage) para la estimación de abundancia y biomasa de copépodos en el Mar Argentino. Informe de investigación. Contribución INIDEP N° 052.
Embleton, K.V., Gibson, C.E., Heaney, S.I., 2003. Automated counting of phytoplankton by pattern recognition: a comparison with a manual counting method. J. Plankton Res. 25, 669–681.
Fernandes, J.A., Irigoien, X., Boyra, G., Lozano, A.J., Inza, I., 2009. Optimizing the number of classes in zooplankton classification. J. Plankton Res. 31, 19–29.

Fernández Aráoz, N., Péres Seijas, G.M., Viñas, M.D., 1991. Asociaciones planctónicas en la zona común de pesca Argentino – Uruguaya en relación con los parámetros ambientales. Primavera 1986. Frente Marítimo 8, 85–99.
Fernández Aráoz, N., Santos, B., Ramírez, F., 1994. Análisis ecológico de la distribución de los copépodos planctónicos de una campaña de primavera, en la Zona Común de Pesca. Frente Marítimo 15, 133–140.
Gaudy, R., Verriopoulos, G., 2004. Spatial and seasonal variations in size, body volume and body proportion (prosoma:urosome ratio) of the copepod *Acartia tonsa* in a semi-closed ecosystem (Berre lagoon, western Mediterranean). Hydrobiologia 513, 219–229.
Gilabert, J., 2001. Seasonal plankton dynamics in a Mediterranean hypersaline coastal lagoon: the Mar Menor. J. Plankton Res. 23, 207–217.
Gislason, A., Silva, T., 2009. Comparison between automated analysis of zooplankton using ZooImage and traditional methodology. J. Plankton Res. 31, 1505–1516.
Grosjean, P.H., Picheral, M., Warembourg, C., Gorsky, G., 2004. Enumeration, measurement, and identification of net zooplankton samples using the ZOOSCAN digital imaging system. ICES J. Mar. Sci. 61, 518–525.
Grosjean, P., Denis, K., 2007. ZooImage users manual. www.sciviews.org/zooimage.
Halliday, N.C., 2001. A comparison of morphometric and geometric methods for estimation of individual zooplankton volumes. Sarsia 86, 101–105.
Hoffmeyer, M., Torres, E.R., 2001. Morphometric variables and individual volume of *Eurytemora americana* and *Acartia tonsa* females (Copepoda, Calanoida) from the Bahía blanca estuary, Argentina. Hydrobiologia 459, 73–82.
Irigoien, X., Fernandes, J.A., Grosjean, P., Denis, K., Albaina, A., Santos, M., 2009. Spring zooplankton distribution in the Bay of Biscay from 1998 to 2006 in relation with Anchovy recruitment. J. Plankton Res. 31, 1–17.
Jeffries, H.P., Berman, M.S., Poularikas, A.D., Katsinis, C., Melas, I., Sherman, K., Bivins, L., 1984. Automated sizing, counting and identification of zooplankton by pattern recognition. Mar. Biol. 78, 329–334.
Manriquez, K., Escribano, R., Hidalgo, P., 2009. The influence of coastal upwelling on the mesozooplankton community structure in the coastal zone off Central/Southern Chile as assessed by automated image analysis. J. Plankton Res. 31, 1075–1088.
Marrari, M., Viñas, M.D., Martos, P., Hernández, D., 2004. Spatial patterns of mesozooplankton distribution in the Southwestern Atlantic Ocean (34°-41°S) during austral spring: relationship with the hydrographic conditions. ICES J. Mar. Sci. 61, 667–679.
Mauchline, J., 1998. The Biology of Calanoid copepods. Advances in Marine Biology, Vol. 33. Academic Press, New York.
Ortner, P.B., Cummings, S.R., Aftring, R.P., 1979. Silhouette photography of oceanic zooplankton. Nature 227, 50–51.
Pájaro, M., 2002. Alimentación de la anchoíta argentina (*Engraulis anchoita* Hubbs y Marini, 1935) (Pises: Clupeiformes) durante la época reproductiva. Rev. Invest. Desarr. Pesq. 15, 111–125.
Postel, L., Fock, H., Hagen, W., 2000. Biomass and Abundance. In: Harris, R.P., Wiebe, P.H., Lenz, J., Skjoldal, H.R., Huntley, M. (Eds.), ICES Zooplankton Methodology Manual. Academic Press, London, pp. 83–192.
Ramírez, F.C., 1969. Copépodos planctónicos del sector bonaerense del Atlántico Suroccidental. Serie Cont. Inst. Biol. Mar. 98.
Ramírez, F., 1970. Copépodos planctónicos del sector patagónico. Resultados de la campaña "pesquería XI". Phycis 79, 473–476.
Ramírez, F.C., 1971. Copépodos planctónicos de los sectores bonaerense y norpatagónico; resultados de la campaña "Pesquería III". Revista del Museo La Plata (Nueva Serie) Sección Zoología, La Plata, 11, pp. 73–94.
Ramírez, F.C., 1981. Zooplancton y producción secundaria. Parte I: Variación y distribución estacional de los copépodos. Contr. Inst. Nac. Invest. Des. Pesq. 383, 202–212.
Ramírez, F., Santos, B., 1994. Análisis del zooplancton de la plataforma bonaerense en relación con algunas variables ambientales: Campañas "Transección" de 1987. Frente Marítimo 15(A), 141–156.
Ramírez, F.C., Sabatini, M., 2000. The occurrence of Calanidae species in waters off Argentina. Hydrobiologia 439, 21–42.
Riccardi, N., Mariotto, L., 2000. Seasonal variations in copepod body length: a comparison between different species in the Lagoon of Venice. Aq. Ecol. 34, 243–252.
Rodriguez, J., Jimenez, F., Bautista, B., Rodriguez, V., 1987. Planktonic biomass spectra dynamics during a winter production pulse in Mediterranean coastal waters. J. Plankton Res. 9, 1183–1194.
Rolke, M., Lenz, J., 1984. Size structure analysis of zooplankton samples by means of an automated image analyzing system. J. Plankton Res. 6, 637–645.
Roff, J.C., Hopcroft, R.R., 1986. High precision microcomputer based measuring system for ecological research. Can. J. Fish. Aquat. Sci. 43, 2044–2048.
Santos, B., Ramírez, F.C., 1991. Variación estacional de las asociaciones de copépodos en una transecta de la plataforma Argentina. Boll. Inst. Oceanogr. Sao Paulo 39, 71–86.
Sieracki, M.E., Benfield, M., Hanson, A., Davis, C., Pilskaln, C.H., Checkley, D., Sosik, H.M., Ashjian, C., Culverhouse, P., Cowen, R., Lopes, R., Balch, W., Irigoien, X., 2010. Optical Plankton Imaging and Analysis Systems for Ocean Observation. In: Hall, J., Harrison, D.E., Stammer, D. (Eds.), Proc. "OceanObs'09: Sustained Ocean Observations and Information for Society: Conference (Vol. 2), Venice, Italy, 21–25 September 2009. ESA Publication WPP-306.
Sokal, R.R., Rohlf, F.J., 1995. Biometry: The principles and practice of statistics in biological research, 3rd ed. Freeman and Company, W.H., New York, USA.
StatSoft, Inc., 2004. STATISTICA 7 (data analysis software system). www.statsoft.com.
Svetlichny, L.S., 1983. Calculation of planktonic copepod biomass by means of coefficients of proportionality between biovolume and linear dimensions of the body. Ekologiya Morya 15, 46–58.
Temperoni, B., Viñas, M.D., Diovisalvi, N., Negri, R., 2011. Seasonal production of *Oithona nana* Giesbrecht, 1983 (Copepoda: Cyclopoida) in temperate coastal waters off Argentina. J. Plankton Res. 33 (5), 729–740.
Uye, S., Sano, K., 1998. Seasonal variations in biomass, growth rate and production rate of the small cyclopoid copepod *Oithona davisae* in a temperate eutrophic inlet. Mar. Ecol. Prog. Ser. 163, 37–44.
Valdés, L., López-Urrutia, A., Cabal, J., Alvarez-Ossorio, M., Bode, A., Miranda, A., Cabanas, M., Huskin, L., Anadón, R., Alvarez-Marqués, F., Llope, M., Rodríguez, N., 2007. A decade of sampling in the Bay of Biscay: What are the zooplancton series telling us? Prog. Oceanog. 74, 98–114.
Viñas, M.D., Gaudy, R., 1996. Annual cycle of *Euterpina acutifrons* (Copepoda: Harpacticoida) in the Gulf of San Matías (Argentina) and in the Gulf of Marseilles (France). Sci. Mar. 60, 307–318.
Viñas, M.D., Ramírez, F.C., 1996. Gut analysis of first-feeding anchovy larvae from the Patagonian spawning areas in relation to food availability. Arch. Fish. Mar. Res. 43, 231–256.
Viñas, M.D., Negri, R.M., Ramírez, F.C., Hernández, D., 2002. Zooplankton assemblages and hydrography in the spawning area of anchovy (*Engraulis anchoita*) off Río de la Plata estuary (Argentina-Uruguay). Mar. Fresh. Res. 53, 1031–1043.
Viñas, M.D., Diovisalvi, N., Cepeda, G.D., 2010. Individual biovolume of some dominant copepod species in coastal waters off Buenos Aires province, Argentine Sea. Braz. J. Oceanogr. 58, 177–181.
Zarautz, L., Irigoien, X., Fernandes, J.A., 2008. Modelling the influence of abiotic and biotic factors on plankton distribution in the Bay of Biscay, during three consecutive years (2004–06). J. Plankton Res. 30 (2), 857–872.