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SHORT NOTE

A method for measuring the size of early euphausiid larvae

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Abstract A simple method to measure early euphausiid larvae is tested on 35 specimens of *Euphausia superba* obtained in the Weddell-Scotia Confluence region (ranging from Calyptopes I to Furcilia III) against the measures obtained on the same specimens with a graduated eyepiece. The arrangement of the test includes two observers, four magnifications (from 7.5× to 62.5×) whenever feasible and two replications of each measurement. A total of 953 measurements were analyzed in an incomplete random blocks ANOVA design not finding significant differences between magnifications, methods, observers and their interactions. It was found that the relative differences between methods were of the same magnitude as the differences between replications (approximately 5%). The proposed method is less demanding on laboratory work, thus allowing the measurement of the large number of specimens needed to estimate size frequency distributions.

Keywords *Euphausia superba* · Early larvae · Measurement method · Relative error

Introduction

The measurement of the size of individuals at different stages in their life cycles is widely used in population dynamics studies. In the case of the early stages (up to Furcilia III) of euphausiids, its application has been restricted to morphological descriptions (e.g., Fraser 1936; Marr 1962; Pertzova 1976; Makarov 1979). Little attention has been paid to their size distributions as a source of information on their biology and life cycles.

This is mainly due to the difficulties associated with measuring small, and possibly curved, specimens under the microscope, which becomes a prohibitively time-consuming operation. The need to process large numbers of observations to estimate frequency distributions prompted attempts to use automated methodologies retrieving information on size of several plankton species (Macinnes et al. 1974). Most automatic systems are focused on pattern representation/feature measurement, feature extraction/selection, classification and abundance estimation. Still, the main disadvantage of them is that they fail in the classification accuracy (Zhang et al. 2000; Hu 2006).

More recently, automated devices allow in situ measurement of plankton size and density as a controlled flux goes trough an optical system but at the cost of taxonomic identification (Grosjean et al. 2004). Other developments include size measurement using multibeam acoustics (Herman et al. 1993) and optical devices mounted on floats (Checkley et al. 2008) that, as all automated devices, have the ability to cover and analyze large expanses of water but cannot fully exploit the detailed information present in plankton samples.

We present here a methodology focused on the measurement of the length of individual specimens from first Calyptopes to third Furcilia (CI to FIII), which can be conducted simultaneously with the routine processing of plankton samples, obtaining the same accuracy than that obtained using a micrometric eyepiece. Measurement with this method is based on the same principle as the silhouette

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photography described by Foote (2000) but replacing the photographic film and digitizing table by a camera lucida and an image scanner. The aim of this work is to compare the results obtained using the camera lucida method and the standard micrometric eyepiece in order to validate the methodology and to obtain an estimate of the measurement errors needed to evaluate the density function estimates (Carroll et al. 2006).

Materials and methods

Thirty-five undamaged specimens of *Euphausia superba* larvae (5 CI, 9 CII, 2 CIII, 9 FI, 8 FII, 1 FIII and 1 FVI) were collected in the area of the Weddell Scotia Confluence and the southern Scotia Sea during February and March 1995 between 56°20′ y 60°00′ S and 48°30′ and 50°30′ W on board the BIP DR EDUARDO HOLMBERG. Samples were taken with a Mongo net (quadruple Bongo) 200 mesh hauled vertically. Details of the sampling were published previously (Marschoff et al. 1998). A mixed ANOVA model was fitted to the log-transformed measurements with fixed factors method and magnifications and the random factors observer and replication. The relative error (E) was defined for each pair of observations of the same specimen as $E = \frac{|x_1 - x_2|}{(x_1 + x_2)/2}$, where x_1 and x_2 stand for measurements of the same specimen under the same conditions.

Measurement

Larvae were placed under a dissecting microscope with a camera lucida attached (Wild M5), and for each larvae, a line was drawn from the anterior end of the carapace to the end of the telson (Fig. 1). The drawings were scanned (300 dpi), digitized and measured in pixels. Conversion factors were obtained processing a graduated objective (analogous to the method used to calibrate micrometric eyepieces).

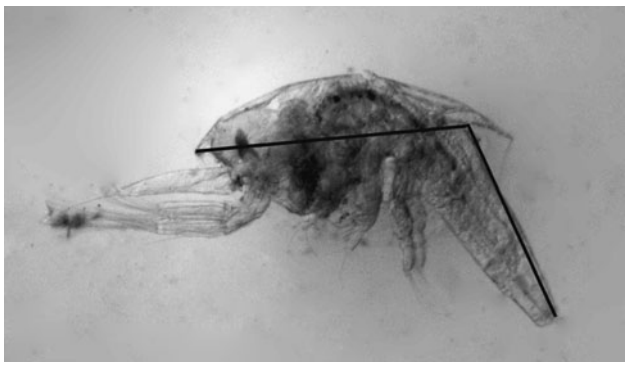

Fig. 1 Calyptopes I of *Euphausia superba* showing the measured line

Dedicated programs to digitize and measure the drawings were built-in FORTRAN to output a list of individual measurements in millimeters.

Each specimen was measured by two different operators using the new method and a micrometric eyepiece, calibrated with the same graduated objective and mounted on the same dissecting microscope. Measurements were taken under all magnifications (7.5×, 15.0×, 31.25× and 62.5×), if practical, and replicated independently to estimate measurement errors.

Results

From the potential 1,120 measurements (2 observers ×4 magnifications ×2 methods ×35 specimens ×2 replications), 953 valid observations were analyzed. The difference arises from large specimens not measured at greater magnifications (110 measurements): damage of specimens due to the repeated manipulation (54) and 3 recording errors with the eyepiece method. Means, standard deviations and coefficients of variation for each specimen are presented in Table 1.

An ANOVA, incomplete random block model was fitted to the log-transformed measurements. No significant differences ($\alpha = 0.05$) were found between observers, methods, magnifications and their interactions. Of greater interest is the comparison of the relative differences between measurements of the same specimen. The averages (across magnifications) of the relative error are presented in Table 2.

Discussion

Size distributions are used as an important part of research programs focused on krill (Siegel 2000a, b) but are currently restricted to larger specimens (late furcilia onwards). In systematic or physiological studies, early larvae have been measured in their hundreds or less (e.g., Fraser 1936; Makarov 1979; Daly and Zimmerman 2004).

The measurement of specimens using a microscope involves dedicated processing of each specimen. Fraser (1936) describes the device he used to strengthen and measure larvae under a microscope. Alternatively, curved specimens might be measured in parts. Both options are prohibitively time-consuming in view of the large number of specimens needed to estimate frequency distributions (Thompson and Tapia 1990; Carroll et al. 2006).

Recently, optical systems had seen large developments mostly aimed to determine abundance, shape and possibly taxonomic recognition at higher levels but yet unable to measure individual specimens together with their

Table 1 Means in millimeters (across observers, magnifications and replications) of the specimens measured by the two methods

No.	Micrometric				Camera lucida			
	Mean	SD	CV	N	Mean	SD	CV	N
1	1.74	0.08	0.05	16	1.75	0.13	0.07	16
2	1.97	0.21	0.10	16	2.05	0.18	0.09	16
3	4.54	0.22	0.05	16	4.25	0.17	0.04	12
4	2.63	0.08	0.03	16	2.65	0.09	0.04	16
5	1.83	0.05	0.03	16	1.83	0.08	0.04	16
6	4.53	0.28	0.06	16	4.50	0.24	0.05	12
7	6.37	0.29	0.05	16	6.38	0.22	0.03	10
8	19.53	1.39	0.07	12	20.85	0.45	0.02	8
9	1.60	0.05	0.03	16	1.61	0.10	0.06	16
10	1.95	0.12	0.06	16	1.94	0.09	0.05	16
11	5.21	0.28	0.05	16	5.40	0.31	0.06	12
12	5.19	0.03	0.01	2	5.09	0.09	0.02	4
13	4.08	0.18	0.04	16	4.16	0.08	0.02	12
14	3.31	0.10	0.03	16	3.33	0.08	0.02	12
15	3.08	0.12	0.04	16	3.06	0.13	0.04	13
16	4.09	0.15	0.04	16	4.14	0.13	0.03	12
17	4.16	0.20	0.05	16	4.17	0.13	0.03	12
18	2.45	0.06	0.02	16	2.48	0.10	0.04	16
19	5.02	0.29	0.06	15	5.09	0.42	0.08	9
20	4.24	0.15	0.04	16	4.27	0.16	0.04	12
21	3.23	0.22	0.07	16	3.35	0.14	0.04	13
22	4.93	0.32	0.07	16	5.09	0.38	0.07	12
23	3.42	0.00	0.00	1	3.33	0.05	0.01	3
24	3.42	0.17	0.05	16	3.51	0.17	0.05	13
25	4.22	0.16	0.04	16	4.19	0.21	0.05	12
26	5.45	0.19	0.03	16	5.48	0.31	0.06	12
27	3.17	0.20	0.06	16	3.15	0.12	0.04	13
28	4.33	0.30	0.07	16	4.49	0.23	0.05	12
29	4.14	0.17	0.04	16	4.19	0.10	0.02	12
30	5.36	0.44	0.08	16	5.73	0.15	0.03	12
31	5.31	0.28	0.05	16	5.53	0.19	0.03	12
32	5.41	0.22	0.04	16	5.47	0.09	0.02	12
33	4.37	0.22	0.05	16	4.19	0.23	0.05	12
34	3.29	0.10	0.03	16	3.33	0.09	0.03	12
35	3.32	0.13	0.04	16	3.38	0.11	0.03	13

SD Standard deviations, *CV* Coefficient of variation, *N* Number of measurements

taxonomic determination at the species level (Hu 2006). The silhouette photography method described by Foote (2000) is based on an image on photographic film that is later measured using a digitizing table to draw the specimens under a dissecting microscope.

Thus, counting and sorting plankton samples under a microscope is still necessary to determine species and stage of euphausiid larvae. The method described here aims to measure and sort specimens in a single manipulation of the sample. It allowed the measurement of more than twenty thousand euphausiid larvae from 18 stations in a four-month-person time, including sorting and classifying whole samples.

Table 2 Mean relative errors (across specimens and magnifications) of the different methods

Observer	Method	
	Micrometric	Camera lucida
I	0.025 (130)	0.030 (104)
II	0.048 (131)	0.067 (105)
Total	0.037 (261)	0.049 (209)

Number of pairs of replications intervening in each mean between parentheses

From the comparison of both methods, it emerges that their differences are of the same order as the differences between replications of the same measurement (both below 5%) (Tables 1, 2) indicating that the camera lucida method yields equivalent results as using a micrometric eyepiece with the advantage that it is possible, with relative ease, to measure large numbers of specimens.

Ethical standards

The authors declare that the experiments comply with the current laws of Argentina.

Conflict of interest The authors declare that they have no conflict of interest.

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