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SHORT REPORT

Enhancing fish diet analysis: equations to reconstruct *Themisto gaudichaudii* and *Euphausia lucens* length from partially digested remains

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Abstract

Themisto gaudichaudii and *Euphausia lucens* are important prey of several fish and squid species of commercial value in the southwest Atlantic Ocean. Although allometric relationships linking size and weight can be used to estimate prey biomass, size is often difficult to obtain as they are usually partly digested. The objective of our work was to provide length–length relationships to estimate body size of both crustaceans from their remains in fish stomachs, as a first step to estimate their weight. Measurements were performed on intact specimens collected in the field. Each of the body dimensions analysed (eye height and width for *T. gaudichaudii*, and carapace length and eye diameter for *E. lucens*) was significantly related to total length in both species. Thus, they appear to be highly reliable predictors of original size and can potentially aid in the identification of crustacean remains, allowing a more accurate analysis of digested contents.

Key words: *Prey size reconstruction, zooplanktonic crustaceans, fish stomach contents, southwest Atlantic Ocean*

Introduction

In fisheries science, accurate estimates of abundance, biomass and production of the different components of the food webs are necessary for the construction and implementation of ecosystem models (Christensen & Pauly 1992). In this kind of study, the characterization of the diet and the estimation of the amounts of resources consumed by individuals are of central importance and usually involve knowing the size and biomass of the prey eaten (Brischoux et al. 2007). Allometric relationships linking size and weight of prey can be used to extrapolate their initial dimensions from uneaten fragments or undigested remains found in the stomach (Härkönen 1986).

Because zooplanktonic crustaceans are key prey of most fish at one stage or another (larvae to adult), knowledge of their biomass is necessary to investigate the relationship between fish feeding and the natural food supply (Ivlev 1961). According to Scharf et al. (1998), size-specific diet information for several predatory fish is critical to determine the extent of size-selective feeding patterns in fish and their role in structuring marine fish populations.

Due to their high abundance and wide distribution in the Argentine Sea (Ramírez 1971; Ramírez & Viñas 1985), the hyperiid amphipod *Themisto gaudichaudii* (Guérin-Méneville, 1825) and the euphausiid *Euphausia lucens* (Hansen, 1905) are important prey of several fish species of commercial value such as hake (*Merluccius hubbsi*), anchovy (*Engraulis anchoita*), mackerel (*Scomber japonicus*) (Sánchez & Prenski 1996; Perrotta et al. 1999; Viñas et al. 1999) and hoki (*Macruronus magellanicus*) (Padovani et al. 2012). Also, *T. gaudichaudii* constitutes the main prey of squid (*Illex argentinus*) (Ivanovic & Brunetti 1994; Mouat et al. 2001). Dry weight–length ($\ln \mathrm{DW}\ (\mathrm{mg}) = -5.31 + 2.38 \times \ln \mathrm{L}\ (\mathrm{mm})$); and volume–length ($\ln \mathrm{V}\ (\mathrm{ml}\ 10^{-2}) = -10.84 + 2.76 \times \ln \mathrm{L}\ (\mathrm{mm})$) regressions have been established for *T. gaudichaudii* by Álvarez Colombo &

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Viñas (1994), while Pérez Seijas (1987) calculated relations between wet weight–subtotal length (♀: $\ln \mathrm{WW}\ (\mathrm{g}\ 10^{-2}) = -4.60 + 2.07 \times \ln \mathrm{STL}\ (\mathrm{mm})$; ♂: $\ln \mathrm{WW}\ (\mathrm{g}\ 10^{-2}) = -5.39 + 2.37 \times \ln \mathrm{STL}\ (\mathrm{mm})$) and volume–subtotal length (♀: $\ln \mathrm{V}\ (\mathrm{ml}) = -14.30 + 4.26 \times \ln \mathrm{STL}\ (\mathrm{mm})$; ♂: $\ln \mathrm{V}\ (\mathrm{ml}) = -13.32 + 3.80 \times \ln \mathrm{STL}\ (\mathrm{mm})$) for *E. lucens*.

Even though the above relationships are very useful when total length of the individuals can be obtained, most of the material recovered from fish stomachs consists of partially digested fragments and the number of entire items that can be readily identified and measured is very often limited. This results in the loss of potentially important diet information. However, hard body parts of crustaceans such as chelae, carapaces or eyes are relatively resistant to digestion and are sufficiently distinct to allow identification to species or genus level (Richardson et al. 2000). Therefore, the present study reports original regression equations derived from intact individuals of *T. gaudichaudii* and *E. lucens* captured in the field, which allow for the estimation of their total length from the size of several structures that are resistant to digestion. Our aim was to provide significant equations for reconstructing size of both species from several body dimensions, as a first step to estimate their weight and biomass. These equations should allow for inclusion of quantitative information of prey items previously unmeasurable, thus improving the accuracy of fish dietary analysis.

Materials and methods

Sample collection and analysis

Zooplankton samples were obtained during three surveys conducted in the Argentine Sea (between 42 and 45°S) in January 2009, 2010 and 2011, as part of the *Merluccius hubbsi* Assessment Program of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). Samples ($n = 17$) were collected by oblique tows from a depth near the bottom to the surface with a 300 μm meshed Bongo net, and immediately fixed in a 5% formalin–seawater solution for further analysis.

In the laboratory, each sample was first mixed thoroughly in a beaker and sieved using a 500 μm mesh to separate small and large mesozooplankton. From the >500 μm fraction (which includes *Themisto gaudichaudii* and *Euphausia lucens*), three aliquots (approximately 10 ml each) were taken at random and stained with Bengal Rose for 24 h to ensure good contrast at the time of scanning. Aliquots were separated into 3 polystyrene cells (127 × 85 mm) and scanned with a commercial colour flatbed scanner (Epson Perfection Photo 4490, Epson Scan software) at a resolution of 1200 dpi. Some manipulation was necessary to avoid the overlapping of individuals. Then, 200 individuals of each species were randomly selected from the resulting 51 images (3 per sample) to be measured with the ImageJ free software (Rasband 1997). The scale set within the ImageJ program was 1 mm to 48 pixels.

Body dimensions taken for *T. gaudichaudii* were: total length (TL, mm) (Figure 1a), eye height (EH, mm) and eye width (EW, mm) (Figure 1b), while for *E. lucens* they were subtotal length (STL, mm), carapace length (CL, mm) (Figure 2a) and eye diameter (ED, mm) (Figure 2b). In the case of *T. gaudichaudii*, TL was measured from the anterior part of the head (excluding the antennae) to the posterior end of the last pair of uropods, according to Sheader & Evans (1975), while EH and EW were measured considering the maximum vertical and horizontal axes, respectively. For *E. lucens*, STL was taken from the tip of the rostrum to the posterior end of the sixth abdominal somite, following Pérez Seijas (1987). We adopted this method in order to relate our equations with the ones proposed by this author. Diameter of the *E. lucens* spherical eye (ED) was measured along the anteroposterior axis ED, EW, EH and CL were taken with the straight line tool in ImageJ, whereas TL and STL were obtained with the segmented line tool, to account for curvature of the individuals. (CL is straight in Fig. 2)

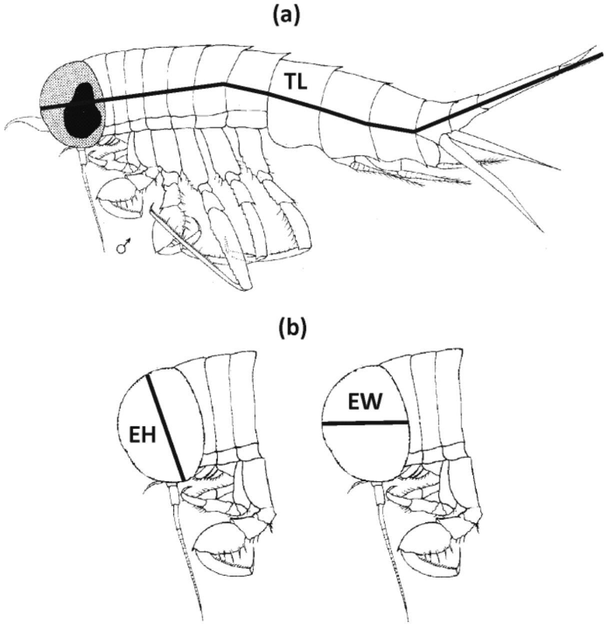


Figure 1. Scheme of *Themisto gaudichaudii* showing the limits of the measurements (a) TL: total length (mm) and (b) EH: eye height (mm) and EW: eye width (mm) (adapted from Ramírez & Viñas 1985).

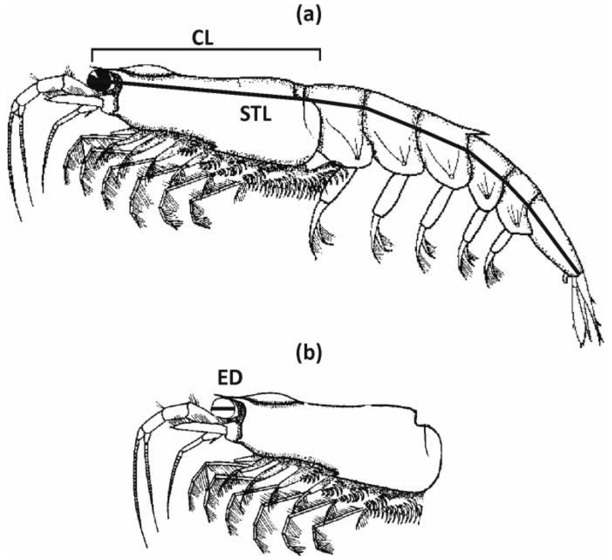


Figure 2. Scheme of *Euphausia lucens* showing the limits of the measurements (a) STL: subtotal length (mm), CL: carapace length (mm) and (b) ED: eye diameter (mm) (adapted from Ramírez 1971).

Data analysis

Regression models (linear, exponential, logarithmic and power) were applied between TL vs. EH and EW for *Themisto gaudichaudii*, and between STL vs. CL and ED for *Euphausia lucens*. The best model was chosen based on the minimum quadratic errors (REGRE software, Gabinete de Biomatemática, INIDEP). Then, least-squares regression equations were generated with the Statistica 8.0 software to predict length from the dimensions mentioned above. Studentized deleted residuals were used to identify potential outliers and influential observations. The critical value was calculated considering a Student's *t*-distribution with a Bonferroni-corrected $\alpha = 0.00025$.

Validation of the approach

To assess the performance of each of the equations, we used a jack-knife procedure as an internal validation technique (CVRL software, Gabinete de Biomatemática, INIDEP). This method excludes one observation, constructs the selected model with the remaining $(n-1)$ observations, and then predicts the response of the excluded observation using this model. This procedure is repeated n times so that each observation in turn is excluded from the model-construction step and its response is predicted. We then studied the relationship between the observed TL (for *T. gaudichaudii*) and STL (for *E. lucens*) values and the jack-knife values ($\text{length}_{\text{jackknife}}$) for each of the dimensions (EW and EH for *T. gaudichaudii*; CL and ED for *E. lucens*) analysed ($\text{length}_{\text{observed}} = \text{a} + \text{b} \times \text{length}_{\text{jackknife}}$) with a Student's *t*-test, testing the null hypotheses Ho: $\text{a} = 0$ and Ho: $\text{b} = 1$ (i.e. $\text{length}_{\text{observed}} = \text{length}_{\text{jackknife}}$).

Results

Length estimation of Themisto gaudichaudii *and* Euphausia lucens *from the field*

TL of *Themisto gaudichaudii* ranged from 2.08 to 18.12 mm, while STL of *Euphausia lucens* varied between 2.47 and 21.5 mm. Regressions relating CL and ED to STL for *E. lucens*, and EH and EW to TL for *T. gaudichaudii*, were all highly significant ($p < 0.01$), with R^2 values ranging from 0.89 to 0.94 (Figure 3). For each of the considered relationships, only one outlier was detected and eliminated. In all cases, the linear model provided the best fit.

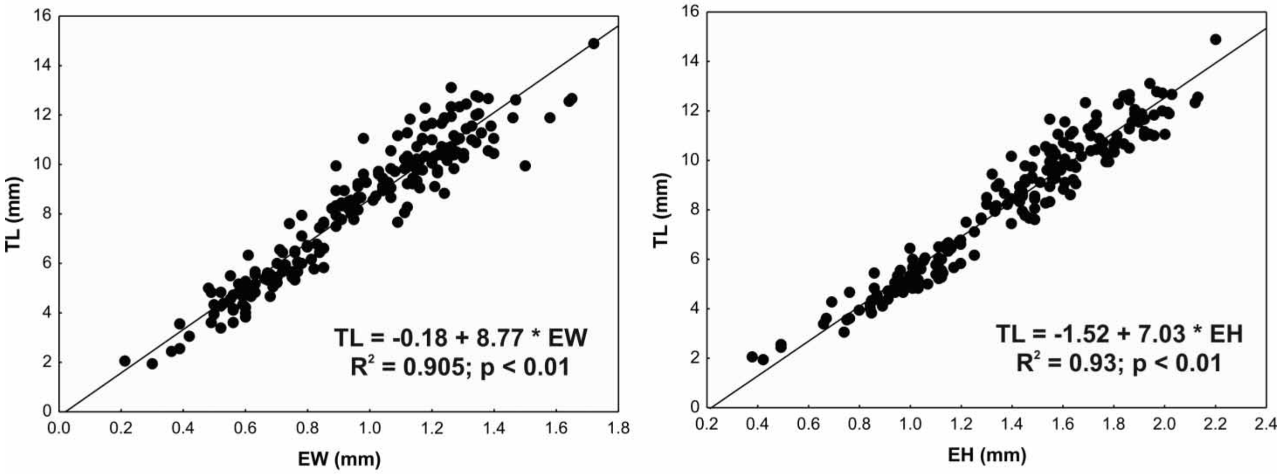
Validation of the proposed equations

There was a good correspondence between the $\text{length}_{\text{observed}}$ and $\text{length}_{\text{jackknife}}$ values for each of the dimensions analysed (Table I), and the null hypotheses (Ho: $\text{a} = 0$ and Ho: $\text{b} = 1$) were not be rejected in all cases. Also, the mean absolute ($\text{length}_{\text{observed}} - \text{length}_{\text{jackknife}}$) and percent relative ($100 \times (\text{length}_{\text{observed}} - \text{length}_{\text{jackknife}})/\text{length}_{\text{observed}})$) errors obtained with the jack-knife method were low for all the studied relationships, demonstrating the validity of the estimated equations.

Discussion

We have shown that the original size of *Themisto gaudichaudii* and *Euphausia lucens* can be predicted from the size of their indigestible fragments, as each of the dimensions analysed was significantly related to total length in both species; thus, they can potentially aid in the identification of crustacean remains recovered from fish stomachs, allowing a more accurate analysis of digested contents. Despite the fact that other authors have previously used eye diameter or carapace length measures as proxies of total length in both species (Ivanovic & Brunetti 1994; Reid et al. 1996; Bocher et al. 2001; Shin & Nicol 2002), their equations were obtained from measurements performed on digested individuals. Therefore, the equations presented in this work constitute the first reference on length–length relationships for both species in the southwest Atlantic derived from intact individuals in zooplankton samples.

(a) *Themisto gaudichaudii*



(b) *Euphausia lucens*

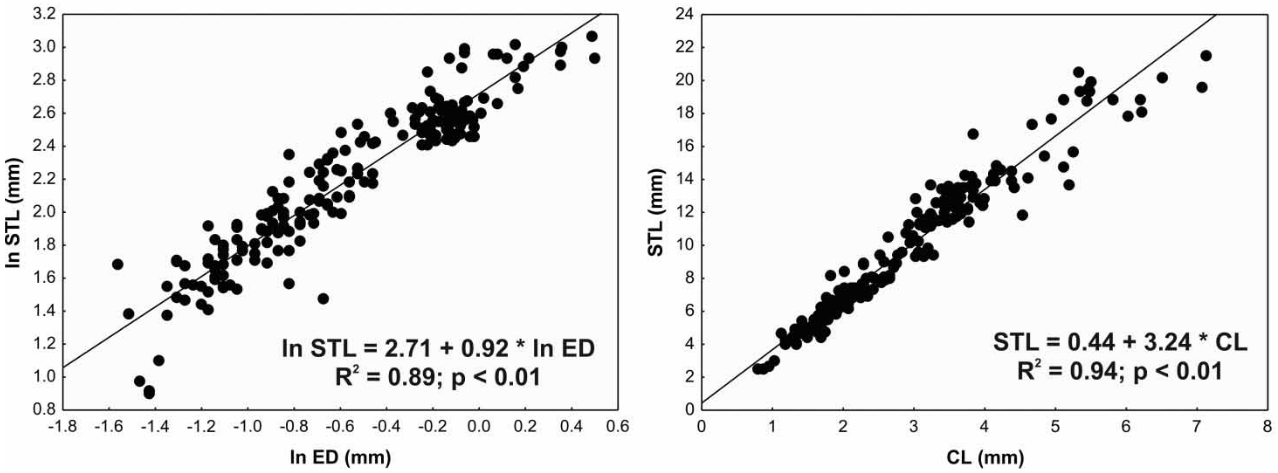


Figure 3. Regression equations relating measurements of (a) EW: eye width (mm) and EH: eye height (mm) to TL: total length (mm) for *Themisto gaudichaudii* and (b) ED: eye diameter (mm) and CL: carapace length (mm) to STL: subtotal length (mm) for *Euphausia lucens*.

Table I. Results of the Student's *t*-tests (significance level: $\alpha = 0.05$) to evaluate the relationship between the observed length values and the jack-knife values, for each of the dimensions analysed in *Themisto gaudichaudii* and *Euphausia lucens*. Mean absolute ($\text{length}_{\text{observed}} - \text{length}_{\text{jackknife}}$) and percent relative ($100 \times (\text{length}_{\text{observed}} - \text{length}_{\text{jackknife}})/\text{length}_{\text{observed}})$) errors obtained with the jack-knife method are presented.

	Ho: a = 0	Ho: b = 1	Mean absolute error	Mean percent relative error
Themisto gaudichaudii				
TL vs. EW	a = 0.013, t = 0.065 p = 0.94	b = 0.99, t = 0.077 p = 0.94	−0.001417	−1.528880
TL vs. EH	a = 0.01, t = 0.063 p = 0.95	b = 0.99, t = 0.068 p = 0.95	−0.000132	−0.569530
Euphausia lucens				
STL vs. CL	a = 0.023, t = 0.13 p = 0.9	b = 0.99, t = 0.16 p = 0.87	−0.0003093	−1.837854
STL vs. ED	a = 0.04, t = 0.13 p = 0.89	b = 0.99, t = 0.15 p = 0.88	−0.003350	−3.479221

Regarding the estimation of size in amphipods, some authors have proposed measurement of pereion segments as a total length proxy in gammarids (Arndt & Beuchel 2006; Nygård et al. 2009). However, and based on preliminary observations that we made from stomach contents of juvenile Argentine hake (*Merluccius hubbsi*) (Temperoni, unpublished data), the eye of *T. gaudichaudii* was the most frequent, well-preserved and distinct remnant other than pereion segments; thus, we consider it to be an adequate and easily measurable morphological trait. Besides, the availability of two equations which relate either eye height or width to total length allows the use of any of them to estimate size, thus accounting for possible shape deformations that the eyes in gut remains may present along the horizontal or vertical axes.

When reconstructing original prey sizes, there are some issues that should be addressed, such as the effect of preservative on length. Some authors have found a shrinkage or weight loss in zooplanktonic crustaceans due to preservation in formalin (Kuhlman et al. 1982; Kapiris et al. 1997), while others have not (Ahlstrom & Thrailkill 1963; Miller 1983; Morris et al. 1988; Thurston & Bett 1995; Põllupüü 2007). In agreement with the latter, we assumed that the fixation in formalin had no shrinkage effect on *T. gaudichaudii* and *E. lucens* because of their rigid exoskeleton. Another issue to consider is the within-species variance of these relationships due to latitudinal variation in size. A positive correlation between both variables has been proposed for amphipods (Watts & Tarling 2011) and euphausiids (Pinchuk & Hopcroft 2007). Therefore, even though our equations may be applied as a total length proxy in related species if no specific equations are found in the literature, they should be used with caution in different areas, taking into account the size-range of the animals used to obtain them.

The equations proposed here could be valuable for studies on trophic ecology of other consumers of both species in the southwest Atlantic and the Southern Ocean. In fact, *T. gaudichaudii* has been repeatedly recorded in the diet of a variety of top predators including not only fish (Schwingel & Castello 1994; Sánchez & Prenski 1996; Perrotta et al. 1999; Viñas et al. 1999; Padovani et al. 2012) and squid (Ivanovic & Brunetti 1994; Mouat et al. 2001) but also seabirds (Reid et al. 1997) and whales (Nemoto & Yoo 1970). *Euphausia lucens* has been recorded in the diet of fishes in the Argentine Sea (Sánchez & Prenski 1996) and the southern Benguela current (King & MacLeod 1976), while other euphausiids are prey of baleen whales (Murase et al. 2002), fur seals (Reid & Arnold 1996) and seabirds (Croxal et al. 1997). Also, the equations can be used as an initial step to estimate first the individual weight and then the biomass of *T. gaudichaudii* and *E. lucens*, with the relationships proposed by Álvarez Colombo & Viñas (1994) and Pérez Seijas (1987), respectively. Therefore, the relative contributions of both species to the diet of many predators can be estimated quantitatively, rather than just mentioning the presence of their fragments, which is essential information for quantifying trophic flows in marine food webs (Richardson et al. 2000).

Automated analysis of zooplankton samples using good quality digitized images is gaining increased interest at the present time (Culverhouse et al. 2006; Di Mauro et al. 2011). The enumeration and measurement of specimens from the zooplankton can be done in a short time, and morphological parameters such as body length can be extracted. As in the present study, some authors have previously measured *Themisto* and *Euphausia* morphological features on high-resolution images with the ImageJ software (Shin & Nicol 2002; Noyon et al. 2011); thus, their use represents a key advantage over the traditional and time-consuming microscope analysis.

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