

## **Length-specific carbon content of the *Daphnia* population in a large subalpine lake, Lago Maggiore (Northern Italy): The importance of seasonality**

Marina Manca, Patrizia Comoli and Teresa Spagnuolo

C.N.R. Istituto Italiano di Idrobiologia Largo Tonolli 50/52, I-28048 Pallanza, Italy

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### **ABSTRACT**

We compared three methods for calculating the standing stock biomass of *Daphnia hyalina galeata* population in Lago Maggiore during a single year. We applied body length/carbon regression equations (LCR) obtained under controlled laboratory conditions and directly from field data on carbon and body length to field data on length-specific abundance. Laboratory-derived equations satisfactorily described our field estimates of length-specific carbon content in spring. As the length-standardized carbon content decreased exponentially along the season, we included time of the year as an auxiliary variable, and tested whether it affected slope, intercept or both. The decrease had no effect on slope but reduced the intercept of the LCR. We also calculated biomass, using a single mean value from the literature. By comparing the results with those obtained by simple conversion of individuals number into carbon we might discriminate between the effect of changes in the size frequency distribution of the population and the decrease with time of the length-standardized carbon content. Although we tested the different models for non-ovigerous females, the results did not change when we included also data on females with eggs in the first three developmental stages, whereas they changed substantially when we included females with embryos. We formulate two hypotheses to explain this result and discuss possible implications for the calculation of the standing stock biomass of *Daphnia* population.

### **1. Introduction**

Accurate biomass estimates of zooplankters are essential for the estimation of secondary production and the construction of models on energy and carbon flux through the zooplankton. The accuracy of biomass estimates obtained using any simple factor to convert from numbers to biomass is limited by the indeterminate growth of cladocerans and the numerous instars of copepods. The relative abundances of different stages and sizes of organisms change along the season, as do the sizes of mature organisms (Culver, 1980). These two facts are particularly important for *Daphnia* populations. In both temperate and tropical lakes, *Daphnia* continuously recruit, so the relation between size and the mass of individuals, whether as carbon or dry weight, changes both with the size structure of the population and

with the season, reflecting different nutritional conditions (Dumont et al., 1975; Bottrell et al., 1976; Culver et al., 1985). As a result, length to biomass regressions can also change over the season (Geller and Müller, 1985). If known, such equations can be applied to reconstruct long-term changes in biomass more accurately whenever length measurements are available.

Among the different estimates of biomass, the analysis of carbon content in live organisms is probably the best (Boersma and Vijverberg, 1994). To determine the effect of seasonality on *Daphnia* carbon/length regression (LCR) we measured the carbon content of live *Daphnia hyalina galeata* s.l. from Lago Maggiore, a large, deep, subalpine lake in Italy, at different times during 1992. In addition, we compared results from field organisms with those obtained under controlled laboratory conditions at nonlimiting food levels. The proximate purpose was to investigate the nutritive status of *Daphnia* in the field (Duncan, 1985; Manca et al., 1994), and particularly to assess the importance of food limitation in the summer numerical decrease of *Daphnia* population (Manca et al., 1994). We previously noted seasonal variation in the calculated intercepts when field data are fitted to a LCR and suggested such variations may indicate food limitation (Duncan, 1985). From that study, we found that the equation obtained in the laboratory fitted the data for organisms from Lago Maggiore quite well in spring, but from summer to fall there was a considerable decrease in the length-specific carbon content. Therefore, it seemed interesting to look at how the results might vary if we calculated the standing stock biomass of *Daphnia* population in Lago Maggiore from published estimates of average carbon content/daphnid (non length-specific), from the laboratory LCR relation, or from an LCR that allows seasonal changes in its parameters. The latter two approaches use data on the abundance and the size (body length) structure of the daphnid in 1992. This study presents these comparisons.

## 2. Materials and methods

From April to October 1992 we collected *Daphnia* from Lago Maggiore with a large-mouth (60 cm diameter) plankton net of 126  $\mu\text{m}$  mesh size by vertical hauls from -50 m to the surface. *Daphnia* were sorted alive in the laboratory, rinsed in filtered, UV sterilized GF/C lake water, measured and immediately transferred into clean ( $\text{CCl}_4$  washed) tin capsules for drying (70°C overnight). We measured carbon content of individuals of known length with a Carlo Erba CHN 1106 Elemental Analyzer. Although we counted eggs and embryos and noted the developmental stage of the individuals analyzed (425), we used only data from non egg-bearing females for the analysis. For the same dates (ten in total) and station we also collected quantitative zooplankton samples with a Clarke-Bumpus plankton sampler (same net, sinusoidal hauls, in the upper 50 m depth). These samples which contained the organisms in at least 1000 l of lake water, were fixed first with absolute alcohol and then in 10% formaldehyde, and used to count and measure *Daphnia* to estimate abundance and the length-frequency distribution of the population. Samples were in general well preserved and ballooning was almost certainly negligible: very few eggs were found outside mother's body in the samples. To have a reliable estimate of the length-frequency distribution of the *Daphnia* population on

each date, we measured 200 individuals (from the top of the head to the caudal portion of the carapace, spine excluded) from fixed samples. If fewer than 200 animals were available, all the specimens in the sample were sized. We calculated the estimated carbon content (in  $\mu\text{g}$ ) of each field-collected individual measured for length using the carbon/length regression formula, and multiplying the mean of all such estimates times the number of individuals/liter.

*Daphnia* for laboratory experiments came from the third generation of pre-acclimated females kept at constant temperature and nonlimiting food conditions until 20 days old (Manca et al., 1994). Over intervals of one and two days we harvested a set number of individuals with a body length between 0.6 and 2 mm and treated them as described above. For neonates, 60 individuals per sample were required, but fewer larger animals were needed. To avoid manipulations which might damage our individuals, and being aware that eggs and embryos might be a source of variability for individual carbon content, we used only non ovigerous females in the intermolt phase.

### 3. Results

#### 3.1 Statistical treatment of the data

We used two allometric equations to calculate *Daphnia* biomass. These were:

1. a length/carbon regression (LCR) equation obtained from animals grown in the laboratory at constant, nonlimiting food conditions;
2. an LCR equation resulting from the analysis of specimens collected from Lago Maggiore at different times in the year.

The equation was in form:

$$\ln C = \ln a + b \ln l; \quad (1)$$

(where:  $C = \mu\text{g}$ ,  $l = \text{mm}$ ).

The statistical estimates for each parameter, and overall summaries were as follows:

Data set	$\ln a$ ( $\pm\text{SE}$ )	$b$ ( $\pm\text{SE}$ )	R-SQ	F	P	d.f.
Laboratory	1.24 (0.03)	2.77 (0.09)	0.97	899.81	<0.001	30
Field	1.35 (0.14)	1.52 (0.25)	0.54	35.76	<0.001	30

(data refers only to non-ovigerous females).

The laboratory equation described field data collected in spring adequately; the field data all lay within the 95% confidence limits of the laboratory equation (1) (Manca et al., 1994). On the other hand, the equation overestimated the carbon content of summer and autumn specimens (Manca et al., 1994). This overestimate resulted because the length-specific carbon content of *Daphnia* declined from spring to autumn. The unitary carbon content in animals of 1.5 mm changed from 11  $\mu\text{g}$  in spring to 7  $\mu\text{g}$  in summer, and in the animals of 2 mm the unitary carbon

content in summer was half that found in spring (12  $\mu\text{g}$  versus 24  $\mu\text{g}$ ). As a consequence of this marked seasonality, the general equation for field data accounted only for 54% of the total variability (R-SQ = 0.54).

The general decline of carbon content with time had no effect on the slope of the LCR but reduced the intercept (Fig. 1). We therefore applied an allometric equation which included time of the year as an auxiliary variable ( $t$ , in days, starting from the middle of April) to correct our estimate for seasonality.

To establish this relation we tested three alternatives:

- a. time as a term affecting the intercept (+  $ct$ ) so the regression model became:

$$\ln C = \ln a + b \ln l + c \cdot t; \quad (2a)$$

- b. time as a term affecting the slope (+  $dt \ln l$ ); in this case the model tested was:

$$\ln C = \ln a + b \ln l + d \cdot t \cdot \ln l; \quad (2b)$$

- c. a combination of both terms ( $ct$ ,  $dt \ln l$ ), in the model:

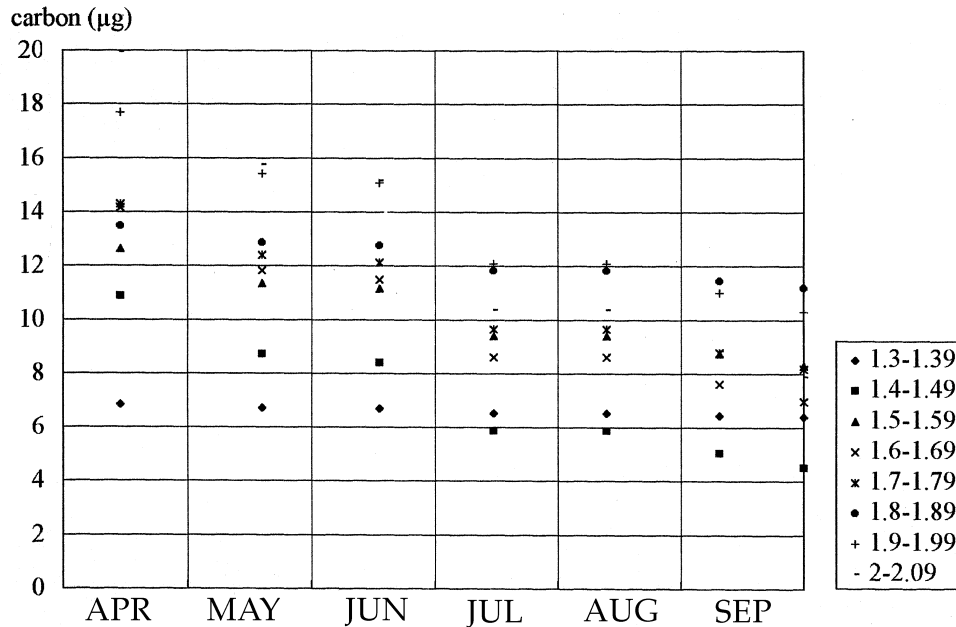
$$\ln C = \ln a + b \ln l + ct + d \cdot t \cdot \ln l; \quad (2c)$$

The application of the three equations to field data gave the following estimates (\* included females with eggs in the first three developmental stages):

Equation	lna ( $\pm$ SE)	b ( $\pm$ SE)	c ( $\pm$ SE)	d ( $\pm$ SE)	R-SQ	F	P	d.f.
(2a)	1.830 (0.137)	1.865 (0.198)	-0.0033 (0.0006)		0.76	46.47	<0.001	29
(2b)	1.233 (0.116)	2.997 (0.422)		-0.006 (0.015)	0.71	34.9	<0.001	29
(2c)	2.354 (0.384)	0.641 (0.862)	-0.006 (0.002)	0.006 (0.004)	0.78	32.88	<0.001	28
(2a)*	1.728 (0.156)	1.920 (0.210)	-0.003 (0.001)		0.71	47.35	<0.001	39

Although equation (2c) had the highest R-SQ (0.78), it also had the lowest F-value. Both results reflect increased number of parameters and decreased d.f. (28). Neither of the parameters ( $c$  and  $d$ ) were significantly different than zero ( $P \leq 0.05$ ). The parameters in both equations (2a) and (2b) were significantly different from zero (95% level of significance). Equation (2b) had a slope very close to the value 3 at which growth would be isometric (Geller and Müller, 1985). However, equation (2a) had higher R-SQ and F values. Therefore, the best equation to describe our data was (2a). Surprisingly, the same pattern was observed when we included females with eggs in the first three developmental stages (as described by Green, 1956) in the analysis (2a\*).

We also compared the results obtained by using our length-specific equations with those resulting from using a constant conversion factor to transform animals into the standing stock of animals (B in  $\mu\text{g C l}^{-1}$ ) (de Bernardi et al., 1988).

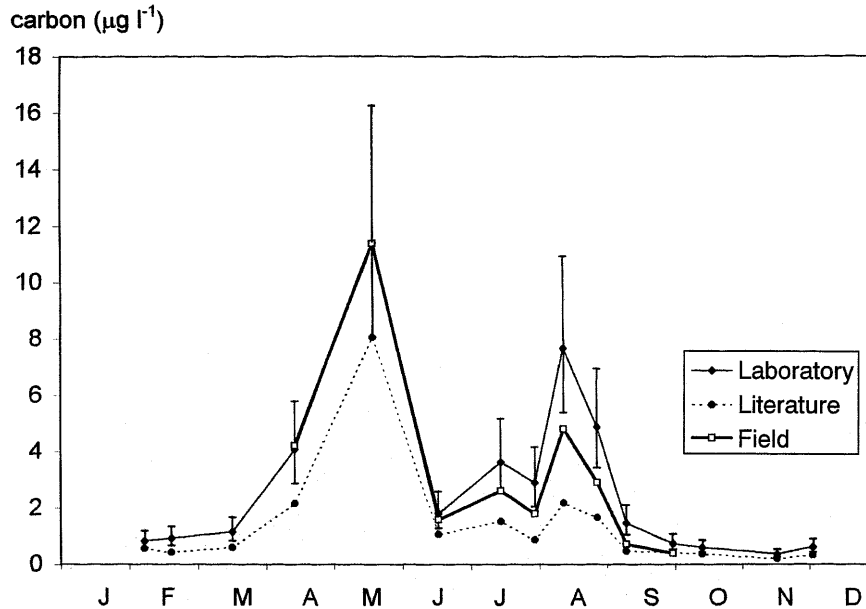


**Figure 1.** Seasonal changes in carbon content of different size classes (body length, in mm) of *Daphnia* population in Lago Maggiore. Each points represent the average carbon content of individuals in each size class

### 3.2 Application of the different equations

Equation (1) is quite similar to those found in literature for the relationship between body length and biomass of *Daphnia* from laboratory and field estimates (Geller and Müller, 1985; Manca, 1996). This equation has two advantages. It was obtained using the entire range of body lengths observed in field and laboratory samples, and can therefore be applied to situations where the distribution of length-frequency data of the population is skewed to the left (as typical for the peak in density during spring or summer, when the major part of *Daphnia* population is made up by immature organisms,  $\geq 1$  mm). Most of the published results from field populations are based on specimens  $\geq 1$  mm, and therefore, can hardly be applied to individuals below this limit. The second advantage is that, because it is based on individuals grown at the same ambient conditions, it has very restricted sources of individual variability, and therefore R-SQ and the level of significance of the parameters estimates are high. Its application allows the researchers to account for changes in the size structure of the population in addition to changes in density.

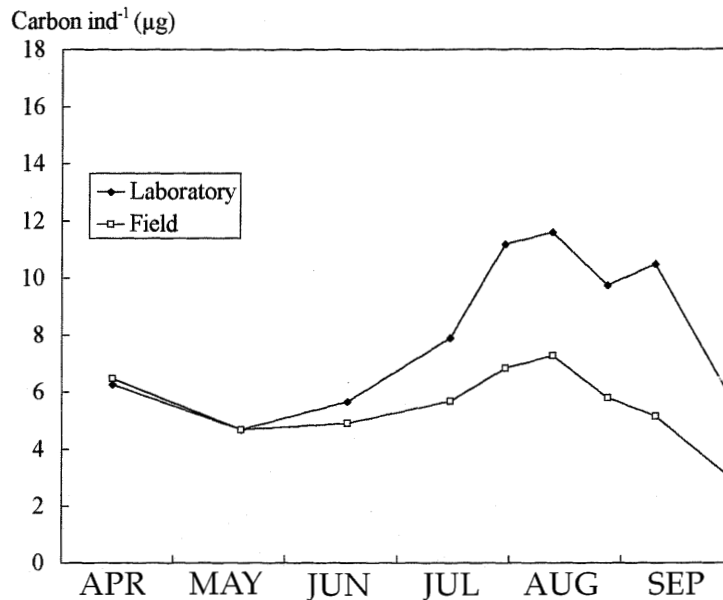
An interesting result is achieved from the computation of the variability of our carbon estimates by applying the equation for the standard error of predicted  $C_i$  for each given value of  $l_i$  (Sokal and Rohlf, 1990). Despite the goodness of our estimates, the range of variability is large, especially for high values (Fig. 2). In general the values obtained by simply using the constant conversion factor of  $3.3 \mu\text{g C/indi}$



**Figure 2.** Standing crop (carbon) of *Daphnia* population in Lago Maggiore resulting from the application of different models. Laboratory and field estimates are based on the length-specific carbon content. Vertical bars refer to the standard error for carbon predicted from laboratory LCR equation (Sokal and Rohlf, 1990) (for explanation of symbols see text)

vidual (de Bernardi et al., 1988) are below the lower limit of estimates obtained with equation (1). However the effect of increased mean body length in late summer might be at least partially counterbalanced by the general decline in the length-specific carbon content (Manca et al., 1994). Therefore, a more reliable estimate is probably obtained by using the equation from field data, which accounts for seasonality with an auxiliary variable. The disadvantage of this approach is that we lack field data for very young and small specimens, since we did not find them, in appropriate amounts in the lake. The advantage of an equation that describes small animals is not so high in summer, when most individuals are large, and the estimate from field data is quite close to that obtained using laboratory data, confirming that the laboratory equation was appropriate to describe the spring situation (Fig. 2). In summer, the decrease in length-specific carbon content results in values which are below the lower limit calculated from equation (1).

To look at the effect of changes in size structure, we expressed our data in terms of average carbon content per individual, taking into account the mean length of individuals at each date (Fig. 3). These values all lay above the conversion factor from literature; although the lower limit of our estimate was very close to the literature value in May. As an effect of the general increase in size, summer values were higher than spring ones. In fact, the size distribution of the Lago Maggiore *Daphnia* population was skewed toward small specimens in spring and toward large specimens in summer (Manca and Comoli, 1995). When we also consider the dependence



**Figure 3.** Seasonal variation in mean individual carbon content of *Daphnia* from Lago Maggiore resulting from the application of different models (for explanation of symbols see text)

of the length-standardized carbon content on time, the situation is different. Spring values remain between 6.5 and 4.7  $\mu\text{g carbon ind}^{-1}$  (in April and May respectively), but values of about 7  $\mu\text{g carbon ind}^{-1}$  are achieved in August, and only in late September do we find very low values, close to those suggested by the conversion factor reported in literature (de Bernardi et al., 1988).

#### 4. Discussion and conclusions

In Lago Maggiore, the *Daphnia* population changes seasonally not only in abundance but also in size structure and in carbon content per unit of length. The length-frequency distribution of the population is skewed toward small specimens in spring, when the population is most abundant and toward large specimens in late summer (Manca and Comoli, 1995; Manca, 1996). The carbon content standardized by length decreases exponentially from spring onward; the slope of the relation of carbon to length is the same for different body lengths whereas the intercept changes. This situation can be synthesized in a model where in addition to a carbon estimate weighted on body length, time of the year is included as a term affecting the intercept's values. However, this model should be applied with caution: although it provides more accurate estimates than those obtained by simple conversion of numbers into carbon or the application of laboratory equations, the range of uncertainty is still large. Even with a relatively tight relation (as in the case of our laboratory equation) the range of variability of our estimate remains high at high population sizes.

The numerical value of  $c$  is probably specific to Lago Maggiore, and possibly to the year of study. Further precaution is necessary when this model is applied to a long-term series of data on *Daphnia* body length. Although it is reasonable to expect that this seasonal decrease in carbon/unitary length is general through the years and is also observed in other lakes (Berberovic, 1990), we should have an independent estimate of food availability. Therefore, further research is needed on this point.

However, even with the above-mentioned limitations, we emphasize the most important result of our study: there appears to be no need to incorporate the product term  $d \cdot t \ln l$ . Time trends are effectively controlled by using a simple adjustment of the intercept to the slope of the LCR. This looks important, if it proves to be general to other habitats, because it would simplify the use of statistical methods for biomass and productivity calculations, and would eliminate the variance that would arise from a product term.

Our study also suggests the importance of the developmental stage of eggs and embryos in the evaluation of carbon. Because the inclusion of females with eggs in the first three developmental stages (as defined by Green, 1956) did not substantially change the LCR equation, egg deposition until this stage appears simply a transfer of a certain amount of carbon from one compartment of mothers' body (lipid drops) to another (brood chamber, as eggs). The situation is different for embryos. There are two possible explanations for this difference: 1) carbon content might be very close to that of neonates and therefore a function of embryos' body length; 2) mothers with embryos might have already prepared a storage of energy reserves for the successive brood.

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