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Factors influencing the biomass of the rotifer *Brachionus plicatilis* in culture

M. Yúfera[1], E. Pascual[1] & J. Guinea[2]
[1] *Instituto de Ciencias Marinas de Andalucía (C.S.I.C.). Apartado oficial, 11510 Puerto Real, Cádiz. Spain;* [2] *Acuicor, S.A. Apartado 570. 11201 Algeciras, Cádiz, Spain*

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Abstract

The contribution of the egg weight and the population size structure to the body mass have been studied in two strains of *Brachionus plicatilis* of different size. A mathematical model was developed in order to obtain a reliable estimate of the dry mass from two single easily determined parameters; the egg/female ratio and the mean lorica length.

Introduction

Body size of *Brachionus plicatilis* varies among strains (Snell & Carrillo, 1984), but a single strain may also show size variation as a response to environmental factors (Serra & Miracle, 1987). Likewise, body dry weight varies in routine cultures with given conditions, depending on the growth phase and the nutritional state, which is monitored by the egg/female ratio (Yúfera & Pascual, 1989). These differences can be very important, since the mean individual dry weight could range between $<0.1\ \mu g$ and $1.0\ \mu g$. Therefore, assigning an approximate value may result in an appreciable error in bioenergetic studies.

This paper tries to describe a general rule explaining the variation in the body weight of *B. plicatilis* under cultivation This is of primary interest in larval feeding studies in which food conversion rate may be determined from gut content (females + eggs). This also interesting for monitoring rotifer mass culture in hatcheries, where *Brachionus* strains may periodically change to others of different origin and/or they may show seasonal replacement of clones. Therefore, the contribution of the egg weight and the population size structure to the body mass in two types (L and S) of *B. plicatilis* have been studied. A mathematical model has been developed in order to obtain a reliable estimate of dry mass from two singles parameters that are easily determined; the egg/female ratio and the mean lorica length.

Materials and methods

Brachionus plicatilis strains S-1 (L-type) and BJ (S-type) were maintained in batch cultures in 10 l beakers at 25 °C temperature and 33‰ salinity. Cultures were fed on either the Eustigmatophyceae *Nannochloropsis gaditana* or the Prasinophyceae *Tetraselmis suecica*.

Dry weight and lorica length were obtained from samples taken in different growth phases, at a wide range of egg/female ratio. Dry weight (DW) was determined by drying samples of 400–2000 individuals at 90 °C to constant weight. Egg dry weight was determined from samples of 200–600 eggs, which were previously separated from the females by ultrasonic treatment. Population mean body size has been determined for different conditions, by measuring the lorica length without spines (Yúfera, 1982) in samples of 50–80 individuals.

To determinate the parameters of the model, data from 38 samples of *B. plicatilis* in the different culture conditions were studied using non-linear regression analysis by a modification of the computer programme proposed by Jolivet (1983).

Results and discussion

Changes in body length and dry weight in relation to egg/female ratio for the two *Brachionus* strain and the different foods are given in Fig. 1. As expected, body dry weight is correlated to the egg/female ratio, while the lorica length showed small variations. In addition, the results are different for each microalgae. Egg dry weight of large rotifer (S-1) was 293 ng with *N. gaditana* and 189 ng with *T. suecica*, while the small rotifer (Bs) eggs weighed 77 and 86 ng respectively (Table 1).

Taking these results into account, it seems clear that the mean individual body weight at a given growth condition is, at least, the result of adding the fraction of egg weight to mean female mass without eggs. Furthermore, both the female and egg weights are determined by the size structure of the population, that is by the mean lorica length. As also demonstrated by other authors (Nagata, 1985; Planas & Estévez, 1989), this mean lorica length is a consequence of the culture conditions. In this case, it is the type of microalgae.

Thus, in the mathematical model formulation the mean individual dry weight is dependent on three variables. These are: the mean female length, the egg/female ratio and the type of strain. We consider that the mean individual dry weight is the sum of the body mean dry weight and the egg dry weight multiplied by egg/female ratio (egg incidence).

The body mean dry weight is considered to be a function of the body condition factor multiplied by the mean length elevated to a certain exponent. This body condition factor is thought to be directly related to the strain and to some degree to the nutritional status. As the nutritional status is in turn closely linked to the egg/female ratio, we have used this variable in the model to describe the starvation vs. the well fed effect on the body condition factor. The mathematical formulation employed is the multiplication of the body condition factor when the egg/female ratio is 1, by an expression of the type

$$A + (1 - \mathrm{A}) * (\text{egg/female ratio})^{\mathrm{B}},$$

where A is the body condition factor when the egg/female ratio is 0, divided by body condition factor when the egg/female ratio is 1.

The mean egg dry weight is considered to be a proportion of the total mean body dry weight when the egg/female ratio is 1.

Then the model is as follows:

$$\mathrm{MDW} = \underbrace{(\mathrm{STF} * \mathrm{BCF} * (\mathrm{RCF} + (1 - \mathrm{RCF}) * (\mathrm{ERF}^{\mathrm{P}_1}))) * ((\mathrm{ML}^{\mathrm{P}_2})/100\,000))}_{\text{Body}} + \underbrace{(\mathrm{EGR} * \mathrm{STF} * \mathrm{BCF} * \mathrm{EFR} * (\mathrm{ML}^{\mathrm{P}_2})/100\,000))}_{\text{Egg incidence}}$$

when:

MDW: Mean dry weight (ng) of one individual in the population (dependent variable).

Table 1. Comparison between estimated and observed egg dry weight.

Strain	Microalgae	EGR	Mean length (μm)	Estimated egg dry weight (μg)	Observed egg dry weight (μg)
S1	*T. suecica*	0.381	237.4	175.7	189.3 ± 46.3
S1	*N. gaditana*	0.539	239.8	256.2	293.0 ± 15.7
Bs	*T. suecica*	0.491	141.7	96.4	86.0 ± 11.3
Bs	*N. gaditana*	0.359	140.2	68.3	77.2 ± 4.7

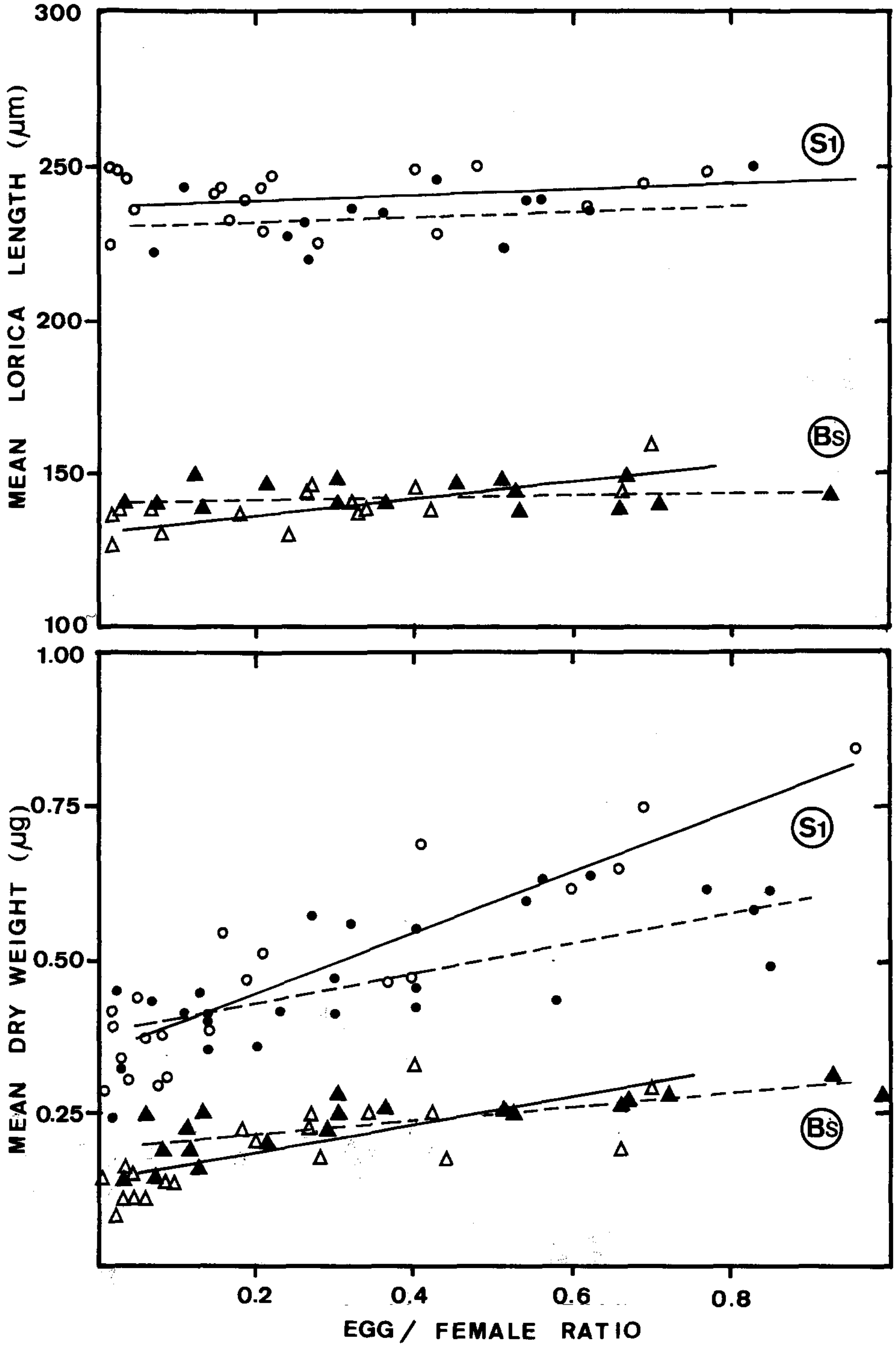

Fig. 1. Mean lorica length and dry weight of *B. plicatilis* in relation to the egg/female ratio. Circles: strain S1; triangles: strain Bs; solid line: *Nannochloropsis*; broken line: *Tetraselmis*.

STF: Strain factor (parameter).
BCF: Body condition factor when EFR is 1.0 (parameter).
RCF: Ratio of the body condition factor when EFR is 0, divided by BCF (parameter).
EFR: Egg/female ratio (independent variable).
P_1: Exponent describing the effect of the egg/female ratio on the body condition factor (parameter).
ML: Mean length (μm) of the population (Independent variable).
P_2: Exponent describing the effect of mean length on the mean body dry weight (parameter).
EGR: Ratio of the mean egg dry weight, divided by the mean body dry weight when EFR is 1 (parameter).

The first part of the model represents the contribution of body mass and the second the egg incidence. Taking into account that in individuals of the same strain there are no appreciable changes in the body shape (Serra & Miracle, 1987; Snell & Carrillo, 1984) we can fix the value of parameter P_2 to 3, as this is the normal and logical exponent in all the organisms without these changes (Omori & Ikeda, 1984). The value of P_1 was fixed to 0.1 because it was highly correlated (0.9992) with the parameter RCF and varied between 0.12 and 0.076 in the final stages of the iterations. The value of STF for strain S-1 was taken as the reference and fixed to 1.

The values of the parameters obtained from the non-linear regression analysis were as follows:

STF: Strain S1 (reference): 1.000 (fixed)
Strain Bs: 2.000
BCF: 3.443
RCF: 0.626
P_1: 0.100 (fixed)
P_2: 3.000 (fixed)
EGR: 0.455

$R^2 = 0.911$

The results of the model are presented in the figs 2 and 3.

In the above model, the estimated strain factor (STF) for Bs is 2.0. This means that the small strain has in fact a body condition factor double that of the large strain. The assumption that the egg weight is proportional to mean body dry weight (implicit in the model) seems to be a logical conclusion as the egg length and width are linearly correlated with the lorica length (Fukusho, 1983; Yúfera, 1987). The mean egg dry weight is estimated in the second part of the equation, and represents 45.5% (100∗EGR) of the mean body dry weight when the egg/female ratio is 1.0, independent of the strain and the diet. When this parameter is set free in the regression analysis, the EGR values obtained for different strains and

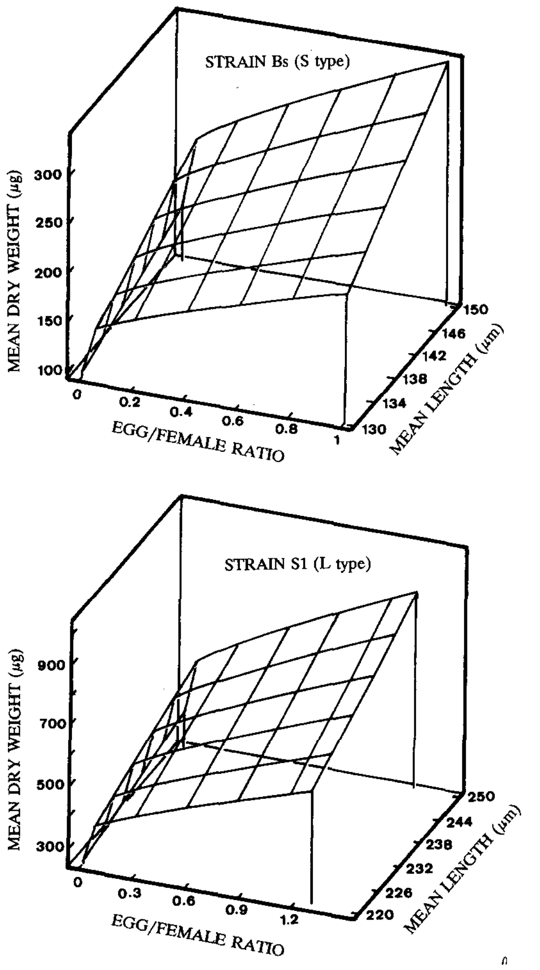

Fig. 2. Tridimensional plot showing the dry weight values estimated by the model.

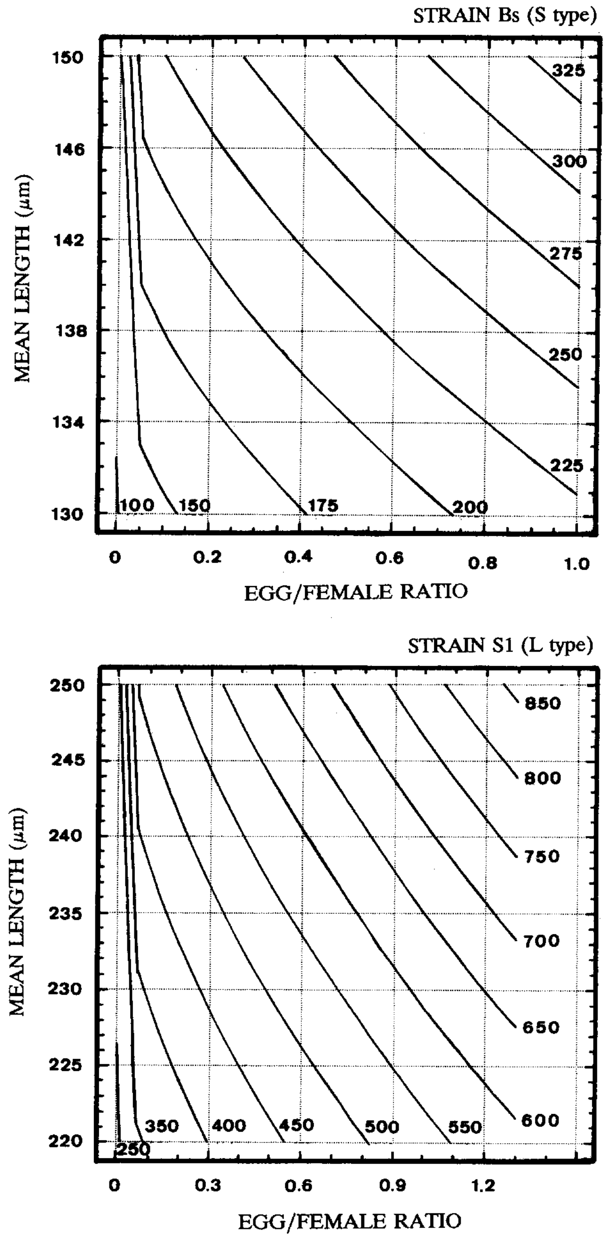

Fig. 3. Isopleths generated by the model describing the influence of egg/female ratio and mean lorica length on the dry weight. Numbers indicates the rotifer dry weight (ng).

phytoplankton species varied between 0.359 and 0.539 (Table 1). In demonstrating the validity of the model (taking into account that the mean egg dry weight is not given to the model explicitly) the expected mean egg dry weight for these strains and foods and the observed values are shown in Table 1. These results indicate that the model is very closely linked to reality because even small differences in the mean egg dry weight are detected when utilizing it. On the other hand, the pronounced decrease of weight (observed and estimated) when the egg/female ratio is very low reflects the starved condition of the animals because egg deposition ceases only below the maintenance ration.

The model may be applicable to different *B. plicatilis* strains, since all of then may be characterized as either S or L type (Fu *et al.*, 1991). In addition, the model may also be useful for other Brachionidae with similar shape if parameters were calculated by non-linear regression analysis from experimental data. A precise comparison with published data of other cultured clones is difficult because dry weight was not given as a function of the egg/female ratio and the mean lorica length of the population. However, the values obtained by Nagata (1985, 1989) on a L-type clone (mean lorica length: ≈245 μm, assuming spine length as 20 μm; neonate dry weight: 320 ng; egg dry weight: 260 ng; adult + 1 egg dry weight: 800 ng) and by James & Abu-Rezeq (1989) on a S-type clone (lorica length range: 90–160 μm; egg ratio 0.4 in chemostat culture; mean body dry weight: 220 ng) compares very well with the model.

In conclusion the model explains quite well the principal factors that, in our opinion, determine the dry weight in a given strain. These are the size structure and growth status of the population, which are in turn influenced by the environmental conditions.

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