

Leonard V. Polishchuk · Jacobus Vijverberg

## Contribution analysis of body mass dynamics in *Daphnia*

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**Abstract** The concept of body mass dynamics can be viewed as part of life history theory, but its potential has remained largely untapped due to a lack of analytical methodology. We therefore propose a method, called contribution analysis, which enables us to decompose a change in body mass into contributions associated with variations in individual egg mass, clutch size, and standard somatic mass (somatic mass adjusted to body length). The advantage of contribution analysis is that various contributions are expressed in the same units (units of mass) and show the amount of resources committed to changes in the individual traits, while the traits themselves are measured in different units and thus incomparable on their own. The method is tuned to study zooplankton, and is applied to examine body mass dynamics in *Daphnia galeata*. We found that when recovering from a poor-resource environment just above the threshold food concentration, *Daphnia* primarily increase their standard somatic mass, that is, restore body condition. When the trophic environment improves further but remains below the incipient limiting level, resources are invested equally to enhance body condition and reproduction in terms of clutch size. Finally, when food is no longer a limiting factor, almost all resources are committed to increase clutch size. While individual egg mass also varies, it never attracts more resources than the shift in the most prioritized trait. We suggest that the significance of this shift in resource

allocation priorities is to keep an adult female alive in a poor environment and thus to allow her to retain her reproductive potential for better conditions in the future. Contribution analysis of body mass dynamics may allow us to detect flexible allocation strategies in a changing natural environment.

**Keywords** Body condition · Clutch size · Life history · Resource allocation · Zooplankton

### Introduction

Life history theory takes a central role in modern ecology (Sibly and Calow 1986; Roff 1992; Stearns 1992), and research on body mass dynamics can be viewed as part of this field. Life history theory describes and explains the allocation of resources to structural growth (related to increase in body volume), storage, and reproduction (McCauley et al. 1990; Kooijman 2000; Shertzer and Ellner 2002). Each of these resource allocation pathways corresponds to a certain component of an individual's body mass—structural growth and storage are associated with elements of somatic mass, and reproduction with reproductive mass. Resources committed to a certain pathway make a change in the corresponding component mass, and all component changes add up to a change in the total body mass. Components of body mass dynamics are therefore coupled with resource allocation pathways, and can be interpreted in terms of life history theory.

The potential of research on body mass dynamics, in particular in zooplankton (Duncan 1985; Boersma and Vijverberg 1994; Manca et al. 1997; Winder and Spaak 2001), has remained largely untapped due to a lack of an appropriate analytical methodology. For example, if we observe that *Daphnia* somatic mass and clutch size (number of eggs per brood) are both going up, how can we compare these changes, and the resource allocation pathways underlying them, given that they are measured

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L. V. Polishchuk (✉) · J. Vijverberg  
Department of Food Web Studies,  
Netherlands Institute of Ecology (NIOO-KNAW),  
Centre for Limnology, Rijksstraatweg 6,  
3631 AC Nieuwersluis, The Netherlands

L. V. Polishchuk  
Department of General Ecology, Biological Faculty,  
M. V. Lomonosov Moscow State University,  
Moscow, 119899 Russia  
E-mail: leonard\_polishchuk@hotmail.com

in incomparable units? Here we present a new approach to analyse body mass dynamics in planktonic animals, which combines direct body mass and egg mass measurements (McCauley 1984; Salonen 1998) with a mathematical technique known as contribution analysis (Caswell 1989; Polishchuk 1995, 1999; Boersma and Vijverberg 1996; Levin et al. 1996; Mehner et al. 1998; Sibly and Smith 1998; Bizina 2000). This approach enables us to decompose body mass variations into component parts (contributions), each dependent on changes in a single trait relevant to body mass, and to compare them in order to find out which trait has priority to be restored when food conditions improve and which gets sacrificed first when food conditions deteriorate. This response to changing food conditions when viewed in the context of life history theory may allow us to identify strategies by which organisms and populations cope with environmental stresses, and to evaluate whether these strategies can be interpreted as evolutionarily adaptive.

The traits we are dealing with are individual egg mass, clutch size and standard somatic mass (somatic mass adjusted to body length). The former two refer to reproduction and the latter reflects an animal's body condition. Our focus is on variation in these traits and on their contributions to the resulting body mass shifts in response to changing trophic conditions. Such shifts can be tracked in a certain lake over time (Duncan 1985; Boersma and Vijverberg 1994; Manca et al. 1997; Winder and Spaak 2001) or among lakes arranged along an environmental gradient (Jeppesen et al. 2000). Typically, studies of body mass dynamics do not consider an individual's growth since successive body mass estimates refer to animals of a certain length, rather than to members of the same cohort in the course of their lifetime. Accordingly, individual growth is beyond the scope of the present study.

In this paper, we develop a methodology of contribution analysis in relation to body mass dynamics, and apply it to examine variation in body mass of the cladoceran *Daphnia galeata* under changing food conditions. The ultimate goal of this paper is to show that body mass dynamics can be analysed in the context of life history theory.

## Methods and materials

### Contribution analysis as applied to body mass dynamics

We start with the standard model of energy allocation within an individual (Shertzer and Ellner 2002), which suggests that net production is partitioned into structural body mass, storage (reserves) and reproductive mass

$$\text{Net production} = \Delta(\text{Structural mass}) + \Delta(\text{Storage}) \\ + \Delta(\text{Reproductive mass}),$$

where symbol  $\Delta$  emphasizes the fact that it is the increments of the corresponding components that add up to

net production. The net production, on the other hand, is equivalent to an increment in the total body mass  $C_t$ , that is

$$\Delta C_t = \Delta(\text{Structural mass}) + \Delta(\text{Storage}) \\ + \Delta(\text{Reproductive mass}). \quad (1)$$

This equation is true, regardless of whether an increment  $\Delta C_t$  is positive (i.e. body mass increases) or negative (i.e. body mass decreases, for instance, due to starvation). Since structural mass and storage together comprise somatic mass, the equation becomes

$$\Delta C_t = \Delta(\text{Somatic mass}) + \Delta(\text{Reproductive mass}). \quad (2)$$

The somatic mass scales as a power function of body length  $L$  (Peters 1983)

$$\text{Somatic mass} = D'L^a$$

where  $D'$  and  $a$  are parameters. It is worthwhile to present the last equation such that it contains body mass of an animal of unit length. This can be achieved by rewriting it as

$$\text{Somatic mass} = (D'L_0^a)(L/L_0)^a = Dl^a, \quad (3)$$

where  $L$  is measured in mm,  $L_0 = 1$  mm,  $l = L/L_0$  is dimensionless, and  $D = D' L_0^a$  thus represents the somatic mass of a 1-mm individual. It is called here the standard somatic mass, after Boersma and Vijverberg's (1994) standard (total) carbon content.

One interpretation of the standard somatic mass in the relationship of body mass to length (that is  $D$  in Eq. 3) is that it is a measure of an animal's 'state of health', or body condition (Bolger and Connolly 1989). The condition is closely related to the trophic situation animals are dealing with, as has been repeatedly observed in fishes (Bolger and Connolly 1989) and in cladocerans (Lampert 1977; Duncan 1985; Taylor 1985; Urabe 1988; Hessen 1990; Urabe and Watanabe 1991; Boersma and Vijverberg 1994; Glazier 1998). Another interpretation is based on the Dynamic Energy Budget (DEB) model (Kooijman 2000), which maintains that somatic mass can be divided into structural mass and storage, and that structural mass is primarily quantified in terms of an animal's body dimensions. The amount of structural mass in standard somatic mass  $D$  should therefore not vary with changing trophic situation (since  $D$  applies to an animal of a fixed body length) and, consequently, all variations in standard somatic mass should be due to variations in storage. These two interpretations of variations in  $D$  conform to each other, since changes in an individual's body condition are often interpreted in terms of varying amounts of reserves (Stearns 1992, p. 86).

The reproductive mass can be expressed as the product of individual egg mass  $C_e$  and clutch size  $E$

$$\text{Reproductive mass} = C_e E$$

Here only egg mass but not ovary contents is regarded as reproductive mass. This is because ovary contents may be resorbed under food shortages and thus may be regarded as part of storage. In addition, in many zooplankton species eggs are attached to the mother's body or placed into a brood chamber, so that the mass of individual eggs can be measured directly while the mass of the ovaries cannot. Substituting the expressions for somatic mass and reproductive mass into Eq. 2 gives

$$\Delta C_t = \Delta(Dl^a) + \Delta(C_e E). \quad (4)$$

The products under the  $\Delta$  sign can be expanded using the formula for the total differential

$$\Delta C_t \cong \Delta D(l^a) + D\Delta(l^a) + \Delta C_e E + C_e \Delta E \quad (5)$$

(for a more general derivation of this equation see Appendix). Since individual growth is not considered here, we assume body length  $L$  and hence  $l = L/L_0$  to be constant. The exponent  $a$  is constant too, for it is usually associated with changes in body length (Tesch 1968). This entails  $l^a = v$  being constant and  $\Delta(l^a) = 0$ . Hence Eq. 5 simplifies to

$$\Delta C_t \cong v\Delta D + E\Delta C_e - C_e \Delta E. \quad (6)$$

The shift in body mass  $\Delta C_t$  is thus decomposed into three components, or contributions (hereafter designated as Con followed by the corresponding variable), namely Con  $D = v \Delta D$ , Con  $C_e = E \Delta C_e$  and Con  $E = C_e \Delta E$ , each of which depends on a shift in only one trait,  $D$ ,  $C_e$  and  $E$ , respectively, while the two other traits are treated as constants. It is worth noting that the trait shifts, taken on their own, are not additive (because the traits are measured in incomparable units); contribution analysis, however, makes them additive by appropriately choosing the coefficients multiplying the trait shifts (these coefficients are partial derivatives, or sensitivities of body mass with respect to a unit trait shift; see Appendix). Moreover, the sum of contributions equals the resulting shift in body mass, although in general Eq. 6 only approximates  $\Delta C_t$  (in the next section we show how to make this approximation exact). Finally, there is a correspondence between the decomposition into contributions (Eq. 6) and the original balance equation (Eq. 1): Con  $D$  corresponds to  $\Delta(\text{Storage})$ , Con  $C_e$  plus Con  $E$  to  $\Delta(\text{Reproductive mass})$ , and  $\Delta(\text{Structural mass})$  becomes zero since body length is constant ( $\Delta(\text{Structural mass})$  corresponds to  $D \Delta(l^a)$  in Eq. 5).

Since contributions are expressed in the same units as body mass, contribution analysis provides a common currency to compare the effect of standard somatic mass, individual egg mass and clutch size on body mass dynamics. Moreover, contributions can be interpreted as the amount of resources committed to, or extracted from, the body's storage (Con  $D$ ), an individual egg (Con  $C_e$ ) and the number of eggs (Con  $E$ ), which add up to comprise the resulting shift in the total body mass.

## Calculating contributions

We use contribution analysis to examine how *Daphnia* of a certain body length allocate available resources in response to changing food conditions. Animals reared at different food levels have different body length, however. This does not affect standard somatic mass and individual egg mass but does affect clutch size, which significantly depends on length (see Results). To correct for the effect of length, we obtained linear regressions of clutch size on body length at each food level and then found clutch size  $E_a$  adjusted to a certain body length  $L_c$

$$E_a = c + bL_c, \quad (7)$$

where  $c$  and  $b$  are food-level specific regression parameters while  $L_c$  is common to all food levels. This common body length  $L_c$  is also used to calculate  $v = (L_c/L_0)^a$ .

Now we need a more specific notation to provide exact formulas for calculating contributions. Let us designate the observed trait shifts from food level 1 to food level 2 by  $\Delta D = D_2 - D_1$ ,  $\Delta C_e = C_{e2} - C_{e1}$  and  $\Delta E_a = E_{a2} - E_{a1}$ , and note that according to Eq. 4 and given  $E = E_a$  the total body mass is

$$C_t = vD + C_e E_a. \quad (8)$$

Then we have: Con  $D + \text{Con } C_e + \text{Con } E_a = v(D_2 - D_1) + E_a(C_{e2} - C_{e1}) + C_e(E_{a2} - E_{a1})$  and  $\Delta C_t = (vD_2 + C_{e2}E_{a2}) - (vD_1 + C_{e1}E_{a1})$ . The sum of contributions equals exactly the shift in body mass, if in Con  $C_e$  and Con  $E_a$  one takes the mean value for the coefficients multiplying the trait shifts. Thus, the exact formulas for contributions are

$$\text{Con } D = v(D_2 - D_1), \quad (9a)$$

$$\text{Con } C_e = 0.5(E_{a1} + E_{a2})(C_{e2} - C_{e1}), \quad (9b)$$

$$\text{Con } E_a = 0.5(C_{e1} + C_{e2})(E_{a2} - E_{a1}), \quad (9c)$$

The errors of contributions were quantified by means of Monte Carlo simulations. 1,000 values of  $C_e$ ,  $E_a$  and  $D$  were produced by random sampling from the normal distributions whose means and standard deviations were set equal to the experimental values of  $C_e$ ,  $E_a$  and  $D$  obtained at four food concentrations, and to their respective standard errors. These random values of  $C_e$ ,  $E_a$  and  $D$  were used to calculate 1,000 contributions of Con  $C_e$ , Con  $E_a$ , and Con  $D$  for each between-concentration shift in body mass. The standard deviation estimated on the basis of this contribution array was taken as the standard error for the contributions calculated on the basis of the experimental  $C_e$ ,  $E_a$  and  $D$ .

## Life table experiment

The experiment was performed with one clone (GAL21) of *Daphnia galeata*, a well-established laboratory clone

from the Netherlands Institute of Ecology. Animals were kept in 140-ml plexiglas flow-through vessels at 17.5°C with a light:dark regime of 16:8 h as used by Doksaeter and Vijverberg (2001), and with a maximum of eight animals per vessel. The flow-through rate was 3.6-fold medium renewal (ca. 500 ml) per day. The vessels and plankton gauze were cleaned every 4–5 days and tubing was replaced after 2 weeks.

Log-phase cells of *Scenedesmus obliquus* algae grown in chemostats were used as food. Fresh food medium was prepared daily. Prior to use the algae were centrifuged and then resuspended in 0.45 µm-filtered lake water.

Prior to the start of the experiment animals were grown at a food concentration of 0.5 mg C l<sup>-1</sup> over three generations to obtain sufficient numbers. The experiment itself was started with newborns from the third brood produced by the third generation.

Four different food concentrations were tested: 0.07, 0.11, 0.18 and 1.00 mg C l<sup>-1</sup>. Our attempt to collect data at a lower concentration of 0.04 mg C l<sup>-1</sup> failed because we were unable to obtain a sufficient number of adults needed for the analysis. The lowest successful food concentration is just above the threshold food concentration (0.03–0.04 mg C l<sup>-1</sup> for *D. galeata*; Gliwicz 1990; Achenbach and Lampert 1997), this and the two intermediate concentrations are below the incipient limiting level (0.26–0.36 mg C l<sup>-1</sup> for a similar-sized *D. longispina*; Lampert 1987), and the highest food concentration used is far above the incipient limiting level. Each food treatment consisted of 9–13 replicates (vessels), and each vessel was started with eight randomly selected neonates that were born within the same time interval of 12 h. The experiment was continued until the animals reached the third adult instar, which took 2–4 weeks depending on food concentration. Increased mortality was observed at the concentration of 0.07 mg C l<sup>-1</sup>, but mortality at the three higher concentrations was low (1–2% day<sup>-1</sup>), which is generally the case in well-designed *Daphnia* cultures (Vijverberg 1989).

We examined the first to third adult instars with eggs, all three instars being in approximately equal proportions at every food treatment. Since growth in mass may be high during an intermolt period (Tessier and Goulden 1982), only adults carrying eggs in developmental stage I (Threlkeld 1979) were dealt with. The clutch size  $E$  (number of eggs in the brood chamber) and body length  $L$  (mm) were recorded for every animal. The body length was measured from the top of the head to the base of the tail spine under the microscope with an ocular ruler.

To assess the amount of carbon contained in adult daphnids ( $C_s$ , µg carbon) and in eggs ( $C_e$ , µg carbon), about half of the individuals from each vessel, taken at random, was dissected to release the eggs from the brood chamber, while the other half was kept intact. The whole animals with eggs and individual eggs were first washed in deionised water in order to exclude external carbon sources and then dried in silver cups

at 60°C before measurements were performed. All animals were burned singly while eggs were burned in groups belonging to one clutch, and the individual egg mass  $C_e$  was then calculated as an average over group. The number of carbon measurements for animals was: 35 (0.07 mg C l<sup>-1</sup>), 57 (0.11 mg C l<sup>-1</sup>), 54 (0.18 mg C l<sup>-1</sup>) and 54 (1.00 mg C l<sup>-1</sup>). The number of carbon measurements for  $C_e$  was: 22 (0.07 mg C l<sup>-1</sup>), 35 (0.11 mg C l<sup>-1</sup>), 39 (0.18 mg C l<sup>-1</sup>) and 36 (1.00 mg C l<sup>-1</sup>). A CALANUS carbon analyser (Salonen 1998) was used for carbon determination.

## Results

### Individual trait estimates

#### Egg mass

The individual egg mass  $C_e$  does not depend on the mother's length at any of the four food levels ( $F_{1,20}=0.004$ ,  $P=0.95$  at 0.07 mg C l<sup>-1</sup>;  $F_{1,33}=0.43$ ,  $P=0.51$  at 0.11 mg C l<sup>-1</sup>;  $F_{1,37}=2.65$ ,  $P=0.11$  at 0.18 mg C l<sup>-1</sup>;  $F_{1,34}=0.05$ ,  $P=0.83$  at 1.00 mg C l<sup>-1</sup>; Fig. 1), which is likely due to a narrow range of the females' sizes since we deal with three adult instars only. Hence, for contribution analysis the individual egg mass  $C_e$  is taken as an average over all individual egg mass estimates per food level. One data point at 0.07 mg C l<sup>-1</sup> (Fig. 1a) and one data point at 0.11 mg C l<sup>-1</sup> (Fig. 1b) show very large values of egg mass (> 1.4 µg C per egg) and are likely to be outliers; however, had they been excluded from calculations, it would have only slightly affected the average egg mass (upon exclusion average  $C_e$  shifts from 0.73 µg C to 0.70 µg C at 0.07 mg C l<sup>-1</sup> and from 1.00 µg C to 0.97 µg C at 0.11 mg C l<sup>-1</sup>), and made no difference to the lack of the effect of the mother's length on egg mass (upon exclusion  $F_{1,19}=0.005$ ,  $P=0.95$  at 0.07 mg C l<sup>-1</sup>;  $F_{1,32}=0.65$ ,  $P=0.43$  at 0.11 mg C l<sup>-1</sup>).

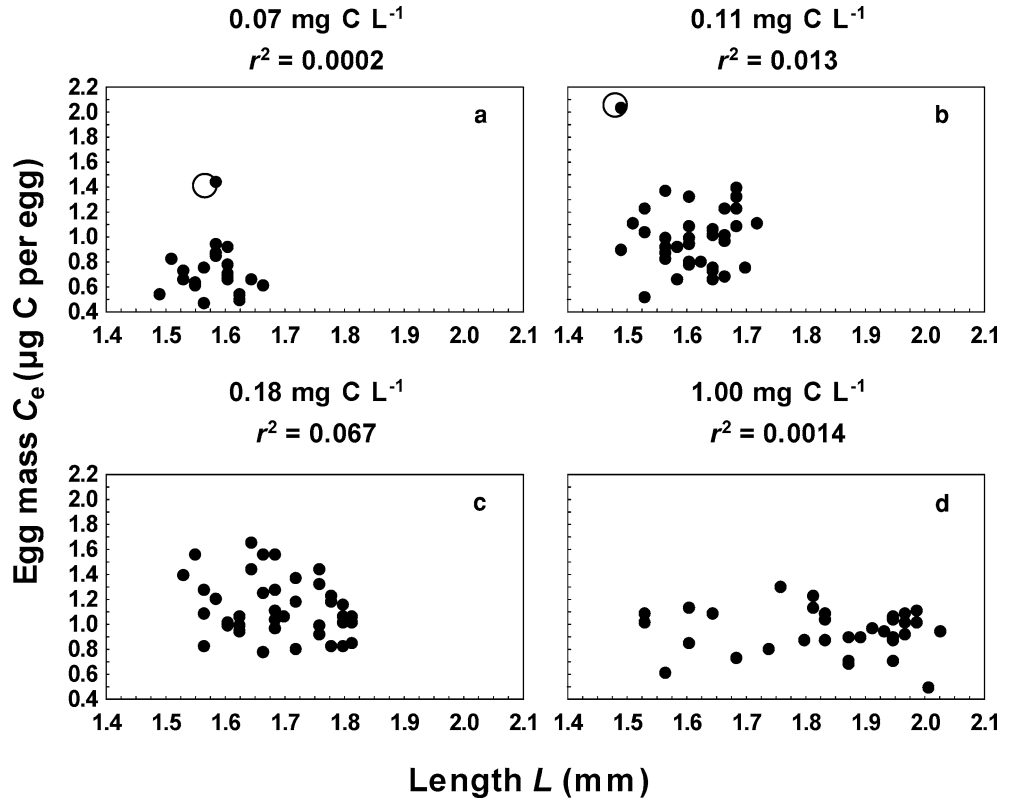
#### Clutch size

Clutch size  $E$  depends upon body length at all food concentrations, as evidenced by the regressions of clutch size on body length per food level ( $F_{1,33}=10.98$ ,  $P < 0.01$  at 0.07 mg C l<sup>-1</sup>;  $F_{1,55}=10.91$ ,  $P < 0.01$  at 0.11 mg C l<sup>-1</sup>;  $F_{1,52}=86.50$ ,  $P < 0.001$  at 0.18 mg C l<sup>-1</sup>;  $F_{1,52}=140.04$ ,  $P < 0.001$  at 1.00 mg C l<sup>-1</sup>). The adjusted clutch size  $E_a$  is calculated by Eq. 7 from the regressions given in Fig. 2, and provided with a standard error (Sokal and Rohlf 1995). The common body length  $L_c = 1.675$  mm is taken to be an average length over all 200 animals examined in this study.

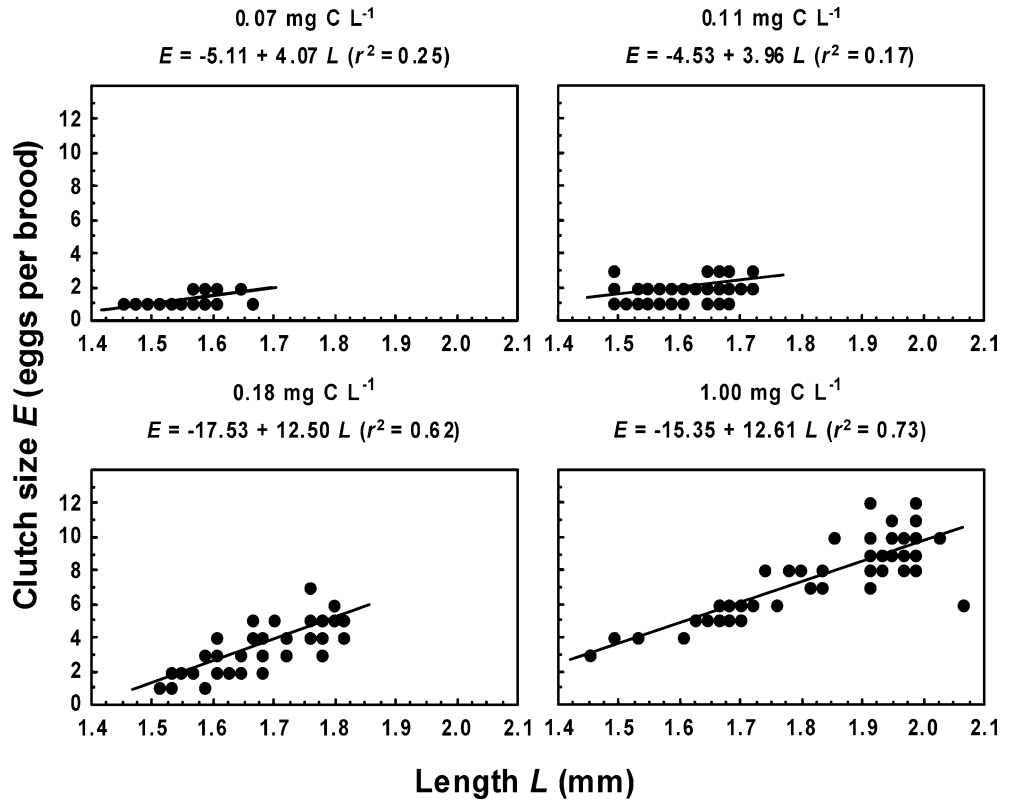
#### Standard somatic mass

For each individual animal, somatic mass  $C_s$  was calculated as the total body mass minus total egg mass, the

**Fig. 1** Individual egg mass in relation to body length at four food concentrations: 0.07 (a), 0.11 (b), 0.18 (c) and 1.00 (d) mg C L<sup>-1</sup>. Encircled in a and b are two likely outliers which, however, only slightly affect the results. For more details see text



**Fig. 2** Clutch size in relation to body length at four food concentrations



latter being the number of eggs present in the brood chamber of a given animal times the average individual egg mass per food level. The somatic mass  $C_s$  was first

regressed on the dimensionless index  $l$  of body length  $L$  to give standard somatic mass  $D$  and body-length exponent  $a$  specifically for each food level. Since  $D$  is the



intercept of an  $l$ - $C_s$  regression, it need not be adjusted to length. However, it needs to be adjusted to a common value of  $a$ , because we are not interested in the behaviour of  $a$ , which is largely associated with length growth, and because such adjustment would make an estimate of  $D$  more accurate. The latter is particularly important in view of the fact that the range of the independent variable in our  $l$ - $C_s$  regressions is relatively small (since our analysis is restricted to three adult instars only) and may not be sufficient to get accurate estimates for the two parameters,  $a$  and  $D$ , at one stroke (Boersma and Vijverberg 1994, and Winder and Spaak 2001 provide further arguments for such adjustment). Hence, we calculated an average value of  $a$  over four food-level specific exponents, which is found to equal 3.07 for original exponents obtained from nonlinear regression (Statistica for Windows 2003) and 3.01 for original exponents obtained from linear regression on log-transformed data.  $D$  at each food level was then worked out from a linear regression of  $C_s$  on  $l^{3.07}$  with the intercept set to zero (Fig. 3); had we regressed  $C_s$  on  $l^{3.01}$ ,  $D$ 's would have differed by less than 5%.

#### The behaviour of the individual traits

The traits do not remain constant while trophic conditions gradually improve, as revealed by one-way ANOVA for  $C_e$  ( $F_{3,128}=18.8$ ,  $P < 0.001$ ; data are log-transformed to ensure the homogeneity of variance: Bartlett's  $\chi^2=4.0$ ,  $df=3$ ,  $P=0.26$ ) and by  $t$ -test on the trait's successive estimates over the four food concentrations used, with a significance level of 0.05 (for indi-

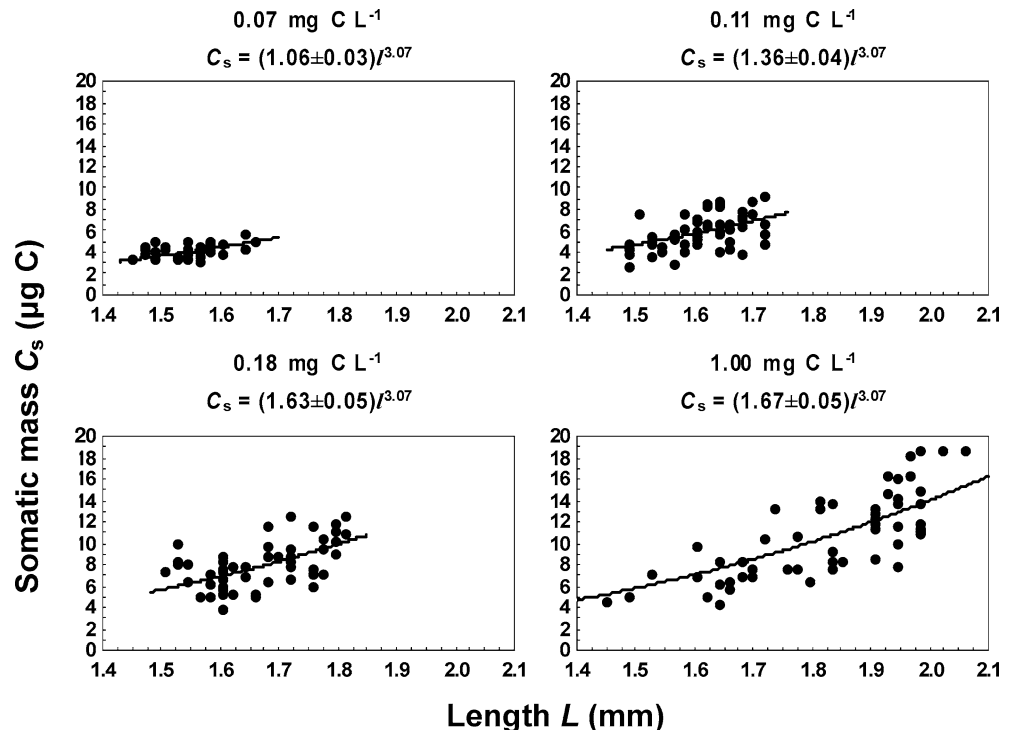
vidual egg mass  $C_e$  all three successive differences are significant; for clutch size  $E_a$  the difference between  $0.07 \text{ mg C l}^{-1}$  and  $0.11 \text{ mg C l}^{-1}$  is marginally significant,  $P=0.052$ , while the two other differences are significant; for standard somatic mass  $D$  the differences at lower food concentrations are significant but the one between  $0.18 \text{ mg C l}^{-1}$  and  $1.00 \text{ mg C l}^{-1}$  is not significant,  $P=0.53$ ; Fig. 4a–c).

The individual egg mass  $C_e$ , adjusted clutch size  $E_a$  and standard somatic mass  $D$  demonstrate a variety of responses to increasing food concentration:  $C_e$  follows a hump-shaped curve;  $E_a$  experiences a consistent trend upwards; while  $D$  exhibits a saturation response (Fig. 4a–c). According to Eq. 8 with  $v=4.875$  ( $=1.675^{3.07}$ ), the food-level specific  $C_e$ ,  $E_a$  and  $D$  yield the total body mass  $C_t$ , whose changes are the subject of contribution analysis. This calculated  $C_t$  differs from the observed total body mass  $C_o$  (Fig. 4d), though the difference is noticeable only at  $1.0 \text{ mg C l}^{-1}$  (since at this food level daphnids grew to a somewhat larger size than  $1.675 \text{ mm}$  to which trait values are adjusted). Paradoxically perhaps, while  $C_t$  is fully determined by the known individual traits, a visual inspection of Fig. 4 does not tell us much as to which of the traits produces a larger effect on the increase in the total body mass over the trophic gradient. Furthermore, the pattern of the effects remains hidden.

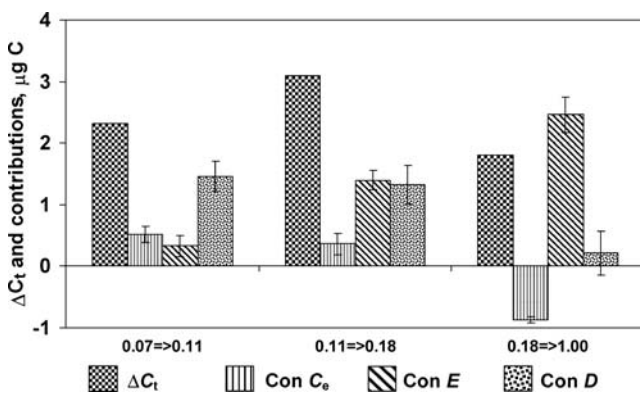
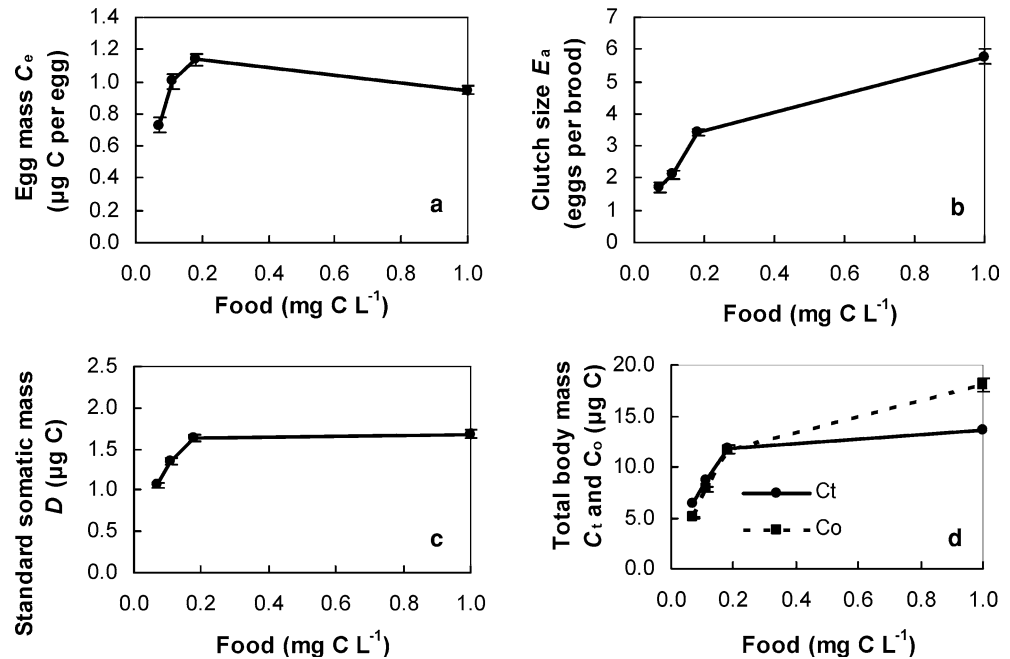
#### Contribution analysis

Contributions calculated according to Eqs. 9a, b, c are shown in Fig. 5. The left-most bar in each cluster rep-

**Fig. 3** Somatic mass in relation to body length at four food concentrations. The equations at the top of each panel are adjusted to hold a common exponent of 3.07. The coefficient in front of  $l^{3.07}$  is standard somatic mass  $D \pm 1$  SE. The  $D$  is expressed in  $\mu\text{g C}$  since  $l=L/L_0$  is dimensionless;  $L_0=1 \text{ mm}$



**Fig. 4** Average individual egg mass  $C_e$  (a), clutch size  $E_a$  adjusted to body length (b) and standard somatic mass  $D$  (c) in relation to four food concentrations (0.07, 0.11, 0.18 and 1.00 mg C l<sup>-1</sup>). Error bars represent  $\pm 1$  SE. These quantities are used as input data for contribution analysis. Also shown are total body mass  $C_t$  (d) calculated on the basis of  $C_e$ ,  $E_a$  and  $D$ , and observed total body mass  $C_o$  ( $\pm 1$  SE), which is different from  $C_t$  because the latter refers to animals of a fixed size of 1.675 mm



**Fig. 5** Contribution analysis of an increase in body mass of *Daphnia galeata* along a trophic gradient. Each shift in the total body mass ( $\Delta C_t$ ) is decomposed into three contributions, with each contribution associated with a shift in a single trait—individual egg mass (Con  $C_e$ ), clutch size (Con  $E_a$ ) and standard somatic mass (Con  $D$ ); the sum of contributions equals  $\Delta C_t$ . Three body mass shifts correspond to transitions between successive food levels—from 0.07 to 0.11, 0.11 to 0.18, and 0.18 to 1.00 mg C l<sup>-1</sup>; each shift is analysed separately. Error bars represent  $\pm 1$  SE calculated by means of Monte-Carlo simulations

resents a change in the total body mass  $C_t$  and three other bars show the contributions to this change of individual egg mass  $C_e$ , clutch size  $E_a$  and standard somatic mass  $D$ , with the sum of contributions being equal to the resultant change in body mass,  $\Delta C_t$ . It is worth noting that a bar above the zero line indicates that body mass goes up or that a contribution is positive and causes body mass to increase, while a bar below the zero line indicates that body mass goes down or that a contribution is negative and causes body mass to decline. We perform contribution analysis successively from an

animal's state at 0.07 mg C l<sup>-1</sup> to its state at 0.11 mg C l<sup>-1</sup>, from its state at 0.11 mg C l<sup>-1</sup> to that at 0.18 mg C l<sup>-1</sup>, and from its state at 0.18 to that at 1.00 mg C l<sup>-1</sup>.

The Monte Carlo contribution errors show that all large contributions are significantly different from zero (Fig. 5). Specifically, out of nine contributions obtained from the analysis of the three between food-level changes, the smallest one (by absolute value) is nonsignificant (this is Con  $D$  for a change between 0.18 mg C l<sup>-1</sup> and 1.00 mg C l<sup>-1</sup>;  $P=0.55$ , calculated from the normal distribution), the next-to-smallest one is marginally significant (this is Con  $E_a$  for a change between 0.07 mg C l<sup>-1</sup> and 0.11 mg C l<sup>-1</sup>;  $P=0.06$ ), and all the rest are significant at a level of 0.05. Clearly, large (and significant) contributions are of major interest but small contributions also need to be noted, even if nonsignificant, because, as we see below, it is the relation between the large and the small that makes the pattern of contributions particularly interesting.

Figure 5 shows that body mass  $C_t$  exhibits a monotonic increase along the trophic gradient. The increment in body mass, however, is differently partitioned between certain body components and respective functions in different portions of the gradient. When just recovering from a poor-resource environment (0.07 to 0.11 mg C l<sup>-1</sup>), adult *Daphnia* of a fixed size devote most of the available resources to restore body condition, that is storage (Con  $D$ ), and less to restore reproduction either in the form of Con  $C_e$  or in the form of Con  $E_a$ . When animals face intermediate but still stringent trophic conditions (0.11 to 0.18 mg C l<sup>-1</sup>), resources are invested equally in enhancing body condition and reproduction in terms of egg numbers (Con  $E_a$ ), but relatively little goes to an increase in

individual egg mass (Con  $C_e$ ). Finally, when food is no longer a limiting factor (0.18 to 1.00 mg C l<sup>-1</sup>), almost all of it is committed to boost reproduction in terms of number of eggs, while an individual egg is even somewhat deprived of resources and body condition remains virtually unchanged. That is, as trophic conditions gradually improve we observe a consistent pattern of resource allocation that can be described as a shift in priorities—animals first restore body condition and then foster reproduction in terms of number of eggs, while keeping individual egg mass relatively constant. The ratio of the contribution to body condition over the contribution to clutch size (Con  $D$ /Con  $E_a$ ) seems indicative of trophic situation: it is 4.4 at poor food conditions, roughly 1.0 at intermediate conditions and about 0.1 when conditions improve beyond the incipient limiting level.

Changes in body mass and corresponding contributions can be traced in the reverse direction, that is from 1.00 mg C l<sup>-1</sup> to 0.07 mg C l<sup>-1</sup>, when body mass declines. The resulting picture of contributions appears if the bars in Fig. 5 are mirrored relative to the  $X$ -axis. We then observe that as food conditions gradually deteriorate, animals first sacrifice reproduction while keeping body condition untouched, then equally cut down on reproduction and body condition and finally are forced to draw from reserves in order to survive.

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## Discussion

Our results suggest that there is no uniform pattern of resource allocation independent of the trophic situation, but rather, a consistent shift in priorities between competing demands for maintaining body condition and producing eggs. What is essential is in which part of the trophic gradient animals happen to occur. When food concentration is far below the incipient limiting level, the main trait to be dealt with is body condition: *Daphnia* immediately restore body condition with increasing food abundance, and allocate less to reproduction. In contrast, when food concentration is largely above the incipient limiting level, available resources are committed to increasing clutch size rather than to further enhancing body condition. When trophic situation changes in the opposite direction, the shift in priorities turns the other way round: reproduction which has been restored last is sacrificed first.

In the context of life history theory, shifts in resource allocation priorities such as those we have observed here have been interpreted as part of an evolutionarily adaptive strategy by which organisms and populations cope with food shortages (Glazier 2002). The significance of the observed shift is to keep an adult female alive in a poor-resource environment and thus to allow her to retain her reproductive potential for better conditions in the future. This is a characteristic feature of the iteroparous reproductive strategy that is typical for

cladocerans (Threlkeld 1987) as well as for many other animals (Roff 1992).

While the priority of maintaining body condition over reproduction at low food levels is not new (Perrin et al. 1990; McCauley et al. 1990; Glazier and Calow 1992; see Glazier 2002 for general discussion), there are two main reasons why contribution analysis may be particularly useful. First, owing to a high between-state resolution (an animal's state here is associated with a certain trophic environment), it allows us to track a full sequence of events—from body condition attracting a major part of resources at one end of the trophic gradient to clutch size attracting a major part of resources at the other end, with an intermediate state when both traits receive a roughly equal amount of resources. Second, owing to a high between-trait resolution, it enables us to identify leading players in the resource allocation theatre. These are body condition and clutch size but not individual egg mass, contribution to which is never the largest by absolute value compared to the contribution to one or to both of the other traits (Fig. 5). This is not to say that the hump-shaped response of individual egg mass to changes in trophic conditions does not have adaptive significance—in fact it has (Tessier and Consolatti 1991; Glazier 1992; Boersma 1995). This is only to say that variations in body condition and in number of eggs attract more resources than variations in individual egg mass, the latter being consistent with optimal offspring size theory (Smith and Fretwell 1974; Eium and Fleming 2000). As a result, the shift in resource allocation priorities is largely determined by the interplay between body condition and number of eggs. Due to the high potential for between-state and between-trait resolution, contribution analysis of body mass dynamics may allow us to detect flexible allocation strategies under changing trophic conditions, that are characteristic of many natural environments.

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## Appendix

A general approach to the derivation of contributions

Consider an output variable  $y = y(x_1, x_2, \dots, x_k)$ , which is a function of input variables  $x_i$ 's ( $i = 1, \dots, k$ ), and let  $y$  increase or decrease by some value  $\Delta y$ . A general mathematical approach to decomposing  $\Delta y$  into contri-



butions, each of which dependent on change in only one input variable, is expanding  $y(x_1, x_2, \dots, x_k)$  in a Taylor series and dropping the second and higher-order terms to yield

$$\Delta y \cong \sum_i \left( \frac{\partial y}{\partial x_i} \right) \Delta x_i, \quad (10)$$

where  $\Delta x_i$  is a change in  $x_i$ , and  $\partial y / \partial x_i$  is the partial derivative of  $y$  with respect to  $x_i$ . The partial derivative represents sensitivity of  $y$  with respect to a unit change in  $x_i$ , while the product of this sensitivity by an actual change in  $x_i$ ,  $(\partial y / \partial x_i) \Delta x_i$ , gives the contribution of that  $x_i$ -change to the resultant  $y$ -change.

According to Eq. 4 in the main text, the basic equation for body mass is

$$C_t = D I^a + C_e E$$

An application of the general approach (Eq. 10) to this equation gives

$$\begin{aligned} \Delta C_t &\cong \left( \frac{\partial C_t}{\partial D} \right) \Delta D + \left( \frac{\partial C_t}{\partial I^a} \right) \Delta(I^a) + \left( \frac{\partial C_t}{\partial C_e} \right) \Delta C_e + \left( \frac{\partial C_t}{\partial E} \right) \Delta E \\ &\cong I^a \Delta D + D \Delta(I^a) + E \Delta C_e + C_e \Delta E \end{aligned} \quad (11)$$

which is Eq. 5 in the main text. Although in general Eq. 11 only approximates  $\Delta C_t$  (the more accurately, the smaller are the changes in the input variables), it can often be made exact through an appropriate choice of the points at which to evaluate partial derivatives (see main text).

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