

Experimental testing of dynamic energy budget models

E. G. NOONBURG,* R. M. NISBET,* E. MCCAULEY,† W. S. C. GURNEY,‡
W. W. MURDOCH* and A. M. DE ROOS§

*Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106, USA, †Ecology Division, Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4, ‡Department of Statistics and Modelling Science, University of Strathclyde, Glasgow G1 1XH, UK, and §Department of Pure and Applied Ecology, University of Amsterdam, Kruislaan 320, 1098 SM Amsterdam, the Netherlands

Summary

1. Dynamic energy budget (DEB) models describing the allocation of assimilate to the competing processes of growth, reproduction and maintenance in individual organisms have been applied to a variety of species with some success. There are two contrasting model formulations based on dynamic allocation rules that have been widely used (net production and net assimilation formulations). However, the predictions of these two classes of DEB models are not easily distinguished on the basis of simple growth and fecundity data.

2. It is shown that different assumptions incorporated in the rules determining allocation to growth and reproduction in two classes of commonly applied DEB models predict qualitatively distinct patterns for an easily measured variable, cumulative reproduction by the time an individual reaches an arbitrary size.

3. A comparison with experimental data from *Daphnia pulex* reveals that, in their simplest form, neither model predicts the observed qualitative pattern of reproduction, despite the fact that both formulations capture basic growth features.

4. An examination of more elaborate versions of the two models, in which the allocation rules are modified to account for brief periods of starvation experienced in the laboratory cultures, reveals that a version of the net production model can predict the qualitative pattern seen for cumulative eggs as a function of mass in *D. pulex*. The analysis leads to new predictions which can be easily tested with further laboratory experiments.

Key-words: *Daphnia*, energy allocation, individual growth, physiological ecology, reproduction

Functional Ecology (1998) **12**, 211–222

Introduction

A large body of literature contains observations relating patterns of growth and reproduction of individual organisms to food supply. Typical data for many species include growth curves, age or size thresholds for the onset of reproduction, and age or size dependence of fecundity. Food-limited growth and reproduction are the outcome of the allocation of finite resources to competing physiological processes, yet it is difficult to infer unambiguously an organism's priorities from the commonly available data (e.g. McCauley, Murdoch & Nisbet 1990a; McCauley *et al.* 1990b).

Dynamic energy budget (DEB) models (Kooijman 1993; Nisbet, Ross & Brooks 1996; and references therein) describe the rules by which individual organisms assimilate and utilize energy from food. In these models individuals are characterized by a small number of 'i-state variables' (e.g. age, length, mass)

and interact with the 'environment' (e.g. food density, temperature, pH). Feeding rates depend on both the i-state and the environment, but the priorities for energy allocation to maintenance, growth and reproduction generally depend only on the i-state. Thus DEB models represent an individual as a 'black box' with one 'input' from the environment (assimilation), and several 'outputs' (growth reproduction, respiration, excretion, etc.). The model relationships between input and output are determined by a set of differential equations in the i-state variables, and for any given model it is a straightforward exercise to predict growth and reproduction in a known food environment (e.g. Kooijman 1986, 1993; Nisbet *et al.* 1989; Gurney *et al.* 1990; Ross & Nisbet 1990). However, the inverse problem, inference of the allocation rules (which represent unknown physiology) from data on growth and reproduction at different food levels, is mathematically much more demanding, and with

limited or noisy data may be impossible. This is because different models can often be parameterized to give equally good fits to any given set of growth and fecundity data (see e.g. Ross & Nisbet 1990 for models of the mussel *Mytilus edulis*).

Although difficult to detect from commonly available data on individuals, differences in allocation rules can have pronounced effects on population dynamics (Gurney *et al.* 1996), with the demography of food-limited populations at equilibrium being determined by the priorities for energy allocation. Thus Murdoch *et al.* (1992) argued that, wherever possible, models of growth and reproduction must be tested directly against experimental data on the performance of individuals, before they are used as the basis for population models. If a model 'fails' the tests, it should be modified (and re-tested). However, this classic modelling philosophy is difficult to implement for the reasons given above. Nisbet *et al.* (1996) argued that to facilitate experimental tests, DEB models should be formulated in a way that makes it as easy as possible to distinguish the effects of (a) model structure (the rules describing partitioning of energy within an organism), (b) model functions (how quantities such as assimilation and maintenance rates vary with the length or mass of the organism) and (c) model parameters.

The primary question addressed in this paper is whether we can derive robust, qualitatively distinguishable predictions from two model families that differ solely in their assumptions on model structure. Most DEB models of ectotherms in the literature fall into one of two families, which we call *net production* and *net assimilation* models and define formally in the next section of this paper. The two groups of models differ in their assumptions concerning the allocation of energy or assimilate to reproduction. Table 1 gives a taxonomically diverse selection of examples of each group.

Daphnia is an ideal organism to test predictions from DEB models, as the literature contains observations of growth and fecundity for several species under a variety of feeding regimes (Elendt 1989; McCauley *et al.* 1990a and references therein; Bradley, Perrin & Calow 1991; Urabe & Watanabe 1991; Glazier & Calow 1992). Furthermore, *Daphnia*

are of both general ecological and applied interest because they are an important component of many freshwater food webs and their fecundity is used in standardized toxicity tests (e.g. Kooijman & Bedaux 1996 and references therein). In this paper, we focus on a data set for *Daphnia pulex* collected by one of the authors (E.M.), but broaden our analysis by also testing the models against two previously published data sets on *D. pulex* (Richman 1958; Paloheimo, Crabtree & Taylor 1982).

In the following section, we introduce simple net production and net assimilation models. The use of simple models allows us to focus on differences in predictions that are caused solely by the different allocation priorities. We show that the two models make qualitatively distinct predictions for an easily observable food-dependent variable, the number of eggs produced by the time an individual reaches a given size, and demonstrate that our *D. pulex* data are inconsistent with the prediction of either model for this variable. We conclude the paper with a discussion of more sophisticated models in the literature, which in general are unable to reproduce the pattern observed in the data, and propose a potential resolution of the inconsistency in the context of net production models.

Theory

FORMULATION OF THE BASIC MODELS

Simple DEB models assume a common currency of energy or biomass; in this paper carbon is chosen as the unit of this currency. Only adult (i.e. reproductively mature) females are considered, and the *i*-state of an adult individual is represented by a single variable, $W(t)$, representing the total carbon committed *as an adult* to somatic tissue by time t . The measured carbon content of an animal, $C(t)$, at time t , is related to $W(t)$ as follows:

$$C(t) = W(t) + w_m, \quad \text{eqn 1}$$

where w_m is the carbon content of an animal at the onset of reproductive maturity. Individuals assimilate

Table 1. Examples from the literature of organisms to which dynamic energy budget models have been applied. We have classified the models as net production or net assimilation according to the form of the allocation rule (see Theory section)

Net production models		Net assimilation models	
Organism	Reference	Organism	Reference
Rotifers	Majkowski & Bramall (1980)	<i>Daphnia</i>	Evers & Kooijman (1989)
<i>Daphnia</i>	Paloheimo <i>et al.</i> (1982)	Pond snails	Zonneveld & Kooijman (1989)
Walleye Pollack, Pacific Halibut	Bledsoe & Megrey (1989)	<i>Daphnia</i>	Gurney <i>et al.</i> (1990)
Striped Bass	Brandt & Kirsch (1993)	Bacteria	Kooijman, Muller & Stouthamer (1991)
Salmonids	Broekhuizen <i>et al.</i> (1994)	Mussels	van Haren & Kooijman (1993)
		General	Kooijman (1993)
		Reptile embryos	Zonneveld & Kooijman (1993)
		Nematodes	Ratsak, Kooijman & Kooi (1993)

carbon at a rate A which in general depends on $C(t)$ and on the concentration of food in the environment. Maintenance rate, M , is assumed to depend only on $C(t)$. If R denotes the rate of commitment of carbon to reproduction, then carbon balance requires that

$$\frac{dW}{dt} = A - M - R. \quad \text{eqn 2}$$

If, as in the *Daphnia* used in the model tests, carbon committed to reproduction is rapidly bound irreversibly in eggs, then we can compute $E(t)$, the cumulative egg production by time t , from

$$\frac{dE}{dt} = R/w_E, \quad \text{eqn 3}$$

where w_E is the mass of an egg (assumed constant in all our models).

Two models that differ only in their assumptions concerning the allocation to reproduction are considered. Full model specification requires rules for all possible combinations of i -state and the food environment, including priorities when food is scarce and assimilation is inadequate to meet maintenance (Nisbet *et al.* 1996). Here, for compactness, only the equations for the (default) situation of adequate food are given, but the effects of short bouts of starvation are considered in the Discussion.

In the *net production* model, the energy required for maintenance is allocated first from assimilation. A fraction $1 - \chi$ of net production (defined as $A - M$) is then assigned to reproduction. This fractional allocation may depend on W , but is assumed to be independent of the food environment. The processes of growth and egg production are then described by the differential equations

$$\frac{dW}{dt} = \chi(A - M), \quad \text{eqn 4}$$

$$\frac{dE}{dt} = (1 - \chi) w_E^{-1} (A - M). \quad \text{eqn 5}$$

In the *net assimilation* model, a fraction $1 - \kappa$ of assimilation goes to reproduction and the remainder, κ , is utilized for growth and maintenance, with maintenance having the first call, so that

$$\frac{dW}{dt} = \kappa A - M, \quad \text{eqn 6}$$

$$\frac{dE}{dt} = [(1 - \kappa) A - p] w_E^{-1}. \quad \text{eqn 7}$$

Here, the constant p represents the rate at which assimilate is consumed for maturity maintenance (Kooijman 1993). In the net production model, maturity maintenance is either absent or included in M . This assumption is addressed in more detail in the Discussion, but note here that it does not alter the qualitative predictions presented below.

The difference between these models is thus that, in the net production model, maintenance is drawn from assimilate before it has been partitioned between growth and reproduction, whereas the opposite is true in the net assimilation model. Hence, in the former, the rate of growth relative to reproduction is independent of the food supply, i.e. these two processes are given equal priority. Because the net assimilation model partitions assimilate first, the rate of reproduction relative to growth depends on the value of p relative to M .

PREDICTIONS OF THE MODELS

In this section predictions are derived for the number of eggs that an individual will produce by the time it reaches some arbitrary mass (w_a), as a function of the food concentration in the environment. Individual performance is affected by food concentration only via food dependence of the assimilation rate.

Dividing dE/dt by dW/dt gives dE/dW , the slope of a graph of cumulative egg production against cumulative adult carbon gain. For the net production model,

$$\frac{dE}{dW} = \frac{(1 - \chi)}{\chi w_E}, \quad \text{eqn 8}$$

and for the net assimilation model,

$$\frac{dE}{dW} = \frac{(1 - \kappa) A - p}{(\kappa A - M) w_E}. \quad \text{eqn 9}$$

Integrating these functions from mass at maturity (w_m) to some arbitrary mass ($w_a > w_m$) gives the cumulative number of eggs produced by the time an individual reaches that mass, as a function of food concentration:

$$E(w_a) = w_e^{-1} \begin{cases} \int_{w_m}^{w_a} (\chi^{-1} - 1) dW & \text{net production,} \\ \int_{w_m}^{w_a} \frac{(1 - \kappa) A - p}{\kappa A - M} dW & \text{net assimilation.} \end{cases} \quad \text{eqn 10a, b}$$

Evaluation of these integrals involves further assumptions on how A , M , χ and (possibly) κ vary with W .

Typical predictions from equation 10 are plotted in Fig. 1(a). The net production model predicts a single line for all food levels, whereas the net assimilation model predicts a different line for each of the three food levels displayed in the figure. The food dependence of $E(w_a)$ is more easily seen in Fig. 1(b), in which we assigned w_a an arbitrary constant value and plotted $E(w_a)$ against food concentration. With the net production model, $E(w_a)$ is independent of food concentration, as is easily seen by the absence of A in equation 10a. The net assimilation model, on the other hand, predicts that, for any value of w_a , $E(w_a)$ decreases as food concentration increases. This result can be understood by noting that as food concentration is lowered, both models predict that the time

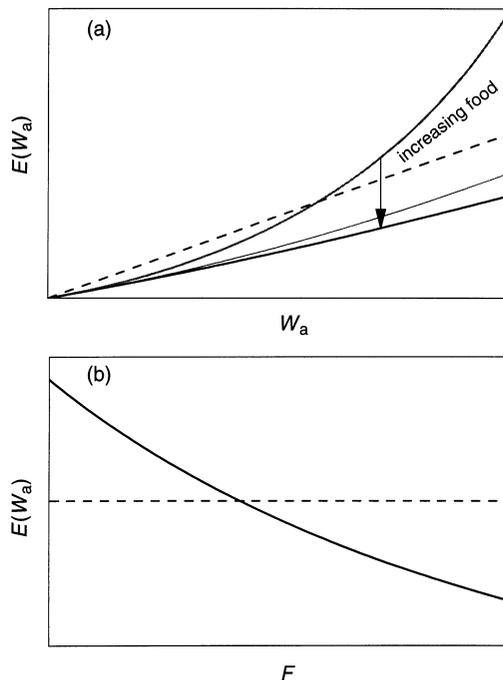


Fig. 1. Typical model predictions for the number of eggs ($E(w_a)$) produced by an individual daphnid as a function of (a) mass (w_a), and (b) food supply (F). Dashed lines represent the net production model, and solid lines represent the net assimilation model. In (a), the net production model predicts a single line, regardless of food concentration; the lowest line for the net assimilation model represents the highest food concentration. In (b), the curves show reproductive output up to a fixed arbitrary mass (w_a). For these simulations, we assumed the assimilation rate follows a type II functional response with maximum ingestion rate related to the surface area of the individual by a constant ξ , and constant assimilation efficiency ε : $A = \varepsilon\xi W^{2/3} F / (F + F_H)$. The maintenance rate is assumed to be proportional to mass: $M = \mu W$. In the net assimilation model, $p = [(1 - \kappa) / \kappa] \mu w_m$. The fractions χ and κ are assumed to be constants.

required to grow from w_m to w_a increases. The food dependence in the net assimilation model reflects that model's assumption that during this time a specified fraction κ of total assimilate is allocated to the *sum* of growth and maintenance, but an increasing proportion of this quantity is consumed by maintenance demands as food concentration decreases. At the same time, the fraction of total assimilate allocated to reproduction remains the same. Thus, the rate of growth relative to the rate of reproduction decreases with decreasing food, with more eggs produced for a given change in mass. According to the net production model, the rate of growth relative to the rate of reproduction is fixed at $\chi / (1 - \chi)$, regardless of the food concentration.

Experimental data

The predictions from equation 10 were tested against data from laboratory-reared *Daphnia pulex* (McCauley *et al.* 1990a). Individual daphnids were raised separately in 30-ml vials containing water with one of four

concentrations of the alga *Chlamydomonas reinhardtii*: 1000, 4000, 8000 or 30 000 cells ml^{-1} . These concentrations correspond to 0.036, 0.14, 0.29 and 1.08 mg C l^{-1} . At 2-day intervals, the individuals were removed from the vials, the length and number of eggs in the brood pouch were recorded, and neonates were removed. They were then placed in vials containing fresh algal mixture. This feeding protocol is referred to as batch culture.

Richman (1958) and Paloheimo *et al.* (1982) report equivalent data for different clones of *D. pulex* raised in batch cultures with different algal concentrations, culture volumes and transfer frequencies. Richman raised isolated individuals in 10-ml vials at four concentrations of *C. reinhardtii*: 25 000, 50 000, 75 000, and 100 000 cells ml^{-1} (estimated to be 0.58, 1.15, 1.73 and 2.30 mg C l^{-1} ; Gurney *et al.* 1990). They measured length and number of eggs produced, and transferred the daphnids to fresh food each day. Paloheimo *et al.* also transferred individuals daily, but used 100 ml of water with 10 000 cells ml^{-1} (4.2 mg C l^{-1}) of *C. reinhardtii*. These authors report only the mean length and cumulative eggs measured each instar for all individuals raised at each food level.

Because the models predict changes in body carbon, whereas length was the only size measurement made in the experiments, a relationship between length and carbon content is needed. The literature contains well-established allometric relationships between these quantities, but there is an open issue as to whether the values of the parameters in the allometric relations are independent of food (Urabe & Watanabe 1991). This turns out to be a good assumption for the clone of *D. pulex* used in our analysis. Some 95 individuals were raised in isolation at three different food levels (0.036, 0.144 and 0.288 mg C l^{-1}) following the same protocols from McCauley *et al.* (1990a). Individuals from different food levels were killed at various lengths immediately following the moult, and the length and dry mass of each individual were measured. Eggs deposited in the brood pouch were subtracted from the dry mass. A multiple regression analysis was performed including length, food level and food \times length interaction terms. In the model containing all three terms, only the intercept and length terms are significant ($P_{\text{length}} = 0.0017$, $P_{\text{food}} = 0.13$, $P_{\text{length} \times \text{food}} = 0.30$). Likewise, with the interaction term omitted, the intercept and length remain the only significant terms in the regression ($P_{\text{length}} = 0.0001$, $P_{\text{food}} = 0.25$). It is concluded that the parameters of the length–mass relationship for the *D. pulex* clone used in this experimental test do not depend on food level.

Analysis

MODEL TESTS ON THE CLONE OF *DAPHNIA PULEX*

In Fig. 2 are plotted log transformed cumulative eggs

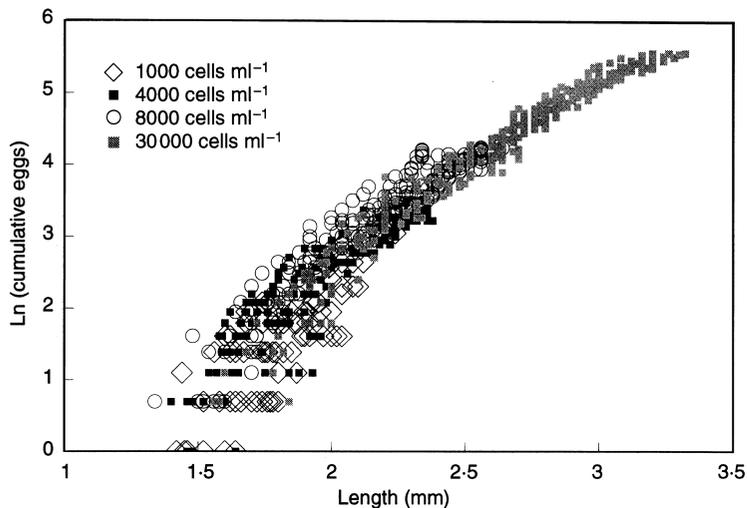


Fig. 2. Cumulative eggs (log transformed) as a function of total length for four food levels. Each point represents one instar from an individual daphnid (see key to symbols on Fig. 2).

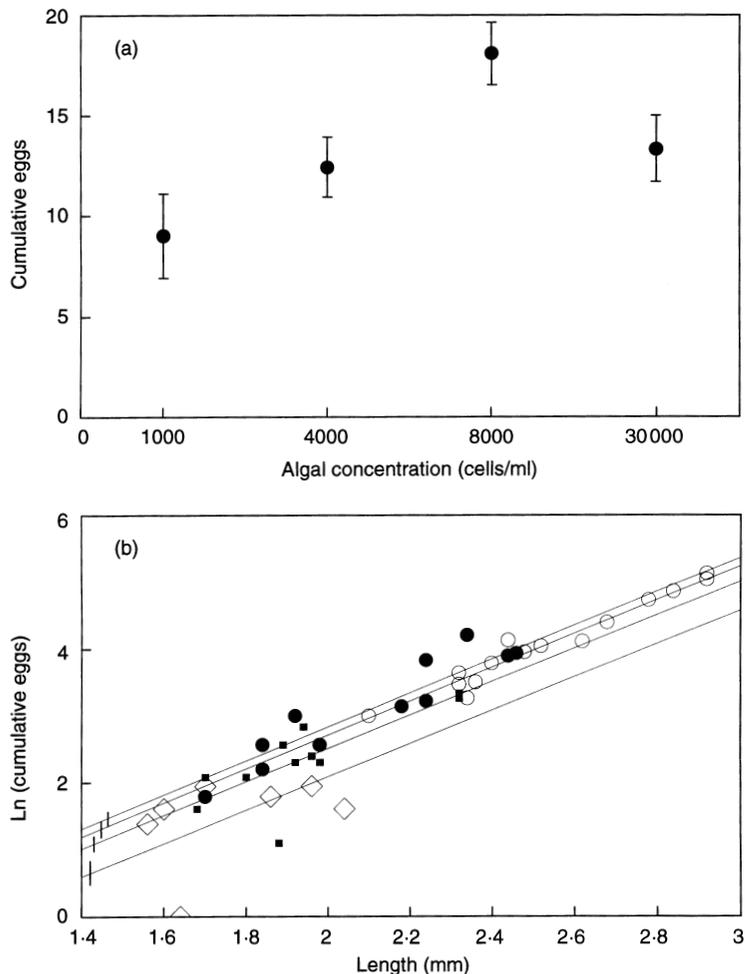


Fig. 3. (a) Mean cumulative eggs \pm one standard error at the instar for which length is closest to 2 mm. Sample sizes from lowest to highest algal concentration are 4, 10, 10 and 15. (b) Cumulative eggs (log transformed) for one randomly selected adult instar from each individual plotted against length, with regression lines from ANCOVA. Error bars show \pm one standard error of the adjusted means: \diamond = 1000 cells ml^{-1} , \blacksquare = 4000 cells ml^{-1} , \bullet = 8000 cells ml^{-1} , \circ = 30 000 cells ml^{-1} .

against length at each instar for all individuals in the data set. At first glance, the data appear to fall approximately on a single curve, as predicted by the net production model. A closer examination, however, reveals that on average, the number of eggs produced by an arbitrary length increases with the three lowest algal concentrations then decreases at the highest concentration. This pattern is more readily apparent in Fig. 3(a). Here, cumulative eggs at the instar closest to 2 mm length are plotted for each algal concentration. This length gives the most representative sample across food densities, but a qualitatively equivalent pattern is apparent over the range of post-reproductive lengths. An analysis of variance of cumulative eggs at \approx 2-mm length with algal concentration as the treatment variable is significant ($P < 0.01$).

By comparing cumulative eggs over the entire range of lengths, we can obtain a more precise test of the apparent pattern. In order to avoid the problem of dependence between multiple observations from each individual, a single adult instar was randomly selected from each individual for the analysis. These data are plotted in Fig. 3(b). The same pattern is apparent as in the previous analysis: at a given length, cumulative eggs increases over the three lowest food levels and decreases at the highest level. An analysis of covariance with length as the covariate demonstrates that this pattern is significant ($P < 0.005$).

The models against which we are comparing the data describe growth and reproduction only after the individual reaches maturity. In the data analysis, however, the total length was used, which is the sum of both juvenile and adult growth. A more appropriate measurement of size for such a comparison is the change in length since the individual reached maturity. Figures 4(a) and (b) display plots equivalent to Figs 3(a) and (b); here, however, the scale on the horizontal axis has been changed to growth since the instar during which the first clutch was produced. The value for change in length was determined for each individual by subtracting the length of the instar in which the first eggs were produced from the length after the moult when the selected clutch was released. Figure 4(a) shows the cumulative eggs produced by the time an individual grew 0.6 mm since its first reproductive instar. Figure 4(b) is a plot of the natural logarithm of cumulative eggs against growth since the first reproductive instar for one randomly selected instar from each individual. These plots suggest that cumulative eggs at a given length may increase with food supply over the entire range of algal concentrations used in our experiment. The corresponding ANOVA and ANCOVA for the two patterns are significant ($P < 0.005$ for both tests). The value used for growth since reproductive maturity includes growth during the beginning of the first reproductive instar, before allocation to the first clutch began, as well as growth at the end of the last

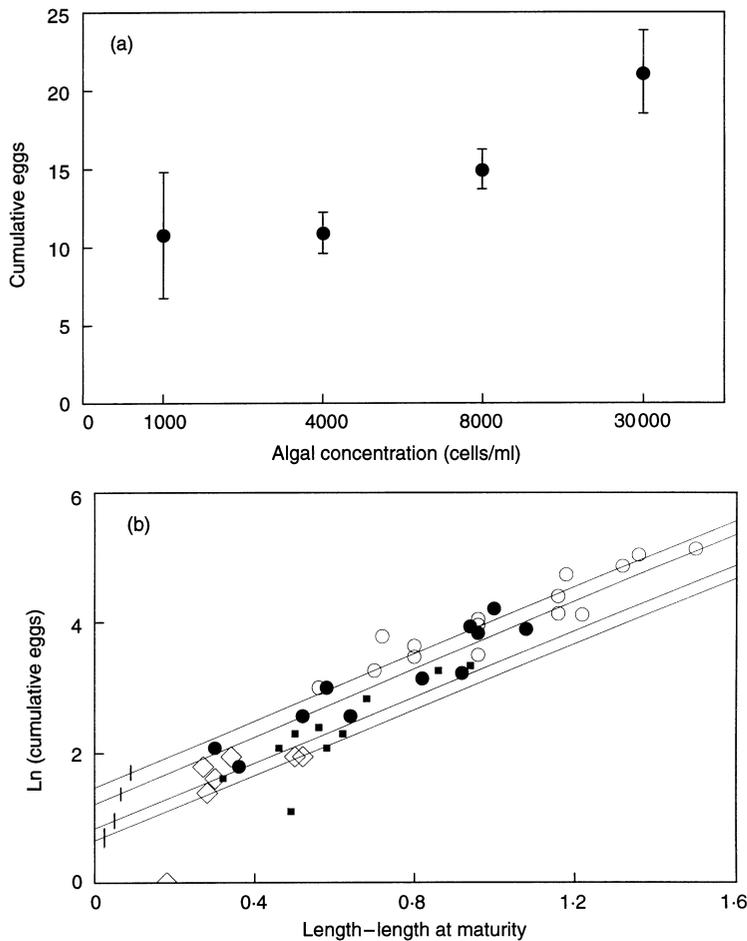


Fig. 4. (a) Mean cumulative eggs \pm one standard error at the instar for which the change in length since reproductive maturity is closest to 0.6 mm. Sample sizes from lowest to highest algal concentration are 4, 8, 10 and 13. (b) Cumulative eggs (natural log transformed) for one randomly selected adult instar from each individual plotted against change in length since the first reproductive instar, with regression lines from ANCOVA. Error bars show \pm one standard error of the adjusted means: \diamond = 1000 cells ml⁻¹, \blacksquare = 4000 cells ml⁻¹, \bullet = 8000 cells ml⁻¹, \circ = 30 000 cells ml⁻¹.

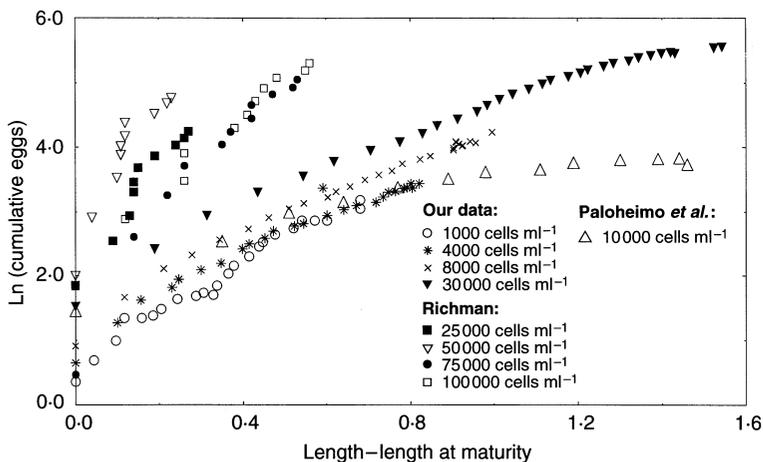


Fig. 5. Mean cumulative eggs (log transformed) as a function of mean length at each instar from Richman (1958; digitized from his Figs 4 and 5) and Paloheimo *et al.* (1982; their Table 3), plotted with means calculated from our data (see key to symbols on Fig. 5).

instar, after allocation to the last clutch ended. Because individuals raised at high food concentration grow more quickly, these quantities increase with increasing food. If the precise carbon content when allocation to eggs began and ended could be determined, all the curves would be shifted up, but the curves for high food would be shifted further. Hence, the actual pattern is more exaggerated than that displayed in Fig. 4.

It should also be noted that the comparison between the pattern in the data and the predictions of the models is influenced by the assumption that the mass of an egg is constant over different food levels. This assumption is required because the models predict the quantity of assimilate allocated to eggs, whereas the data consist of only the number of eggs. In fact, Boersma (1995) showed that in *D. galeata* the carbon content of each egg increases with increasing food supply, implying that more assimilate is packaged in an egg at the higher algal concentrations. Gurney *et al.* (1990) were forced to make a similar assumption when fitting a model of growth and reproduction in *D. pulex* to data. If this observation were to be incorporated in the analysis of the data, the pattern of increasing allocation to eggs at a given mass would become even more pronounced. On the other hand, Enserink, de la Haye & Maas (1993) found that the carapace length and lipid index of *D. magna* neonates decreased with increasing food. Here, constant w_E for *D. pulex* was taken as the simplest assumption in the absence of a clear pattern of variation with food supply.

MODEL TESTS ON OTHER DATA SETS

The data from Richman (1958) and Paloheimo *et al.* (1982) expand the range of food concentrations against which the predictions of the models can be tested. In terms of daily total carbon available to each daphnid, Richman's lowest food level is comparable to our third highest food level, while his highest food level is approximately 50% higher than our highest food level. The protocol followed by Paloheimo *et al.* provided individuals with a daily food supply an order of magnitude greater than the highest concentrations used by us or Richman.

Figure 5 displays these two data sets along with the mean trajectories from our data. Because only the means at each instar were reported by Richman and Paloheimo *et al.* we subtracted the mean length at which the first clutch was produced from the mean length at each instar to obtain an estimate of growth since maturity for each food level. The increasing trend for cumulative eggs at mass observed in our data continues for Richman's two lowest food levels; however, data from his two highest food levels fall below the curves for our highest food level. This reversal continues with the data from Paloheimo *et al.* in which daphnids received the highest food supply. These data fall below the curve for our highest food level.

Discussion

FAILURE OF BOTH SIMPLE MODELS

The pattern found in the data is inconsistent with the prediction of either the net production or the net assimilation model. The two basic models make qualitatively distinct predictions for the relationship between the number of eggs produced by the time an individual reaches any arbitrary size and food supply. The net production model predicts that cumulative quantity of eggs at a given mass is independent of food supply, whereas the net assimilation model predicts that this should be a decreasing function. The data, on the other hand, suggest that cumulative quantity of eggs at a given mass in fact increases with increasing algal concentration for daphnids raised in batch cultures in which the algal supply is low enough that an individual starves during part of the time between transfers.

The data sets from Richman (1958) and Paloheimo *et al.* (1982) are more difficult to interpret in the context of this discussion. Because the feeding protocol and the size of the algal cells vary between the experiments, the food levels cannot be compared precisely. Furthermore, these authors report only the mean length and cumulative eggs for each food level, so we cannot account for individual variation in size at maturity. However, daphnids in both sets of experiments received a food supply comparable to or greater than the highest concentration treatment in the experiments reported in this paper. Nevertheless, the data show a peak at the second lowest food level used by Richman. The curve obtained from the experiments of Paloheimo *et al.* in which daphnids were raised with nearly continuous, high-concentration food supply, falls below most of the data from lower food batch cultures.

In the derivation of the predictions for the two models, mass at maturity was treated as a constant independent of food supply. Constant w_m is a prediction of both models when formulated for both juvenile and adult stages, and follows from the assumption that the onset of reproductive maturity occurs when a juvenile has allocated a fixed amount of assimilate to reproductive tissue. The net assimilation model requires the further assumption of a specific form for maturity maintenance as a function of mass. We follow Kooijman 1993 (see also Thieme 1988), who specifies p so as to preserve the prediction of constant w_m while maintaining mathematical consistency at the transition to reproductive maturity. This assumption is perhaps the most disputed part of the net assimilation model as presented here. However, we have confirmed by simulation of the model including the juvenile stage that eliminating or changing the value of maturity maintenance does not alter the qualitative prediction for the food dependence of $E(w_a)$.

The inability of either basic model to predict the peak observed in the data for cumulative eggs at mass

as a function of food supply suggests that some essential biological detail is missing from the models. As the batch cultures allow the daphnids to reduce the algae to a low concentration between transfers, particularly at the lowest food levels, we suspect that the pattern may be due in part to fluctuations in the food supply. In the following section we build on the insight gained from the basic models by examining models formulated with the possibility of temporary starvation in mind.

MORE COMPLEX MODELS

In the Theory section the simplest possible models were formulated in order to highlight the contrasting predictions generated by two different allocation rules. One obvious weakness of the models as formulated above is the absence of any explicit representation of energy storage. This omission is of potential importance for our analyses since the batch feeding regime in our experiments allowed adult individuals to clear all of the food from their containers within the first day after they were transferred. The daphnids were then forced to survive from storage for the remainder of the 2 days between transfers. Similarly, although Richman transferred his daphnids every day, he kept them in 10-ml containers; the total quantity of algae available to the daphnids was higher than in the experiments reported here, but the small size of the containers probably allowed the individuals to clear the food before each transfer, at least in the experiments at lower food densities. The protocol of Paloheimo *et al.* (1982), in which individuals were raised at a high food concentration in 100-ml containers and transferred daily, is closest to providing a continuous food supply for the daphnids, and the assimilation rate probably never fell below the maintenance rate. The three sets of data we used therefore represent a spectrum of food dynamics from frequent starvation to continuous surplus.

In the Appendix, we show how the two basic models have been modified to account for energy utilization during periods of starvation. The models require two additions in order to be applicable to situations with dynamic food. First a storage compartment, in which individuals reserve some energy for periods of low food availability, must be inserted in the basic model structure. The second requirement is a special allocation rule for starvation conditions, as the rules used in the basic models make biologically unreasonable predictions when the assimilation rate falls below a critical value. Depending on the form assumed, the modified rule may reduce allocation to growth or reproduction during starvation. Maintenance demands, of course, must always be met for an individual to remain alive.

By itself the insertion of a storage compartment in either of two locations suggested in the literature does not alter the qualitative predictions of the models to

resemble the pattern observed in the data. In fact, insertion of a storage compartment immediately 'upstream' of reproduction, as proposed by Ross & Nisbet (1990) for the net production model, adds a delay in egg production, such that the prediction for variation with food density of cumulative eggs at mass becomes similar to the prediction of the basic net assimilation model even under non-starvation conditions. Inserting a storage compartment through which all assimilate is channelled before it is utilized for any physiological process, as suggested by Kooijman (1986, 1993), has no qualitative effect on our previous predictions for the net assimilation model. Details of the calculations supporting these statements are given in the Appendix.

One additional feature of the Ross & Nisbet (1990) model does have the potential to explain our results: the assumption of a critical minimum storage level below which reproduction ceases. In a constant food environment, models incorporating this assumption predict that cumulative quantity of eggs at a given mass decreases with increasing food concentration, as in the basic net assimilation model. Under fluctuating food conditions, such as those prevailing in batch cultures, it is also possible to obtain an *increasing* rela-

tionship between cumulative eggs as a function of mass and algal concentration at transfers, with the same set of parameter values in the allocation functions. This occurs if, in batch culture, an individual reduces the food density to the level at which the assimilation rate is less than the maintenance rate, and the combination of egg production and maintenance demands reduce storage below the critical level for reproduction. The intertransfer time is then composed of four distinguishable periods (Fig. 6a and b).

1. Immediately after the individual is placed in fresh food, it begins to build up reserves to the level at which reproduction begins. During this period, the individual grows, but does not reproduce.
2. When storage surpasses the critical minimum level for reproduction, the daphnid begins to allocate assimilate to eggs in addition to growth.
3. The individual eventually reduces the algae to the concentration at which assimilation is insufficient for maintenance, and growth stops. Egg production continues as long as storage exceeds the critical level.
4. Storage falls below the critical minimum level for reproduction, and it is used only for maintenance.

The precise form of the relationship between cumulative eggs at a given mass and food supply depends on the relative length of time spent in each of these four periods. Under the 2-day transfer regime, this model predicts that individuals raised at high food supply rapidly build up storage material, with which they continue reproducing after growth halts; and that they begin reproducing again soon after each transfer. At low food supply, storage is depleted to a lower level during the fourth period, so that the duration of the first period, in which the individual grows but does not reproduce, increases with decreasing algal concentration. Lengthening the first period or shortening the third period increases the ratio of growth to reproduction in food-stressed individuals, allowing them to produce fewer eggs by the time they reach a given mass. Whether this ratio decreases with increasing food depends on the values of the parameters that determine the switches between the four periods, as well as the details of the transfer protocol.

The same argument can also explain a reversal of the trend as the food supply increases. When the food supply is sufficient, the assimilation rate never falls below the maintenance rate, and storage mass remains above the threshold for egg production. Thus, the delay caused by placing allocation to reproduction in the storage compartment before it can be utilized for egg production dominates the dynamics. At high food concentration, growth more quickly outpaces egg production than at low food concentration, and the number of eggs produced by the time an individual reaches a given mass decreases with increasing food supply. This argument could be tested, since it implies that the increasing trend of cumulative eggs with algal concentration should be reversed in daphnids raised under

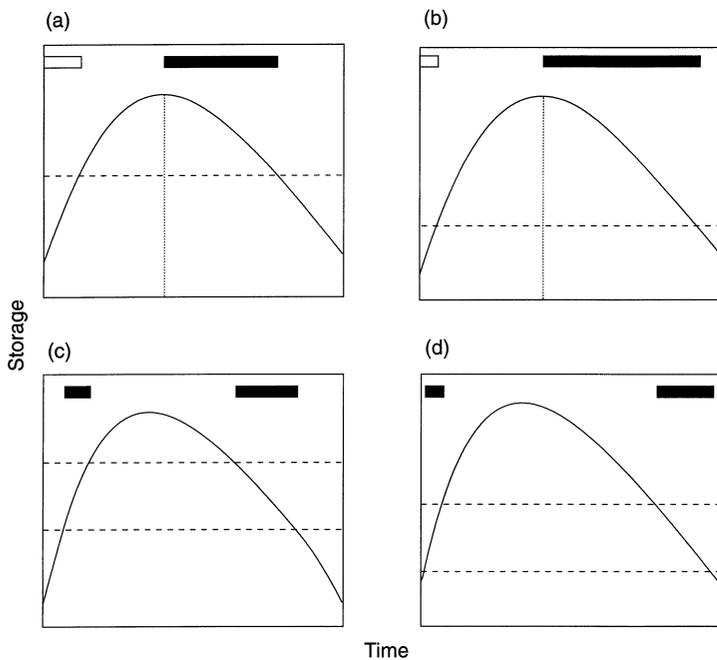


Fig. 6. Storage dynamics during one intertransfer period from simulations of the net production and net assimilation models. Open bars above each diagram indicate fractions of this period in which the individual grows but does not reproduce. Solid bars indicate fractions with reproduction but no growth. Net production model: (a) low food, (b) high food. Horizontal dashed line indicates critical minimum storage level for reproduction. Vertical dotted line indicates the point at which the assimilation rate falls below the maintenance rate. As food supply increases, the time during which an individual grows but does not reproduce decreases, and the time during which the individual reproduces but does not grow increases. Net assimilation model: (c) low food, (d) high food. Dashed lines indicate storage thresholds for reproduction (lower line) and growth (upper line). As food supply increases, the time during which the individual grows and reproduces increases at the expense of time during which it only reproduces. See Appendix for details of the models.

a constant food regime. In fact, the results of Richman (1958) and Paloheimo *et al.* (1982) provide some support for this prediction. These researchers transferred individuals more frequently to higher concentrations, so that at the highest food levels, the daphnids may have spent little time in starvation. As displayed in Fig. 5, the increasing trend for cumulative eggs at mass as a function of food supply peaks and then decreases at the highest algal concentrations.

The allocation rule in the net assimilation model has also been modified for application to fluctuating food conditions (see Appendix). Unlike the net production model, this model assumes that assimilate for both reproduction and growth is drawn from storage; hence, starvation conditions are defined by critical storage densities, below which growth and reproduction cease. In this case, the intertransfer time is divided into five periods (Fig. 6c and d):

1. When the daphnid is placed in fresh food, it neither grows nor reproduces, but only replenishes reserves.
2. Storage density reaches the first critical level, and the individual begins to produce eggs with assimilate in excess of maintenance requirements, but does not allocate any assimilate to growth.
3. When storage surpasses a second critical level, the daphnid resumes growth as well as reproduction.
4. As the food level falls, storage once again falls below the critical level at which growth stops, but reproduction continues.
5. Storage is eventually depleted to the point at which it is used only for maintenance. Here, there is no period in which the individual grows but does not reproduce; however, an individual may reproduce while it is not growing (i.e. during periods 2 and 4). The slope of $E(w_a)$ with respect to food supply depends on the rate of reproduction during these two periods. In the Appendix, we discuss the conditions for which $E(w_a)$ may increase with increasing food supply.

We have been unable to find parameter values that satisfy these conditions for the net assimilation model as formulated by Kooijman (1993). However, the dynamics of DEB models in fluctuating environments are remarkably subtle, and there remains the possibility that modifications of the net assimilation model might be consistent with the observations discussed in this paper. For example, evidence has been obtained that modifications of the functional form of maturity maintenance and storage dynamics may lead to substantial changes in model predictions. Further theoretical investigation of DEB dynamics in variable environments is a high priority for future work.

Conclusions

The analysis described in this paper has led to the derivation of simple, easily tested qualitative predictions from the basic allocation rules of two commonly

applied energy budget models. By testing these predictions against data from *D. pulex*, it has been shown that neither basic model correctly predicts the observed pattern of fecundity and growth over a range of food levels. It was also demonstrated that a more detailed version of the net production model can produce the correct pattern in the number of eggs produced as a function of mass. According to this modified model, the observed pattern at low food concentration in batch culture is the result of frequent, brief episodes of starvation.

The full version of the net production model has the benefit of being able to produce a range of patterns which depend on the feeding regime; however, like the quantitative predictions for growth and fecundity curves, these predictions are quite sensitive to parameter values. In particular, the slope of $E(w_a)$ depends critically on the value of the parameter relating the storage threshold to the mass of the individual. It is somewhat less sensitive to values for the rate at which storage is utilized for reproduction and the parameters in the partitioning function (see Appendix). This sensitivity makes the model more difficult to test, but it is the only explanation we have found so far for the pattern observed in the data.

We conclude by re-emphasizing that although low-food batch culture represents a very special, and unnatural, food environment, it shares many features with the environment experienced by individuals in natural populations (low average food density, rapid fluctuations). Thus we need to understand energy allocation in such environments as a prerequisite to developing physiologically structured population models and predicting the demography of laboratory and field populations. The detailed tests of the basic models presented here are part of a broader search for generalizable theory.

Acknowledgements

We thank E. Muller and S. A. L. M. Kooijman for valuable discussions of the manuscript, and V. Leung for technical assistance. The research was supported by grants DEB-9319301 from the United States National Science Foundation, R819433-01-0 from the United States Environmental Protection Agency, and from the Natural Sciences and Engineering Research Council (Canada). E.G.N. was supported by a graduate training fellowship from the University of California Coastal Toxicology Program and grant GER93-54870 from the United States National Science Foundation.

References

- Bledsoe, L.J. & Megrey, B.A. (1989) Chaos and pseudoperiodicity in the dynamics of a bioenergetic food web model. *American Fisheries Society Symposium* **6**, 121–137.
- Boersma, M. (1995) The allocation of resources to reproduction in *Daphnia galeata*: against the odds? *Ecology* **76**, 1251–1261.

- Bradley, M.C., Perrin, N. & Calow, P. (1991) Energy allocation in the cladoceran *Daphnia magna* under starvation and refeeding. *Oecologia* **86**, 414–418.
- Brandt, S.B. & Kirsch, J. (1993) Spatially explicit models of striped bass growth potential in Chesapeake Bay. *Transactions of the American Fisheries Society* **122**, 845–869.
- Broekhuizen, N., Gurney, W.S.C., Jones, A. & Bryant, A.D. (1994) Modelling compensatory growth. *Functional Ecology* **8**, 770–782.
- Elendt, B.P. (1989) Effects of starvation on growth, reproduction, survival, and biochemical composition of *Daphnia magna*. *Archiv fur Hydrobiologie* **116**, 415–433.
- Enserink, L., de la Haye, M. & Maas, H. (1993) Reproductive strategy of *Daphnia magna*: implications for chronic toxicity tests. *Aquatic Toxicology* **25**, 111–124.
- Evers, E.G. & Kooijman, S.A.L.M. (1989) Feeding and oxygen consumption in *Daphnia magna*: a study in energy budgets. *Netherlands Journal of Zoology* **39**, 56–78.
- Glazier, D.S. & Calow, P. (1992) Energy allocation rules in *Daphnia magna*: clonal and age differences in the effects of food limitation. *Oecologia* **90**, 540–549.
- Gurney, W.S.C., McCauley, E., Nisbet, R.M. & Murdoch, W.W. (1990) The physiological ecology of *Daphnia*: a dynamic model of growth and reproduction. *Ecology* **71**, 716–732.
- Gurney, W.S.C., Middleton, D.A.J., Nisbet, R.M., McCauley, E., Murdoch, W.W. & de Roos, A.M. (1996) Individual energetics and the equilibrium demography of structured populations. *Theoretical Population Biology* **49**, 344–368.
- van Haren, R.J.F. & Kooijman, S.A.L.M. (1993) Application of a dynamic energy budget model to *Mytilus edulis*. *Netherlands Journal of Sea Research* **32**, 119–133.
- Kooijman, S.A.L.M. (1986) Population dynamics on the basis of energy budgets. *The Dynamics of Physiologically Structured Populations* (eds A. J. Metz & O. Diekmann), pp. 266–297. Springer-Verlag, Berlin.
- Kooijman, S.A.L.M. (1993) *Dynamic Energy Budgets in Biological Systems*. Cambridge University Press, New York.
- Kooijman, S.A.L.M. & Bedaux, J.J.M. (1996) Analysis of toxicity tests on *Daphnia* survival and reproduction. *Water Research* **30**, 1711–1723.
- Kooijman, S.A.L.M., Muller, E.B. & Stouthamer, A.H. (1991) Microbial growth dynamics on the basis of individual budgets. *Antonie van Leeuwenhoek* **60**, 159–174.
- Majkowski, J. & Bramall, L. (1980) Sensitivity of bioenergetic growth models of animals to changes in the energy balance parameters. *Journal of Theoretical Biology* **85**, 643–655.
- McCauley, E., Murdoch, W.W. & Nisbet, R.M. (1990a) Growth, reproduction, and mortality of *Daphnia pulex*: life at low food. *Functional Ecology* **4**, 505–514.
- McCauley, E., Murdoch, W.W., Nisbet, R.M. & Gurney, W.S.C. (1990b) The physiological ecology of *Daphnia*: development of a model of growth and reproduction. *Ecology* **71**, 703–715.
- Murdoch, W.W., McCauley, E., Nisbet, R.M., Gurney, W.S.C. & de Roos, A.M. (1992) Individual-based models: combining testability and generality. *Individual-Based Models and Approaches in Ecology* (eds D. L. DeAngelis & L. J. Gross), pp. 18–35. Routledge, Chapman and Hall, New York.
- Nisbet, R.M., Gurney, W.S.C., Murdoch, W.W. & McCauley, E. (1989) Structured population models: a tool for linking effects at individual and population level. *Biological Journal of the Linnean Society* **37**, 79–99.
- Nisbet, R.M., Ross, A.H. & Brooks, A.J. (1996) Empirically based dynamic energy budget models: theory and an application to ecotoxicology. *Nonlinear World* **3**, 65–106.
- Paloheimo, J.E., Crabtree, S.J. & Taylor, W.D. (1982) Growth model of *Daphnia*. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 598–606.
- Ratsak, C.H., Kooijman, S.A.L.M. & Kooi, B.W. (1993) Modeling the growth of an oligochaete on activated sludge. *Water Research* **27**, 737–749.
- Richman, S. (1958) The transformation of energy by *Daphnia pulex*. *Ecological Monographs* **28**, 273–291.
- Ross, A.H. & Nisbet, R.M. (1990) Dynamic models of growth and reproduction of the mussel *Mytilus edulis*. *Functional Ecology* **4**, 777–787.
- Thieme, H.R. (1988) Well-posedness of physiologically structured population models for *Daphnia magna*. *Journal of Mathematical Biology* **26**, 299–317.
- Urabe, J. & Watanabe, Y. (1991) Effect of food concentration on the assimilation and production efficiencies of *Daphnia galeata*. *Functional Ecology* **5**, 635–641.
- Zonneveld, C. & Kooijman, S.A.L.M. (1989) The application of a dynamic energy budget model to *Lymnaea stagnalis*. *Functional Ecology* **3**, 269–278.
- Zonneveld, C. & Kooijman, S.A.L.M. (1993) Comparative kinetics of embryo development. *Bulletin of Mathematical Biology* **55**, 609–635.

Received 26 September 1996; revised 26 March 1997; accepted 4 September 1997

Appendix

As noted in the Discussion section, it is necessary to consider energy reserves, i.e. storage, in modelling the effects of fluctuating food conditions experienced in the laboratory batch cultures. Here, we discuss two examples of net assimilation and net production models with storage.

NET PRODUCTION MODEL

Ross & Nisbet (1990) proposed the following structure for incorporating a storage compartment in the net production model. When the assimilation rate is greater than the maintenance rate, maintenance demands are met immediately from assimilate, as in the basic model. The remaining assimilate is then divided between growth (increase in core mass) and storage. Carbon allocated to reproduction is drawn from the storage compartment at a rate proportional to the amount by which the storage mass exceeds some critical mass. This critical mass is assumed to be some fraction δ of the mass of the core tissue. Hence, a second condition, which determines whether the individual utilizes reserves for reproduction, is that the storage mass is greater than this critical mass. The equations describing the allocation when both of these conditions are met are

$$\left. \begin{aligned} \frac{dS}{dt} &= (1 - \chi)(A - M) - \rho(S - \delta W) \\ \frac{dW}{dt} &= \chi(A - M) \\ \frac{dE}{dt} &= \rho(S - \delta W) w_E^{-1} \end{aligned} \right\} A > M, S > \delta W. \quad \text{eqn 11}$$

Under constant, ample food supply (i.e. both conditions are met), the storage compartment merely

acts as a buffer between assimilation and egg production. The rate at which storage is utilized for reproduction is a constant (ρ), independent of the assimilation rate. The growth rate, on the other hand, is directly proportional to the assimilation rate, which in turn is proportional to the food concentration in the environment. A consequence of this structure is that the storage compartment adds a delay between assimilation and egg production, which becomes more pronounced relative to the growth rate at high food concentration. This model therefore predicts that as the food concentration increases, the rate of growth relative to egg production in non-starvation conditions increases, and $E(w_a)$ decreases as in the basic net assimilation model.

The situation changes when we consider a fluctuating food environment. The two conditions, $A > M$ and $S > \delta W$, operate independently. Thus, when the assimilation rate falls below the maintenance rate, the individual halts growth, but continues to construct eggs from reserves as long as the storage mass is greater than the critical mass. However, under these conditions, the difference between maintenance and assimilation is taken from storage. The equations are then

$$\left. \begin{aligned} \frac{dS}{dt} &= (A - M) - \rho(S - \delta W) \\ \frac{dW}{dt} &= 0 \\ \frac{dE}{dt} &= \rho(S - \delta W) w_E^{-1} \end{aligned} \right\} A < M, S > \delta W. \quad \text{eqn 12}$$

When storage falls below the critical level, allocation to reproduction stops, and storage is utilized only for maintenance. Thus,

$$\left. \begin{aligned} \frac{dS}{dt} &= A - M \\ \frac{dW}{dt} &= 0 \\ \frac{dE}{dt} &= 0 \end{aligned} \right\} A < M, S < \delta W. \quad \text{eqn 13}$$

During recovery from starvation (which occurs in batch culture experiments when the daphnid is placed in fresh algal mixture), the individual begins to grow with the fraction χ of net production as soon as the assimilation rate is greater than the maintenance rate; however, it does not resume egg production until the storage mass has been restored to its threshold level.

The alternation between high and low algal concentrations experienced by the daphnids raised in batch culture therefore exposes them to two periods in which the relative rates of growth and reproduction fluctuate. First, as the food density initially declines, the individual stops growing but continues to construct eggs from storage. Second, until the daphnid

restores its reserves, it grows but does not reproduce. The relative lengths of time that the individual spends in these two periods depends on the frequency of transfers, the volume and concentration of the food medium, the values of the parameters ρ and δ , and the parameters and functional form for χ . Thus the form of food dependence of the relationship between cumulative eggs and mass depends on precise parameter values. We have confirmed by numerical calculations that at low food levels the variation may take the form exhibited by our data.

NET ASSIMILATION MODEL

This model is due to Kooijman (1986, 1993). All assimilate immediately enters a storage compartment in pseudo-equilibrium with the blood. The stored energy is then utilized at a rate U for maintenance, growth and reproduction, and the allocation rules depend on the magnitude of U relative to the maintenance rate, M . When the fraction κ of the utilization rate (U) is greater than the maintenance rate (well-fed organisms), the dynamics take a form which is a natural extension of the equations presented in the text:

$$\left. \begin{aligned} \frac{dS}{dt} &= A - U \\ \frac{dW}{dt} &= \kappa U - M \\ \frac{dE}{dt} &= [(1 - \kappa)U - p] w_E^{-1} \end{aligned} \right\} \kappa U > M. \quad \text{eqn 14}$$

Kooijman (1993; see also Nisbet *et al.* 1996) derives a form for U from considerations of reserve homeostasis.

Because the storage compartment is located 'upstream' of all partitioning in this model, it does not influence the *relative* rates at which assimilate is allocated to growth and reproduction. Hence, the addition of storage by itself does not alter the qualitative prediction for $E(w_a)$ as a function of food supply.

When the default rate at which storage is utilized for growth and maintenance falls below the maintenance rate (food-stressed organisms), growth stops and reproduction is reduced, so that

$$\left. \begin{aligned} \frac{dS}{dt} &= A - U \\ \frac{dW}{dt} &= 0 \\ \frac{dE}{dt} &= [U - (M + p)] w_E^{-1} \end{aligned} \right\} \kappa U < M. \quad \text{eqn 15}$$

Different forms of the model arise depending on our assumptions concerning U for food-stressed organisms (Kooijman 1993). One possibility is that both growth and reproduction stop, and storage is used only for maintenance (i.e. $U = M + p$) in these circumstances. Because this switch stops growth and reproduction

simultaneously, it cannot alter the relative quantities of assimilate allocated to these processes. A second possibility is that growth stops but reproduction continues, until storage reaches some second critical level below which it is used only for maintenance. In this case, food concentration influences both the utilization rate and the length of time during which an individual living in batch culture reproduces but does not grow. These quantities determine the relative rates of growth and reproduction, and therefore the slope of $E(w_a)$ as a function of food supply.

In batch cultures, $E(w_a)$ will be an increasing function of food supply if cumulative reproduction during the two parts of the intertransfer interval in which the individual reproduces but does not grow (see Discussion, Fig. 6) increases with food supply. Conditions under which this occurs are difficult to derive; however, we may obtain an approximation based on the model as formulated by Kooijman (1993). By assuming that utilization is proportional to the stored energy density s ($= S/W$), Kooijman derives the following equation for storage dynamics:

$$\frac{ds}{dt} = \nu W^{-1/3} (f - \alpha s), \quad \text{eqn 16}$$

where ν and α are constants and f is the scaled functional response. During the two periods with reproduction but no growth, utilized reserves in excess of maintenance demands are used solely for reproduction; hence, reproduction is proportional to the integral of stored energy density.

Consider individuals of the same initial mass and storage density upon transfer to fresh food. With a type 2 functional response, individuals transferred to higher initial food concentrations experience higher concentrations throughout the inter-transfer interval. During the first period of reproduction without growth, $f > s$ and $ds/dt > 0$. Because growth has not yet occurred, ds/dt increases with f , and the length of this period decreases with increasing initial food concentration. Thus, in order for reproduction to increase with food supply during these two periods, the decrease in reproduction during the first period must be offset by a greater increase in the second period. The length of the second period does increase with food concentration, because both f and W are larger. On the other hand, with a type 2 functional response, the difference in f during the second period is less than during the first for any given difference in the initial concentration. Furthermore, the increase in the length of this period due to greater mass is proportional to the cube root of the difference in final mass, whereas it is reduced by the greater growth owing to an increase in maintenance, which is proportional to mass. Both of these effects act to reduce the increase in the length of the second period relative to the decrease in the first, further limiting the possibility of $E(w_a)$ being an increasing function of food. Indeed, we have been unable to find a set of parameter values which give such an increasing relationship in numerical simulations of this model.