

THE ALLOCATION OF RESOURCES TO REPRODUCTION IN *DAPHNIA GALEATA*: AGAINST THE ODDS?¹

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Abstract. Variation in offspring size is a common phenomenon in many organisms. In cladoceran zooplankton large offspring are known to have a high starvation resistance. One could, therefore, expect offspring to be large at low food levels, whereas at higher food levels the production of more, but smaller, offspring would yield the highest parental fitness. However, in *Daphnia galeata* I found that individual offspring were smallest at a low food level and largest at intermediate food levels. Moreover, in contrast to the predictions made by several theoretical models, I found that large mothers produced larger offspring. The first discrepancy between data and theory could be explained by the existence of a maximum offspring size, combined with the difficulty of *Daphnia* to produce one egg less than was done in reality. The dependence of offspring size on maternal size could be explained by the higher likelihood of intraspecific competition when large (=old) females are present, and hence higher starvation risks for the offspring. It was found that embryonic respiration was lowest at the lower food levels, and hence the smaller individual egg size seemed to be compensated by lower carbon losses during development.

Key words: *Daphnia*; egg number; egg size; even; reproduction.

INTRODUCTION

Variation in offspring size and number has been described for many organisms, and the underlying processes have often been discussed (e.g., Stearns 1976, Winkler and Wallin 1987). Lack (1954) hypothesized that parents should produce that number of offspring that results in the highest parental fitness, i.e., the highest number of surviving young. Lack assumed that the probability of survival decreased with increasing clutch size as a result of competition between the young. Smith and Fretwell (1974) introduced parental limits in a model of optimal size and number of offspring by assuming that the total amount of energy per breeding attempt is fixed. This would mean that when the energy expended on the production of one individual offspring increases, the total number of offspring decreases, creating a trade-off between propagule size and number. Furthermore, Smith and Fretwell describe a function of offspring size and offspring fitness, characterized by diminishing fitness returns with an increase in effort per offspring. Maximizing parental fitness will then lead to a single optimal investment per offspring for any given environment. As the total effort per breeding attempt does not influence the fitness function, changes in total resource acquisition or in the proportion of resources allocated to reproduction, are not expected to change this optimal investment per offspring.

Organisms living in environments with changing

food conditions may use their resource acquisition as an estimate of the environmental circumstances that their offspring will encounter after birth. If, as assumed in the Smith and Fretwell (1974) model, offspring fitness is related to the amount of parental investment per offspring, this ability of the parents to evaluate the environmental conditions for their offspring may lead to changes in optimal offspring size with changes in the environment.

In planktonic cladocerans like *Daphnia*, adults and juveniles show a large overlap in their use of resources. Moreover, the temporal overlap between adults and their offspring is usually high. As a result, parents may assess the conditions that their offspring will encounter, and thus adjust the offspring size according to their own resource availability. Furthermore, a trade-off between the size and number of the offspring has been found in many cladocerans (e.g., Ebert 1993).

In cladocerans eggs vary considerably in size during the season (Green 1956, Kerfoot 1974, Brambilla 1980, Lampert 1993). Besides the above-mentioned influence of resource acquisition, temperature and changing patterns of predation were also discussed as factors influencing egg size (Kerfoot 1974, Culver 1980, Brambilla 1982, Arts and Sprules 1988, Macháček 1991, Lampert 1993). In general a reduction in egg size occurs when fish predation is high. This has been interpreted as a mechanism to reduce the predation risk by positively size-selective predators, since smaller and lighter eggs lead to smaller newborns. However, in *Daphnia* the size of the offspring is also positively correlated with the size of the mothers (Green 1956, Gliwicz and Guisande 1992, Ebert 1993, Enserink et al. 1993). Since

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fish predation usually reduces the average size of zooplankton, the reduction in egg size may well be caused by a reduction in the size of the mothers (Lampert 1993). However, Vanni (1987) reported that selected size classes of females of *Diaphanosoma birgei* and *Bosmina longirostris* produced smaller eggs in the presence of fish as compared with animals in fishless conditions. Moreover, Macháček (1991) and Stibor (1992) reported a direct effect of the presence of fish on the size of the eggs produced by *Daphnia galeata* and *Daphnia hyalina*, although neither of these authors corrected for the possible effect of the reduced size of the mothers. In laboratory experiments Gulbrandsen and Johnsen (1990) found that temperature also influenced the size of the eggs. The largest newborns were produced at the lowest temperature, but here again this could have been a result of differences in size of the mothers.

In the laboratory, the relationship between food concentration and offspring size has received considerable attention (Taylor and Gabriel 1985, Lynch 1989, Tessier and Consolatti 1991, Cox et al. 1992, Glazier 1992, Guisande and Gliwicz 1992). In general, within the genus *Daphnia*, offspring that are heavier and larger have a greater resistance of starvation (Tessier and Consolatti 1989, Gliwicz and Guisande 1992, Enserink et al. 1993). One can therefore predict that optimal offspring size is large under low food conditions when starvation risks are higher. The results of the different studies on the relationship between food concentration and offspring size are, however, not unequivocal. Taylor and Gabriel (1985) observed no significant differences in egg mass at two different food levels (0.2 and 1.0 mg C/L) for *D. pulicaria* and *D. pulex*. In another study Taylor (1985) reported that egg mass in *D. pulex* was significantly greater at the lower food level, whereas Lynch (1989) reported a marked shift to larger eggs at higher food levels in *D. pulex*, with egg masses lower than the ones reported by Taylor and Gabriel (1985). Glazier (1992) reported a decrease in egg mass with an increase in food concentration for one clone of *D. magna*, but egg masses seemed to decrease at the lowest food levels in another clone. Tessier and Consolatti (1991) and Guisande and Gliwicz (1992) also found a maximum offspring mass for *D. pulicaria* at the intermediate food concentrations. However, egg mass in one clone of *D. pulex* increased with food concentration (Tessier and Consolatti 1991). In short, the results of the different studies on the relationship between resource level and investment per offspring seem to differ considerably.

Glazier (1992) formulated a theoretical model to explain the differences in the empirical data. The difference between his model and that of Smith and Fretwell (1974) is essentially found in the boundary conditions, i.e., in the Glazier model offspring size does not decrease below a minimal viable offspring size at high food levels, whereas at low food levels the offspring

size decreases again, because the total amount of energy available for reproduction is so low that the mothers are forced to produce smaller offspring. This decrease in offspring size should in theory only occur when only one egg is produced, which was not the case in the studies of Tessier and Consolatti (1991). Hence, their explanation for the decrease in size is a different one. They suggested that the higher relative amount of nitrogen in newborns produced at the lowest food levels is indicative of more viable offspring and hence a lower minimum mass is required for successful egg development. However, Ebert (1994) argued that the high nitrogen to carbon ratio is essentially caused by a low carbon content as a result of a lower amount of lipids. As lipid content of juvenile daphnids is positively correlated with survival (Tessier et al. 1983) this higher ratio seems to indicate lower, rather than higher offspring quality.

Recently, Ebert (1994) suggested that a lower individual carbon content of eggs at lower food levels is essentially a problem of resource allocation in small clutches. From a model derived from the Smith and Fretwell (1974) model, combined with the assumption that an upper limit exists for offspring size, Ebert made three predictions. (1) Offspring size variation will be larger among smaller clutches, since the differences in individual offspring size will be more pronounced when only a few eggs are produced. (2) As a result of the existence of a maximum egg size, offspring sizes at lower food levels will be smaller than those produced at intermediate levels. At higher resource levels the offspring sizes will decrease again, since starvation risks are lower. (3) Offspring size distributions should be skewed towards smaller sizes at the lower food levels.

In this study I investigate the role of food conditions determining offspring size in *Daphnia galeata*. Especially at low food levels, as was mentioned above, discrepancies exist among sources of empirical data. The model of Ebert (1994) essentially involves these low food levels, and hence the predictions of this model will be tested. I use different food sources to test the robustness of the offspring size–food level relationships. Moreover, I investigate the relationship between egg mass and newborn mass in order to test the hypothesis that small eggs, produced at lower food levels, are of higher quality.

MATERIALS AND METHODS

Animals

D. galeata was collected from Tjeukemeer, a shallow eutrophic lake in the northern part of The Netherlands (Beattie et al. 1979), using a 120- μ m mesh tow net. In the laboratory individual animals were placed in 100-mL test tubes, and fed a 1:1 mixture of *Chlamydomonas globosa* and *Scenedesmus obliquus* with a total algal carbon content of 1 mg C/L. The incubation temper-

ature was 17.5°C, and the light-dark cycle was 16 h light, 8 h dark. The animals were removed when they produced newborns. These newborns were reared to maturity on the same medium. Neonates produced by the first generation of newborns were used for the experiments described below.

Experimental design

Different amounts of *Chlamydomonas globosa* and *Scenedesmus obliquus* were added to 0.45- μ m mesh filtered Tjeukemeer water to obtain four food levels. (5000, 10 000, 20 000, and 100 000 cells/mL, corresponding to algal carbon contents of 0.13, 0.25, 0.50, and 2.5 mg C/L, respectively). Three series were made up: (1) a series with pure *C. globosa* (CHLAM) media, (2) pure *S. obliquus* (SCENE) media, and (3) a series with *C. globosa* and *S. obliquus* in a 1:1 ratio (CH-SC).

The experiments were all carried out simultaneously, at the same temperature and the same photoperiod as the acclimation phase. For each of the 12 treatments 40 neonates were collected within 12 h of birth, and placed individually in 100-mL test tubes. In order to allow more general conclusions I randomly assigned five clones to be used in the experiments. The animals were selected in such a way that the clonal composition of each treatment was equal. Each individual was transferred daily to a clean tube with fresh medium. The number of eggs per animal were counted and the length of the individuals was recorded for every adult instar. The animals were measured from the upper edge of the eye to the base of the tail spine. The number of newborns was recorded, the length of the newborn individuals was measured, and subsequently they were removed from the tubes. The animals were discarded when they reached the fourth adult instar, because under natural conditions, judging by the size, animals rarely survive beyond this stage.

Carbon content

For each adult instar a number of individuals were taken at random and were dissected to remove the eggs from the brood pouch. If possible the carbon content of three individual eggs was determined for each female. Eggs were sampled in developmental stage 1, as defined by Threlkeld (1979) [stages 1 and 2 according to Gulbrandsen and Johnsen (1990)]. In this stage the egg membranes are as yet not cast off, and should hence allow the best possible measurement of parental investment in reproduction. In addition, the carbon content of a number of newborns produced by the different adult instars was determined within 24 h of birth. The carbon content of individual eggs and individual newborns was measured using a UNICARB Carbon analyser, originally developed by Salonen (1979), an apparatus sensitive enough to determine the carbon content of eggs and newborns individually. The carbon analyser has a lower limit of 0.05 μ g C, and a sensitivity of 0.005 μ g C.

The model of Ebert (1994) predicts larger variation in offspring size between mothers when only a few eggs are produced as compared with the larger clutch sizes. Moreover, offspring size distributions are predicted to be more skewed to the left when reproduction is low. Hence, I computed the standard deviation per clutch size and the skewness of the offspring distribution per clutch size, pooled over all food levels. However, since the length of mothers and offspring sizes are correlated it is necessary for both analyses to correct for the length of the mother first. Moreover, it was also reported that the instar number of the mother affects these relationships (Ebert 1993). Hence, I also corrected the offspring carbon contents for clutch number.

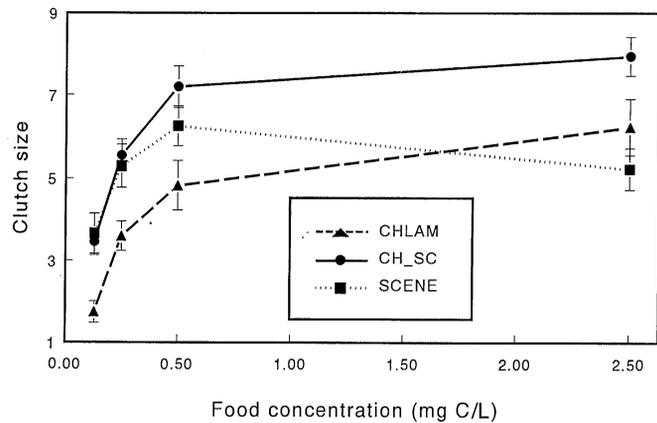
Since measurements of the carbon content of eggs and of newborns are mutually exclusive, I estimated carbon losses from egg to newborn by making completely random egg–newborn pairs. The number of these pairs was limited by the series with the lowest number of observations, which were usually the egg carbon measurements. This was done for every food type–concentration treatment separately, and was repeated 10 times. The losses and standard errors were then averaged. Carbon loss per individual was converted to respiration rate (in microlitres of oxygen per milligram per hour) following Glazier (1991). Although the use of mass losses overestimates respiration due to the inclusion of nonrespiratory losses such as the shedding of egg membranes (Glazier 1991), these values for respiration are still useful for comparative purposes. The significance of the differences in respiration rates between the different treatments was tested with an analysis of variance. First, I tested whether the 10 series of random linking differed; then, in order to avoid pseudoreplication, only one series, randomly chosen from the 10 was analyzed further for food type and concentration effects.

RESULTS

Egg number

The number of eggs produced by the first adult instar increased with increasing food levels, except for the highest *Scenedesmus* concentration (Fig. 1). This resulted in a significant food type and food concentration effect and a significant statistical interaction term (Table 1). The decrease in egg numbers in the animals fed with *Scenedesmus* was not significant (Tukey posthoc comparison). Animals reared on the mixed-algal media had the largest clutch sizes, although pairwise comparisons showed that, with the exception of the highest food level, the differences between animals fed with a mixed algal diet and those fed with *Scenedesmus* were not significant. The animals fed *Chlamydomonas* always produced significantly fewer eggs than animals that were cultured in the mixture.

FIG. 1. Mean number of eggs produced by the first adult instar of *Daphnia galeata* at different food levels and food types. The food types used were *C. globosa* (CHLAM: —▲—), *S. obliquus* (SCENE:■.....), and a 1:1 mixture of the two species (CH_SC: —●—). Error bars indicate the 95% confidence intervals of the mean.



Carbon content of eggs and newborns

Carbon content of the eggs was low on the lowest food level (0.13 mg C/L), higher on the intermediate food levels, and lower again on the highest food level (2.50 mg C/L), irrespective of the food type, although the food level at which egg size was maximal seemed to differ between the food types (Fig. 2a.). The eggs produced by the animals cultured on the mixed media usually had the highest carbon content, and the egg carbon was lowest in animals reared on the *Chlamydomonas* media. These differences between the food types were, however, not significant (Table 1). Fig. 3 shows the full data set of individual egg carbon plotted against the length of the mother. The average carbon content of the eggs increased with the length of the mother ($r = 0.44$; $P < 0.001$). The line in Fig. 3 indicates the average carbon content of the 10% heaviest eggs per 0.1 mm size class of the mother, and is an indication of the maximum egg size. This maximum egg size also increased with the size of the mothers ($r = 0.88$; $P < 0.001$).

The carbon content of newborns followed the same pattern as the carbon content of the eggs: low at the lowest food levels, higher at the intermediate levels, and lower again at the highest levels (Fig. 2b), resulting in significant food, concentration, and interaction effects (Table 1). As expected I found that the average carbon content of newborns was lower than the average carbon content of the eggs. However, the ratio between egg carbon and newborn carbon was not constant (Fig. 4). The offspring produced at the lowest food concen-

trations were all close to the line of equal carbon content of eggs and newborns, indicating that the relative losses of carbon during the development at these levels were lower than at the higher food levels. This was also confirmed by the relative carbon losses converted to respiration rates for all treatments: respiration was indeed lowest at the lowest food concentrations (Fig. 5). No significant differences were found between the 10 replicate series (Table 2a). Within the randomly chosen series I observed a significant food type and food concentration effect and a marginally significant interaction (Table 2b). Posthoc comparisons revealed no significant differences in respiration rates between food concentrations in the animals cultured on the food mixture, whereas animals fed with the mono-algal diets exhibited significant differences among the food levels.

Egg number and offspring carbon

The trade-off between the size and the number of offspring is basic to the Smith and Fretwell (1974) model. This would manifest itself as a negative correlation between these two. I analyzed both the individual egg carbon vs. egg number correlations, as well as the individual newborn carbon vs. egg number (Table 3). To overcome the problem of the increase of total reproductive effort with female length (see also van Noordwijk and de Jong 1986), I computed partial correlation coefficients between offspring size and number with the length of the mother and the instar number kept constant. In order to avoid pseudoreplication average egg carbon and newborn carbon per clutch were

TABLE 1. Summary table of the results of analyses of variance of the effect of food type and food concentration on selected life history characteristics.

Trait	Food			Conc			F × C		
	df	F	P	df	F	P	df	F	P
Size at maturity	2, 447	43.9	<0.001	3, 447	112.2	<0.001	6, 447	5.5	<0.001
Egg number	2, 448	67.2	<0.001	3, 448	127.9	<0.001	6, 448	8.5	<0.001
Egg carbon	2, 182	1.9	0.151	3, 182	2.9	0.036	6, 182	0.9	0.583
Newborn carbon	2, 460	25.9	<0.001	3, 460	5.6	<0.001	6, 460	2.6	0.016

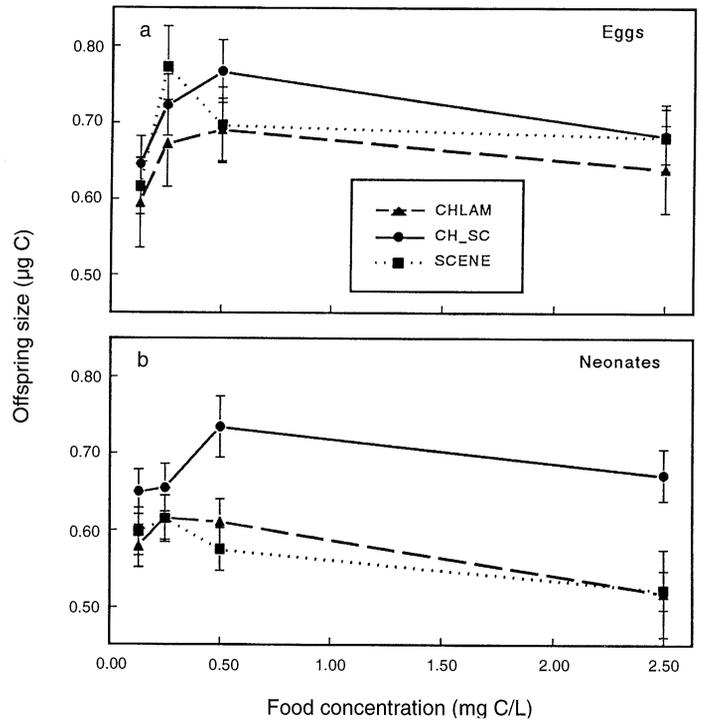


FIG. 2. Carbon content of (a) eggs and (b) neonates of animals at different food types and concentrations. The food types used were CHLAM (---▲---), SCENE (.....■.....), and CH_SC (—●—). Error bars indicate the 95% confidence intervals of the mean.

used for this calculation. Overall partial correlation coefficients were significantly negative for both eggs ($r = -0.26$; $P < 0.001$), and newborns ($r = -0.18$; $P < 0.001$). However, when I split up the animals over the different food levels, most correlations, although negative, were nonsignificant (Table 3).

Skewness and variation

The relationship between skewness and variation of the residual offspring distributions with the number of eggs was analyzed by computing Kendall's tau. The values of skewness and the standard deviation of these distributions were weighted according to the number of animals within each clutch size class. Since the computation of the skewness of a distribution is highly inaccurate when the number of observations is low,

clutch size classes with < 4 observations were omitted from this computation. The standard deviations showed no pattern with clutch size for either egg carbon ($\tau = 0.02$; $P = 0.91$) or newborn carbon ($\tau = 0.08$; $P = 0.58$) (Table 4). Although none of the values computed for skewness differed significantly from 0 (Sokal and Rohlf 1981) (Table 4), I found that the skewness of the egg carbon distributions was significantly correlated with clutch size ($\tau = 0.36$; $P = 0.03$), indicating that egg carbon distributions were more skewed to the left at the lower end of the clutch size spectrum. However, this pattern could not be found in the newborn carbon distributions ($\tau = -0.13$; $P = 0.40$).

DISCUSSION

The present study shows that there is indeed a trade-off between the size of the offspring and the number

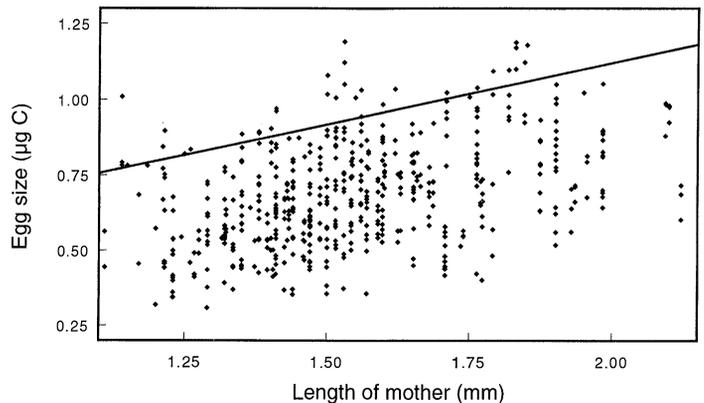
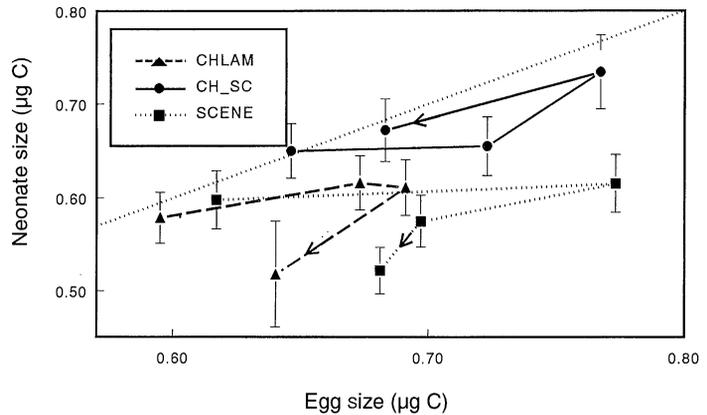


FIG. 3. Scatter plot of all individual egg carbon values vs. maternal length. The line indicates the average of the largest 10% of the eggs of each 0.1 mm size class of the mothers, and is an indication of the maximum egg size.

FIG. 4. Relationship between egg carbon and newborn carbon at the different food types (CHLAM: —▲—, CH_SC: —●—, and SCENE:■.....). Arrows indicate the direction of increasing food concentrations. Confidence limits for egg carbon are not shown for reasons of clarity.



of offspring in *Daphnia galeata*, an essential prerequisite of the Smith and Fretwell (1974) model. Overall correlations of offspring size with number were significantly negative for both egg and newborn carbon content, although when split up over each food source and food level most correlations were weak. This was most likely caused by the low number of degrees of freedom left after correction for length of the mother, clutch number, and the interaction between the two. The Smith and Fretwell model predicts one optimal egg size under a given circumstance. However, I found that offspring size is dependent on the size of the mother, as was also reported by others (e.g., Gliwicz and Guisande 1992). Most authors dealing with offspring size variability consider only within-clutch variation (Crump 1981, Parker and Begon 1986, McGinley et al. 1987), and not among-clutch variation within one mother. However, in cladocerans a dependency of offspring size on mother size, and hence among-brood variation in the same environment, seems to be widespread (e.g., Lampert 1993). Nonetheless, only a few authors have tried to explain this variation. Glazier (1992) proposed selection for early fecundity to be a

possible mechanism, whereas Ebert (1993) suggested that sib competition might be an important selective force. Lampert (1993) gives a third explanation for the offspring size-maternal size relationship. He hypothesizes that the relationship is an adaptation to fish predation: if fish predation is high, small individuals will dominate, producing even smaller offspring. A smaller size would result in reduced predation by fish. Hence adaptation to predation by fish could cause smaller individuals to produce smaller eggs. If predation pressure decreases the animals will grow larger, and will be able to produce large and many offspring, and the population will grow rapidly. Here, I propose another hypothesis that dovetails with Lampert's hypothesis but attacks the problem from the large end: usually high fish predation results in a reduction of the average size of the zooplankton individuals (e.g., Lammens et al. 1985, Vanni 1987). Thus, if individuals of *Daphnia* are large (=old), predation by fish is likely to be low at that time. Other factors being constant this implies a higher probability of intraspecific competition, and hence a depression of resource levels. Since larger eggs have higher survival rates when resources are scarce

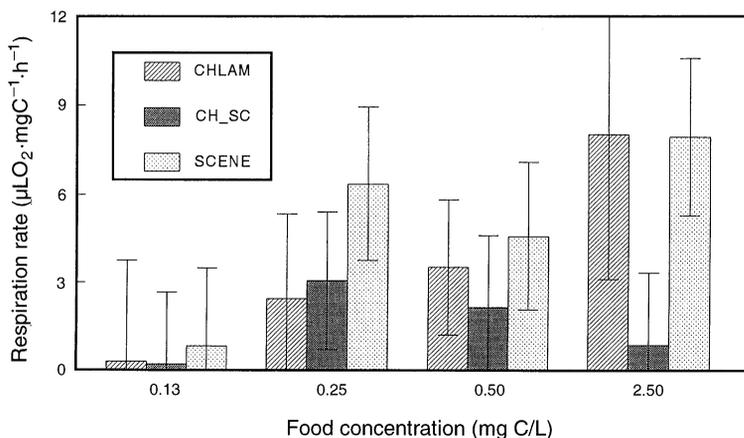


FIG. 5. Respiration rates (in microlitres of oxygen per milligram carbon per hour) during embryonic development of *D. galeata*, as derived from the carbon losses during their embryonic period. Error bars indicate the 95% confidence intervals of the mean.

TABLE 2. ANOVA table of the effects of Replicate series (RS), Food type (FT), and Food concentration (FC) on the respiration rates. (a) Different replicate series included; (b) one randomly chosen series, in order to avoid pseudoreplication.

Effect	MS	df	F	P
a)				
RS	0.000029	9	0.3	0.973
FT	0.006010	2	63.4	<0.001
FC	0.005249	3	55.4	<0.001
RS × FT	0.000023	18	0.2	0.999
RS × FC	0.000023	27	0.2	0.999
FC × FT	0.001675	6	17.7	<0.001
RS × FT × FC	0.000031	54	0.3	0.999
Error	0.000095	5,980		
b)				
FT	0.000501	2	5.4	0.005
FC	0.000416	3	4.5	0.004
FT × FC	0.000177	6	1.9	0.077
Error	0.000093	598		

(e.g., Tessier and Consolatti 1989), large animals that produce large eggs would have a selective advantage. An increase in size of the offspring with increasing competition pressure has indeed been observed for a wide number of species (Brockelman 1975, Christiansen and Fenchel 1979, Parker and Begon 1986, Sargent et al. 1987, but see Guisande 1993). Moreover, if fish predation pressure is low invertebrate predation is likely to become higher. Smaller individuals are generally more vulnerable to invertebrate predation than larger animals. As larger eggs yield larger offspring invertebrate predation would provide an additional selective advantage to animals that produce larger eggs when they are large (see also Meester, *in press*). In contrast to being large, being small (=young) provides no information on the presence or absence of fish or competition, thus the selective forces to produce egg sizes different from the ones favored by the environmental food conditions are less likely to be important if the

animals are smaller. In addition, the production of kairomones by predators might directly influence the size of the offspring. The presence of fish causes a decrease in offspring size (Vanni 1987), whereas invertebrates cause offspring sizes to increase (Stibor and Lüning 1994).

The highest carbon contents were found at the intermediate food levels for both eggs and newborns. This pattern is similar to the ones observed by Tessier and Consolatti (1991) and Guisande and Gliwicz (1992). Further, as was proposed by Tessier and Consolatti (1991), my results suggest that there is a difference in quality of the offspring produced. The eggs produced under low food conditions lost relatively less carbon and thus resulted in newborns that had a similar carbon content as the eggs, thus compensating at least partly for their smaller initial size. The disadvantage of the random pairing of eggs and newborns is that all among-individual variation in egg or newborn carbon content was manifested as carbon losses. Moreover, newborn daphnids increase in mass considerably in a 24 h period, which may also increase the variability in carbon loss estimates. Consequently, the standard errors of this method were large, even resulting in confidence intervals extending below zero. As the pairs were made randomly, linking a light egg with a heavy newborn would yield these gains in carbon. The carbon losses I computed during the development of the embryos represent an average respiration rate of 3.4 $\mu\text{L O}_2\text{mg}^{-1}\text{C}\cdot\text{h}^{-1}$, which is close to the average value found by Glazier (1991), who directly measured respiration in embryos of *Daphnia magna*. It is lower than the average respiration rates for free-swimming cladocerans of $\approx 10 \mu\text{L O}_2\text{mg}^{-1}\text{C}\cdot\text{h}^{-1}$ (Bohrer and Lampert 1988).

The differences in respiration rates between the different food levels and food sources are as yet difficult to explain. It should be reiterated, however, that the

TABLE 3. Correlation coefficients between carbon content of eggs vs. clutch size and carbon content of newborns vs. clutch size. Partial correlations (Part.) were computed with length of the mother and the instar number held constant.

Food*	Con†	Egg carbon					Newborn carbon				
		r	P	Part.	P	N	r	P	Part.	P	N
CHLAM	0.13	-0.55	0.02	-0.53	0.11	17	-0.15	0.37	-0.27	0.12	40
	0.25	0.24	0.33	-0.09	0.29	18	-0.10	0.50	-0.33	0.03	47
	0.50	-0.34	0.15	-0.19	0.54	20	0.26	0.10	0.39	0.02	40
	2.50	-0.11	0.75	-0.67	0.19	10	-0.21	0.26	0.14	0.48	31
CHLSC	0.13	-0.01	0.99	-0.55	0.12	16	-0.15	0.34	-0.27	0.09	44
	0.25	-0.01	0.99	-0.15	0.63	20	-0.33	0.03	-0.63	0.001	43
	0.50	0.64	0.003	0.10	0.77	19	0.32	0.04	-0.09	0.60	43
	2.50	0.35	0.20	-0.06	0.89	15	0.10	0.52	-0.03	0.86	43
SCENE	0.13	-0.34	0.21	-0.33	0.24	15	0.14	0.43	-0.11	0.56	36
	0.25	0.48	0.07	-0.14	0.74	15	0.32	0.04	-0.37	0.03	41
	0.50	0.36	0.13	0.16	0.64	17	0.68	0.001	0.37	0.04	36
	2.50	-0.07	0.83	-0.68	0.13	11	0.28	0.15	0.35	0.11	27

* CHLAM = media with pure *Chlamydomonas globosa*, CHLSC = media with *C. globosa* and *Scenedesmus obliquus* in a 1:1 ratio, SCENE = media with pure *S. obliquus*.

† Con = algal carbon contents in milligrams per litre.

TABLE 4. Analysis of the residuals of individual egg carbon and newborn carbon, from the linear regression of egg carbon and newborn carbon with the length of the mother and the instar number of the mother as independent variables. Skewness of the residual distribution with standard error of the skewness, and the standard deviation of the residual distribution are shown.

Clutch size	Egg carbon				Newborn carbon			
	Skewness	SE	SD	N	Skewness	SE	SD	N
1	-0.225	0.717	0.151	9	0.836	0.491	0.177	22
2	-0.375	0.512	0.110	20	0.221	0.309	0.107	60
3	-0.285	0.564	0.160	16	0.618	0.370	0.130	41
4	0.241	0.434	0.129	29	0.451	0.279	0.138	74
5	-0.222	0.580	0.100	15	-0.240	0.327	0.127	53
6	0.585	0.441	0.141	28	0.535	0.299	0.151	64
7	0.137	0.597	0.114	14	0.079	0.383	0.131	38
8	0.453	0.491	0.143	22	0.353	0.354	0.187	45
9	0.907	0.794	0.122	7	0.487	0.481	0.138	23
10	0.089	0.637	0.119	12	0.110	0.550	0.181	17
11	-0.602	0.794	0.099	7	-0.293	0.616	0.120	13
12	-0.578	1.014	0.210	4	0.798	0.717	0.119	9
13	0.100	2	-0.308	1.225	0.016	3
14	0.049	1.014	0.132	4	0.384	1.225	0.107	3
15	0	-0.131	0.913	0.101	5
16	0	0.182	1.225	0.016	3
17	1	0.499	1.014	0.137	4
18	0.097	2	0.143	2
19	0.561	1.014	0.100	4	1
20	1	-0.147	1.014	0.045	4

respiration rates computed in this study are an overestimation, as a result of the inclusion of nonrespiratory losses. Glazier (1991) estimated that two-thirds of the embryonic carbon losses were a result of actual respiration, but it is unknown whether non-respiratory losses were the same in different treatments. Respiration in free-swimming animals is known to increase with food level (e.g., Bohrer and Lampert 1988). This same pattern was observed for embryos in the two mono-algal series. However, since respiratory losses in free-swimming animals are linked to feeding, it is difficult to envisage that the same processes play a role during the embryonic development. Moreover, eggs produced by animals on the mixed food did not lose more carbon at higher food levels. It is likely that, since lipid content of eggs is influenced by the food level at which the eggs are produced (Tessier et al. 1983), eggs at the lower food levels contained such a low amount of lipids that there was simply little to lose. Moreover, a higher proportion of the lipids present is expected to be incorporated into structural elements, as compared to a higher amount of triacylglycerol in eggs produced at higher food levels (Tessier et al. 1983). Furthermore, daphnids acclimatized to lower food levels might show some adaptation to these conditions (Stuchlíková 1991) by lowering their metabolic rates, which might also result in lower carbon losses. Differences in lipid composition of the eggs might also explain the differences in carbon loss between the mono-algal diets and the mixed diet, as the lipid composition is dependent on the maternal food source (Müller-Navarra 1993).

Most animals that were cultured at the lower food levels still produced 2–4 eggs per clutch. It seems feasible that the energy could be redirected into one egg

less, thus increasing the mass of the offspring, and hence their starvation resistance. If one assumes that the reproductive effort in a given instar is the maximum reproductive effort, i.e., all carbon available for reproduction is used for reproduction, it is possible to compute the individual carbon content of the eggs had there been one egg less in the clutch (Fig. 6, dashed lines). Obviously, the individual carbon content of the eggs increases for all food types and concentrations. The largest increase is at the lowest food levels, because of the lower number of eggs at low levels. The dotted line in Fig. 6 at 1 $\mu\text{g C}$ is arbitrarily chosen as the highest value for the egg carbon content possible. Although an upper limit for offspring size seems plausible as a result of morphological constraints, such as the diameter of the oviducts or the space in the brood pouch, or as a result of physiological constraints, such as the ability of the oocytes to incorporate material, these constraints have only been hypothesized, but not actually observed in cladocerans (e.g., Perrin 1989, Glazier 1992, Ebert 1994). It is, however, clear that there must be a maximum egg size for every egg-producing organism. Averaging the heaviest 10% of the eggs per size class (Fig. 3) will certainly underestimate this upper limit, but should give some indication of the maximum offspring size. I decided, however, not to give the lines of maximum offspring size a positive slope with increasing food concentration (Fig. 6) as could be expected since animals are larger on the higher food levels, because the magnitude of this slope would be difficult to estimate. However, the conclusions drawn from a constant maximum offspring size are essentially the same, and hence I decided to use a maximum egg size, which was independent of the food concentration.

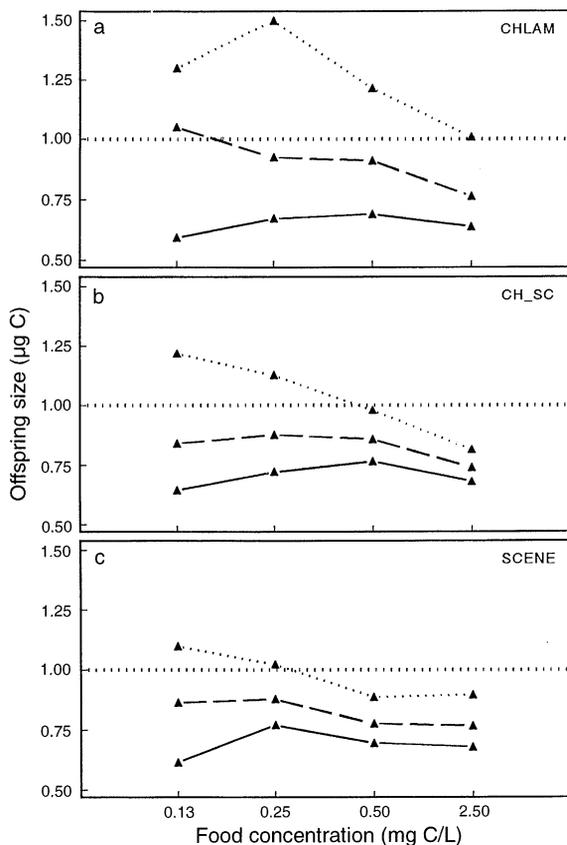


FIG. 6. Egg carbon content (—), egg carbon content had there been one egg less (---), and egg carbon content had there been two eggs less (.....), for (a) CHLAM treatments, (b) CH_SC treatments, and (c) SCENE treatments. The dotted line at 1 µg C indicates the maximum egg carbon content.

The values for the hypothetical egg carbon distribution had there been one egg less stay mostly below the line of the maximum offspring size, and hence it could be argued that at the lower food levels animals should produce one egg less. However, is it a feasible option for *Daphnia* to produce one egg less? Fig. 7 shows the clutch size-frequency distribution of all treatments. It is clear that even-sized clutches were observed in a larger number of cases, especially at the lower end of the distribution. This difference is highly significant ($\chi^2_1 = 51$; $P < 0.001$). The reason for the high frequency of even clutches is most likely the paired nature of the ovaries. The number of the eggs produced is determined in the first part of the instar before the eggs enter the brood pouch (Bradley et al. 1991, Ebert and Yampolsky 1992), but little is known about the division of energy between the two ovaries (Zaffagnini 1987). The observation that even-numbered clutches occur at a higher frequency than clutches with an odd number of eggs suggests that the available energy is divided equally between the two ovaries. Slight relative differences between the ovaries might explain the observation that the differences between even and odd numbers becomes nonsignificant when > 10 eggs are produced ($\chi^2_1 = 0.34$). If the excess of even clutches is due to the paired ovaries, reducing the number of eggs by one might be difficult for daphnids. If one computes the individual carbon content of eggs had there been two eggs less (Fig. 6, dotted curves) it becomes clear that these carbon contents are higher than the maximum offspring size. Moreover, all animals which originally produced two eggs are left out since they do not reproduce at all in this case. Hence, given the assumption that egg number is determined in pairs and that maximal egg carbon content is ≈ 1 µg C for *D. galeata*, then the individual egg carbon content measured represents the highest possible value

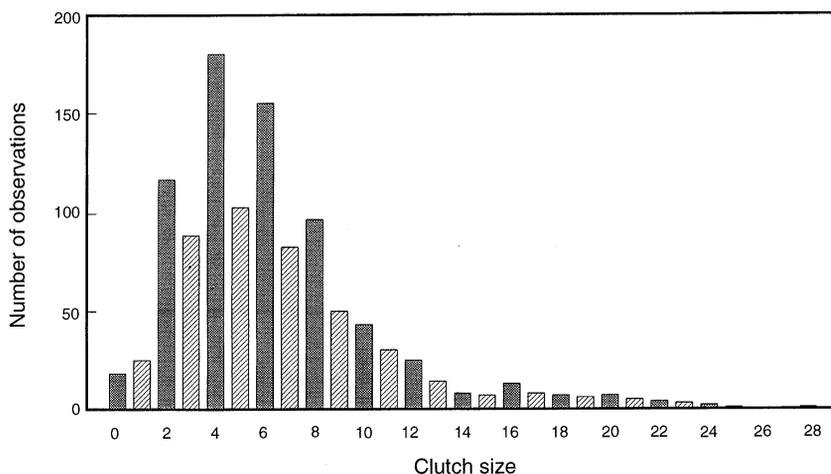


FIG. 7. Clutch size frequency distribution based on pooled observations of the first four adult instars reared at four different food levels and three food types.

for this species. The lower egg carbon at the lowest food levels could thus be caused by constraints on exact egg number determination on the one hand, and a maximum egg size on the other hand.

The size distributions of the eggs were more skewed to the left at the lower end of the clutch size spectrum, as was predicted by Ebert (1994), which again suggests that egg size in *Daphnia* is restricted by a maximal size. However, contrary to the prediction made by Ebert (1994) I found no pattern in the standard deviations. The prediction is not as straightforward as it seems anyway. When egg production is low, the effect of producing one egg more or one egg less is much stronger than when egg production is higher (see Fig. 5), thus resulting in a higher variability between mothers at the low end of the clutch size distribution. However, the introduction of a maximal egg size would decrease the variability again, since not every egg size can be realized. It is unclear how these two processes are balanced, and hence it is impossible to make any statements whether the variance should increase or decrease. However, the skewness at low ends of the clutch size spectrum and the shape of the curve relating offspring size to resource level fulfills the predictions made by Ebert (1994), and this model seems to provide a suitable framework for questions related to size and number of offspring.

Although differences were detected between the food types, the patterns of individual offspring size with food level were similar for all three diets. Hence the relationship between food level and offspring size seems to be robust over different food sources. My observation that the exact food level at which the largest offspring were produced seemed to be dependent on the food source (Fig. 2) could explain the conflicting reports on the relationship between food level and offspring size reported in the literature, especially when only two food levels are considered. I conclude that the combination of a maximum egg size, and the apparent preference of daphnids to produce eggs in pairs can explain the shape of the relationship between offspring size and resource level.

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