

Family planning in *Daphnia*: resistance to starvation in offspring born to mothers grown at different food levels

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Summary. We observed a shift in maternal investment per offspring in clonal cultures of two *Daphnia* species. Mothers grown at high food levels produced large clutches of smaller eggs but their offspring could not survive long under starvation conditions. Genetically identical mothers grown at low food levels produced small clutches of larger eggs, and their offspring, albeit low in numbers, were able to survive long periods of starvation. Our data show that *Daphnia* mothers are capable of assessing food level and use this information in adjusting their fractional per-offspring allocation of reproductive resources.

Key words: Cladocera – *Daphnia* – Food limitation – Per-offspring investment – Resistance to starvation

A trade-off between propagule size and number of offspring (Williams 1966; Smith and Fretwell 1974) is known in many plant and animal species (Wilbur 1977; Winn 1988), but the relative importance of maternal and genetic effects on the relation between effort per offspring and total reproductive effort is not known (Temme 1986). Such a trade-off has been observed in wild populations of asexually reproducing cladocerans, in the form of a shift between large clutches of small eggs and small clutches of large eggs. This shift has been believed to be due to environmental rather than genetic effects since the time of Agar (1913, 1914). Intraclonal variability and the inheritance of variable egg size and clutch size proportions has been shown (Lynch and Ennis 1983; Tessier and Consolatti 1989). However, no experimental proof has been supplied to demonstrate that the shift from a large clutch of smaller eggs at high food levels to a small clutch of

larger eggs at low food levels may occur without any genetic change such as a temporal succession of clones.

Food supply is highly variable in aquatic habitats and subsequent generations of planktonic herbivores must often live at different food levels. Their life history traits are, therefore, flexible so they are able to take the advantage of any food improvement by faster body growth, earlier maturation, and larger number of eggs per clutch, which is most clearly exhibited by parthenogenetically reproducing animals such as *Daphnia* (Stearns 1976; Lynch 1980a, b). Fitness would depend not only on the number of offspring, but also on offspring survival, and this would hinge on the size of an egg, which reflects the amount of resources allocated to an individual neonate (Tessier and Consolatti 1989; Tessier and Goulden 1982; Tessier et al. 1983).

Whereas the ability of an individual *Daphnia* to increase clutch size in response to an increase in food level has been shown in many laboratory and field studies (Bottrel et al. 1976), it remains questionable whether an individual can reduce egg size when food is abundant. Since the weight of an individual *Daphnia* egg is difficult to assess, the data conflict, and there is even more evidence that poorly fed mothers produce smaller eggs than mothers in high-food environments (Tessier and Goulden 1982; Green 1956; Brambilla 1980; Lynch 1989). Most authors perceive it as a trade-off behavior to cope with changing food levels (Tessier and Consolatti 1989). However, the phenomenon has also attracted alternative explanations related to changes in ambient temperature (an increase in egg size as a function of increasing temperature: Brambilla 1982; Perrin 1988; Gulbrandsen and Johnsen 1990) and changing patterns of predation (a decrease in egg size as a function of increasing risk of predation by visually oriented planktivores: Lynch 1980a; Kerfoot 1974; Culver 1980; Arts and Sprules 1988).

Moreover, although the association between egg size and nutritional history of mothers is known at the population level from field observations (Brambilla 1980; Kerfoot 1974), the possibility exists that it may be related to clonal replacement (temporal succession of clones) rather than to plasticity of an individual genotype (clone), and an

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individual's aptitude in assessing food levels and, thus, its ability to predict food conditions which await the next generation.

In our experiment, we dealt with genetically identical animals cloned from a single individual. The neonate resistance to starvation assessed in hours of survival in a food-free medium, was found to be a more precise measure of the per-offspring investment than the egg and neonate size, weight, carbon, protein and lipid content, assessed in fractions of a microgram.

Materials and methods

Experimental animals were grown and starved in the same membrane- or millipore-filtered lake water stored for 24 h to eliminate chemical cues of predation from the lake habitat. We used two *Daphnia* species similar in body size, *Daphnia pulex* Forbes and *Daphnia hyalina* Leydig (0.72–1.81 and 0.61–1.43 mm, body length of neonate and of 6-day-old adult, respectively) to avoid large interspecific differences in life history traits as well as in the relative amount of maternal lipid reserves in neonates (higher in larger species: Goulden et al. 1987).

The source individuals of both species came from well-established clones maintained in membrane-filtered lake water and fed unialgal food (*Scenedesmus acutus* Meyen) from chemostat culture at a concentration of 0.2 mg C·l⁻¹. More than 50 egg-carrying females per species from the third generation were isolated in batch cultures and kept at the same food concentration for 12 h to allow their first brood's offspring to be released. These offsprings were collected and used as experimental animals.

Experimental animals of each species (150 neonates 2–14 h old) were distributed randomly into six 250-ml chambers in a flow-through system (Stich and Lampert 1984), 25 animals of each species per chamber. They were grown at three different food concentrations of 0.04, 0.2 and 1 mg C·l⁻¹ at high flushing rate (21 day⁻¹ chamber⁻¹) of food suspensions to minimize the influence of grazing. As soon as an egg-carrying female was found in a chamber (after 7–8, 10–12, and 14–17 days in 1, 0.2 and 0.04 mg C·l⁻¹ food level, respectively), all the animals of this chamber were transferred to individual 12-ml beakers with an appropriate food suspension (0.04, 0.2, or 1 mg C·l⁻¹).

Each of these animals was inspected every 3 h and transferred to a beaker with fresh food suspension. The freshly hatched neonates of each brood were successively transferred to new beakers containing 0.2 µm membrane-filtered sterilized lake water with no food added (or immediately used for body weight, carbon, protein and lipid content estimates: Guisande and Gliwicz in press). These neonates were inspected for mortality every 3 h and transferred to fresh beakers every 6 h. This was continued until all animals had died and the mean time of survival under starvation conditions could be estimated for different broods of mothers grown at different food levels. The temperature was 20 ± 1°C and light illumination was 300 ± 30 lux at each experimental step.

Table 1. *Daphnia pulex* and *D. hyalina* mean (± 1 SD) clutch sizes (cs) at the time of first brood, second brood and later broods at three different food levels (1, 0.2 and 0.04 mg C·l⁻¹), and numbers of neonates of these broods used for estimates of survival time under starvation (*n*)

Food level	1 mg C·l ⁻¹		0.2 mg C·l ⁻¹		0.04 mg C·l ⁻¹	
	cs	<i>n</i>	cs	<i>n</i>	cs	<i>n</i>
<i>D. pulex</i>						
First brood	5.0 ± 1.1	71	1.3 ± 0.4	8	1.2 ± 0.4	14
Second brood	5.5 ± 1.2	4	1.5 ± 0.5	2	1.0 ± 0.0	5
Later broods	4.5 ± 0.5	2	1.5 ± 0.5	2		0
<i>D. hyalina</i>						
First brood	4.3 ± 1.2	26	2.0 ± 0.8	18	1.2 ± 0.4	10
Second brood	5.0 ± 1.4	20	2.3 ± 0.5	2	1.1 ± 0.4	6
Later broods	3.8 ± 1.0	45	1.8 ± 0.7	8		0

Results

In both species we found that largest neonates were born to mothers grown at the lowest food levels, and the smallest neonates to mothers grown at the highest food levels. In both species clutch size was greatest in mothers grown at the highest food level of 1 mg C·l⁻¹, and smallest in mothers grown at the lowest food level of 0.04 mg C·l⁻¹ (Table 1).

The effect of the food level at which mothers had been grown on the neonate size was most evident in the first brood neonates. In *D. pulex*, the slopes of the neonate dry weight and body carbon on food level regressions were both different from 0 at *P* = 0.05 and *P* = 0.007, respectively (*df* = 21, ANOVA). In *D. hyalina* (no data for body mass or body carbon), the slope of the neonate body length on food level regression was different from 0 at *P* < 0.001 (*df* = 52, ANOVA). Surprisingly, neither lipid nor protein content of freshly hatched neonates was found to be negatively (or positively) related to the mothers' food level (Guisande and Gliwicz in press).

The food level at which mothers had been grown was also important to the neonates' resistance to starvation. In both species, we found longest survival time in neonates born to mothers grown at the lowest food levels and shortest survival time in neonates born to mothers grown at the highest food levels (Fig. 1). The slope of the regression of neonate survival on food concentration (ln) was found to be different from 0 (ANOVA) regardless of whether all the available data were used (neonates from all different broods as in Fig. 1) or data set restricted to the neonates of the first brood (*D. pulex*: *P* < 0.001, *df* = 91, *D. hyalina*: *P* < 0.001, *df* = 52). The greater resistance to starvation of neonates born to mothers grown at lower food concentrations was related to their larger size and higher carbon content (e.g. in *D. pulex* the slope of the regression of survival on body carbon was different from 0 at *P* < 0.001, *df* = 21, ANOVA).

Despite the fact that all the neonates had come from a single clone and their mothers had been grown in the same experimental chambers, their survival time was highly variable, especially in the neonates born to mothers grown at low food levels (Fig. 2). Only a small part of this variability could be related to different clutch sizes, which at low food levels were close to 1 in both species (Table 1).

A more important source of variability in neonate survival was probably the origin of neonates derived from successive broods. Neonates of later broods were more resistant to starvation than the first-brood neonates, a

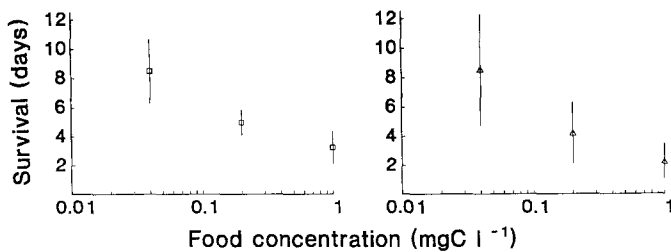


Fig. 1. Mean (± 1 SD) time of survival under starvation conditions in neonates of *Daphnia pulicaria* (left) and *D. hyalina* (right) born to mothers grown at three different *Scenedesmus* food concentrations: 0.04, 0.2 and 1 mg C \cdot l $^{-1}$. Survival on ln food concentration regression statistics for *D. pulicaria*: $a=3.22$, $b=-1.58$, $df=106$, $P<0.001$; and *D. hyalina*: $a=2.11$, $b=-1.74$, $df=129$, $P<0.001$. *D. pulicaria* freshly hatched neonate dry mass (animals dried to constant weight at 60°C) was 2.5 ± 0.1 , 2.3 ± 0.4 and 1.9 ± 0.2 μ g, and body carbon (animals UNDR-infrared-gas-analyzed after combustion in a Pregl-Roth oven, see Gliwicz 1990) was 1.7 ± 0.1 , 1.5 ± 0.1 and 1.0 ± 0.1 μ g at lowest, intermediate and highest food level, respectively. *D. hyalina* freshly hatched neonate body length (no body weight nor body carbon data available) was 713 ± 31 , 706 ± 11 and 664 ± 4 μ m at lowest, intermediate and highest food level. No difference was detected in the body length of the neonates of the first, the second and later broods (Table 1)

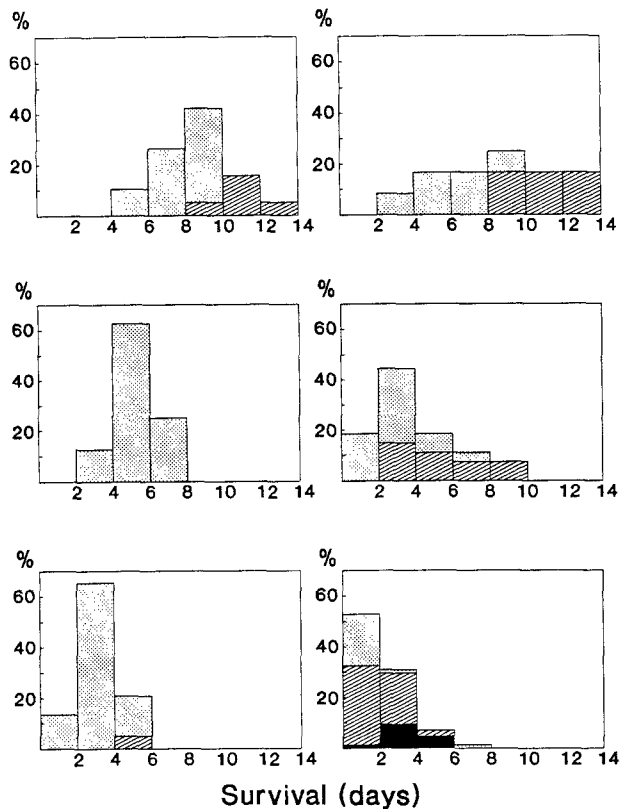


Fig. 2. Distribution (%) of *Daphnia pulicaria* (left) and *D. hyalina* (right) neonates with different survival times under starvation conditions. Neonates had been born to mothers grown at the lowest (0.04, top), intermediate (0.2, middle) and the highest (1 mg C \cdot l $^{-1}$, bottom) food level. They had come from the first brood (light shaded) and later broods (darker shaded). In *D. hyalina* grown at the highest food level (bottom right), the neonates of the second to the fourth brood are darker-shaded, and neonates of the fifth and later broods are darkest-shaded

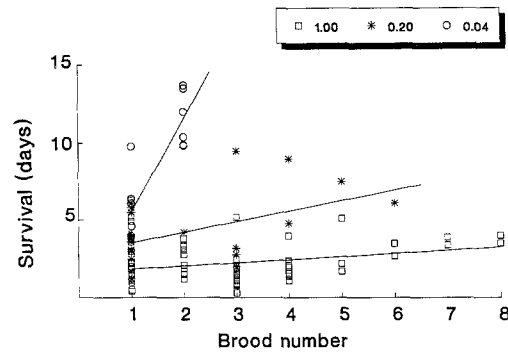


Fig. 3. Survival of *Daphnia hyalina* neonates of different broods born to mothers grown at the lowest (0.04, circles), intermediate (0.2, asterisks), and the highest (1 mg C \cdot l $^{-1}$, squares) food levels. All three regression slopes differ from 0 (ANOVA) at $P<0.001$ ($df=14$), $P=0.012$ ($df=26$), and $P=0.005$ ($df=89$), respectively. They are also different from each other (ANCOVA) at $P<0.001$ ($df=133$). Similar differences were observed in *Daphnia pulicaria* at $P=0.03$ ($df=106$, ANCOVA)

tendency which seemed to be strongest in *D. hyalina* neonates born to mothers grown at low food levels (Fig. 2, right). Figure 3 shows that this is exactly the case. The resistance to starvation increases faster from the first to later broods in neonates produced by mothers grown at lower food levels.

Unfortunately, no third and later brood neonates were produced by *D. pulicaria* and *D. hyalina* grown at the lowest food level of 0.04 mg C \cdot l $^{-1}$, since most mothers died soon after producing the first brood, and the remainder died after producing a second brood. Judging by the steep regression slope (Fig. 3), the third and later brood neonates of *D. hyalina* mothers grown at the lowest food level might have survived starvation even longer, which implies that the slope of the regression in Fig. 1 (right) could be underestimated.

The increase in the effort per offspring from the first to later broods seems to be greater when mothers "assess" that food remains insufficient for their first brood neonates to survive. This results in a further decline in total reproductive effort as the time between brood releases is longer at low food levels, a phenomenon also known in other *Daphnia* species (e.g. Orcutt and Porter 1984). The time between first and second clutch was 3.5 ± 0.3 and 4.0 ± 0.7 days at low food (0.04 mg C \cdot l $^{-1}$), and 2.7 ± 0.3 and 2.5 ± 0.1 days at high food levels (1 mg C \cdot l $^{-1}$) for *D. pulicaria* and *D. hyalina*, respectively (different in both species at $P=0.008$, Wilcoxon signed rank test).

Discussion

If it is assumed that the neonates' resistance to starvation depends on egg size and on the amount of maternal reserves allocated to an individual offspring (Tessier et al. 1983; Tessier and Consolati 1989), the results of our experiment contradict those of other authors who found that eggs of *Daphnia* grown at low food level were smaller than those from higher food levels (Tessier and Goulden 1982; Green 1956; Lynch 1989), and that *Daphnia* neonates

of mothers from low-food cultures have lower lipid reserves or lower dry mass than those of mothers from higher food concentrations (Goulden and Henry 1984; Tessier and Consolatti 1991; respectively). The reason for this contradiction may be that other authors used batch cultures where food fluctuated in a daily rhythm due to grazing and sinking (e.g. Tessier and Consolatti 1991) rather than because they applied a different range of food levels (e.g. 0.2–3 mg C·l⁻¹ in study by Lynch 1989) which might not have been perceived by *Daphnia* mothers as low enough to increase the per-egg investment.

Actually, one of the two experimental species used by Tessier and Consolatti (1991) was *D. pulicaria*, which produced the largest neonates at the intermediate, not at the highest, food level. Thus, their study also showed an increase in per-egg investment from the highest to the intermediate food level at which *D. pulicaria* mothers were grown. This investment was decreased, however, at the lowest food level, which might suggest that there is a critical food level at which a dilemma of “many small or a few large eggs” is replaced by a dilemma of “a few small eggs or none”. Although the three food concentrations at which Tessier and Consolatti (1991) grew *D. pulicaria* mothers were similar to those in our experiments (0.05, 0.25 and 1 mg C·l⁻¹, assuming that carbon is 50% of dry mass of the food alga *Ankistrodesmus falcatus*), the lowest concentration could well be on average significantly less than 0.05 mg C·l⁻¹ due to grazing and sinking over the 24 h between changing food media in the beakers.

Our results are also inconsistent with those field data that show a decrease in egg size in food-limited *Daphnia* populations (e.g. Brambilla 1980).

Our results are consistent, however, with two general notions which have been commonplace in the ecological literature for decades (Wasenberglund 1908; Lack 1954; Hutchinson 1967; Smith and Fretwell 1974; Stearns 1976). First, effort per offspring should be treated separately from total reproductive effort. Second, at low food levels, the per-egg investment should be higher than at high and nonfluctuating food levels, even though the number of offspring would be reduced due to delayed maturation, smaller clutch size and longer time between clutches.

This trade-off between egg size and egg number is often willingly accepted when reproductive strategies are compared in different genera (Goulden et al. 1982), congeneric species (Tessier and Goulden 1987), single-species populations from different habitats (Gliwicz and Rowan 1984), or clones (Lynch 1980b). However, plankton ecologists are reluctant to admit that it may also operate in the absence of genetic differences.

Our results are also consistent with a general assumption that higher quality offspring are produced by older parents (Stearns and Koella 1986; Semlitsch and Gibbons 1990). This has also been reported in *Daphnia* by other authors, who observed that first clutch neonates were considerably smaller than those from later broods (Green 1956; Lynch 1989; Enserink et al. 1990; Ebert 1991; W. Lampert, unpublished manuscript).

The ability to assess food level at which a fractional per-offspring allocation of reproductive resources must be made by an individual mother may be an important source of the intraclonal variability in egg size, neonate resistance

to starvation and, thus, individual fitness in cladocerans. Further studies would be needed to evaluate its relative importance as compared to other sources of variability associated with food limitation, such as maturation time (McCauley et al. 1990), different sizes at birth in first and later clutches (Green 1954), the number of preadult instars (Ebert 1991) and egg developmental time (we found it to be significantly longer in our *Daphnia* grown at low food: Guisande and Gliwicz in press). One of the important questions that should also be explored is whether the per-offspring investment would further increase at even lower food levels, or, as in Tessier and Consolatti's (1991) experiment, it would instead decrease when insufficient resources can be stored for even one larger egg produced over a longer time.

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