

FAMILY PLANNING IN *DAPHNIA*: WHEN IS CLUTCH SIZE DETERMINED?

D. EBERT^{*1} and L.Yu. YAMPOLSKY^{**}

^{*} *Institut für Zoologie, Universität Basel, Rheinsprung 9, Basel CH-4051, Switzerland*

^{**} *N.I. Vavilov Institute of General Genetics, Russian Academy of Science, Gubkin str. 3, Moscow GSP-1 V333, Russia*

ABSTRACT. The total time period to produce the eggs of one clutch in *Daphnia* is two instars (6-7 days at 20° C). Previous reports suggested that the number of eggs can be reduced during egg provision in the ovaries, as a response to decreasing food level. We used *Daphnia magna* and *D. pulex* to study the time lag between a decrease or an increase in the food concentration and a subsequent change in the number of eggs deposited into the brood chamber. The number of eggs in a clutch decreased when females were starved at least for 0.6 adult instar durations before egg laying. The same time lag was found for increasing the number of eggs as a response to increased feeding conditions. The results show that initially oocytes are produced in excess. But the actual number of eggs in a clutch is determined according to the feeding conditions experienced by a female daphniid about half an instar before she deposits the eggs into her brood chamber. This mechanism is discussed as an adaptation to rapidly changing environments.

Key words: *Daphnia*, clutch size, food availability, parallel egg production

Most aquatic habitats, as they are experienced by short living plankton organisms, are temporally unstable. Fluctuations in food, temperature, salinity or other environmental factors can often be considerably shorter than the generation time of an organism. This is important for the reproductive success of an organism because many life-history traits, such as number and size of eggs, strongly depend on the environmental conditions (e.g. Perrin 1989, Gliwicz and Guisande 1992, Glazier 1992). The expression of a phenotypic trait in one environment might however be disadvantageous in another (Stearns 1989, 1992). In fluctuating environments the organisms have to predict future situation to express the life-history traits best suited to the new conditions. A way to avoid this problem is to shorten the time period between the moment when a decision to express certain phenotype is made and the moment of actual development of the trait.

In planktonic crustacean *Daphnia* clutches of parthenogenetic eggs can be produced every adult instar. The production of a single clutch takes two instars (Makrushin 1981, Zaffagnini 1987), i.e., 6-7 days at 20° C. During the second of these two instars nourishment of the eggs takes place. If clutch size would be fixed at the

moment the clutch is initiated, a sudden decrease of food availability might lead to an under-nutrition of eggs, resulting in too small neonates. If feeding conditions improved during the provision of a clutch, the mother would produce fewer eggs than the environmental conditions would allow. Both situations are disadvantageous. Alternatively, a female could produce more oocytes than the current environment supports. The number of eggs could be determined in a later stage during egg production and the number and size of eggs could be adjusted to the environmental conditions shortly before the eggs are laid. The flexible clutch size model would allow a rapid response to environmental conditions. A third possibility could be to produce only as many oocytes as the current environment allows. In the case of improving conditions this clutch cannot be enlarged, but may be reduced in the case of declining conditions.

Existing evidence shows that clutch size is not fixed, but can be reduced half an instar before the eggs are laid. Bradley et al. (1991 a,b) demonstrated that cutting down the food supply at different intermoult stages reduced the size of the next clutch when starvation started before the middle of the intermoult period. If starvation started later, the clutch sizes did not change any more.

¹ Present address: Department of Zoology, ABRG University of Oxford, South Parks Road, Oxford, OX1 3PS, UK
E-mail: EBERT@VAX.OXFORD.AC.UK

Egg production in *Daphnia*

Each reproductive event, i.e. the production of a clutch, takes in total three adult instars (Makrushin 1981, Zaffagnini 1987). During the first instar oogenesis takes place and oocytes move into the ovaries. During the second instar vitellogenesis occurs in the oocytes. At the beginning of the third instar the eggs are deposited into the brood chamber from which fully developed young will be released at the end of this instar. The development from eggs to neonates is clearly visible through the transparent carapace and different developmental stages can be used to calculate when the eggs have been laid and when the next portion of eggs is going to be laid (Threlkeld 1979). Since eggs are laid every adult instar, successive clutches overlap in production and egg development. If feeding conditions allow regular clutch production, two sets of eggs (the next two clutches) are present in the ovaries, whenever a clutch is present in the brood pouch. We name these clutches according to their order of being initiated, the first clutch (the one in the brood chamber), the second clutch (the one which is provisioned inside the ovaries) and the third clutch (the newly initiated clutch). Using the developmental stages of eggs/embryos in the first clutch, one can calculate when the second and the third clutch will be laid and when these clutches were initiated. This is important for understanding the provision state of the eggs in the ovaries.

Using this technique we try to answer two questions: 1) When is clutch size in *Daphnia magna* and *D. pulex* determined? 2) Are *Daphnia* females able to increase clutch size as a response to increasing environmental quality during provision of eggs? To answer these questions we split samples of *Daphnia* females, which shared the same environment for all their life, into starved and wellfed treatment groups and recorded the change of egg number of the following clutches. Stages of the developing eggs were used as an egg-age-indicator to estimate the timing of clutch size determination as a fraction of intermoult time.

Material and Methods

For three experiments samples of *Daphnia magna* or *D. pulex* were taken from ponds and immediately transferred into the laboratory. Egg bearing females were categorized according to the developmental stages of the eggs in their brood chambers using a dissecting microscope (Threlkeld 1979). Five egg stages were distinguished in experiment 2. In experiments 1 and 3 we distinguished additionally early and late

stages in the age classes 1 and 2. Eggs in the stage 1-early showed no sign of differentiation at all, while the stage 1-late was characterized by some degree of differentiation. The eggs in stage 2-early had no visible head bulb; in stage 2-late the head bulb was visible. Late and early egg stages appeared to be of about equal time length (Ebert, in preparation).

All females were placed individually in glass tubes containing 40 ml of filtered (0.22 mm) pond water of the same temperature they came from. One group of females was fed with yeast (well-fed group, 40 000 cell/ml·day), the others were starved. Important for the experimental design was not the exact actual quantity of the food, but increase or decrease of the feeding conditions in comparison with the previous state. Glass tubes were observed for newborn daphniids. Newborns of the next two or three clutches were counted and removed.

Experiment 1: 180 *Daphnia pulex* were collected from an artificial pond in Bol'shiye Koty (Lake Baikal, Siberia) in June 1991. Sample size of each egg stage group was 20 for starved and 10 for well-fed females. Egg stages 3 and 4 were pooled because in *D. pulex* it is hard to separate eggs of these egg stages within the living mother. Water temperature was 16° C.

Experiment 2: 100 (2 treatments × 5 egg stages × 10 replicates) *Daphnia magna* were collected from a sample taken in Moscow Zoo in July 1991. Water temperature was 22° C.

Experiment 3: *Daphnia magna* were taken in August 1991 from the same pond in Moscow Zoo as those in Experiment 2. Only females with eggs in stages 1-early, 1-late, 2-early, 2-late and 5 were selected, because the previous 2 experiments had shown that the time period of interest (i.e. when the clutch size is determined) is covered by this egg age distribution. Sample size varied from 5 to 20 within each group, because only very few females were found carrying eggs. Water temperature was 25° C.

Field samplings in all three experiments were done at a time when the populations were in food limited conditions (low mean clutch sizes) to make sure that both switch to well feeding and starvation show a significant effect. To compare the results of the three experiments we transformed the egg stages into egg age classes according to the stage duration given by Threlkeld (1979). This was done by using the midpoint of the stage duration plus the durations of the previous egg stages. The egg age allowed to estimate the time when the clutch in the brood pouch has been laid and, more important, to estimate how long will it take until the next portion of eggs is deposited into the brood pouch.

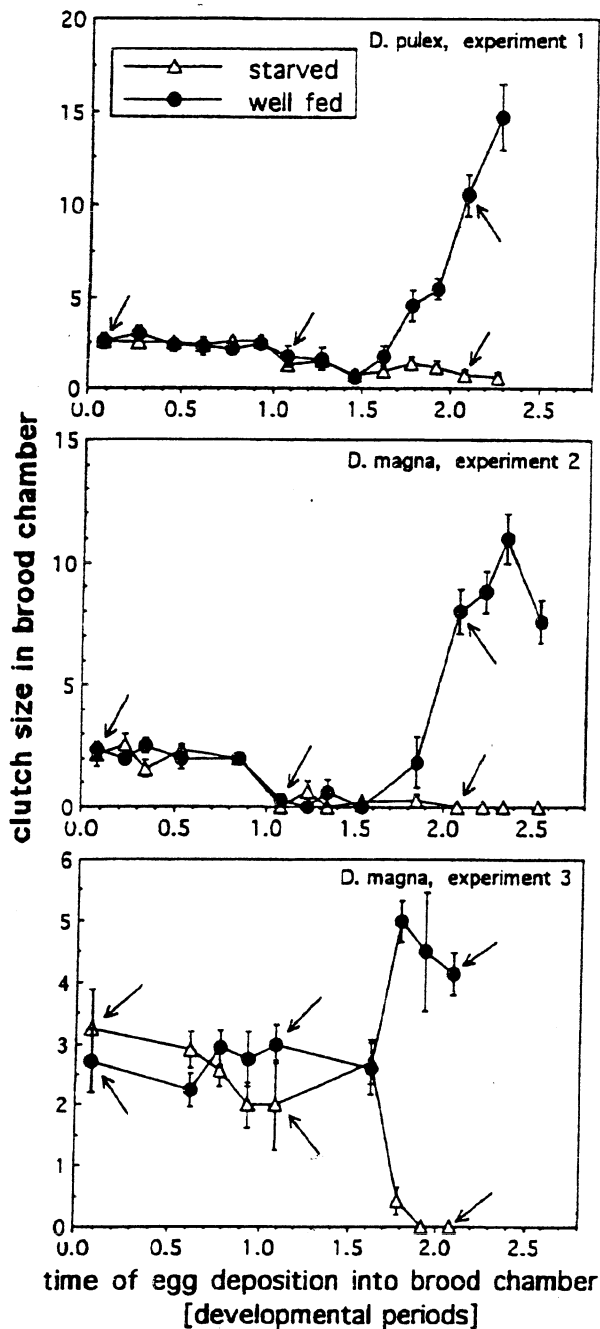


Figure 1. The response of clutch size (± 1 standard error) versus the time of egg deposition into the brood chamber for well-fed and starved treatment groups in three experiments with *Daphnia pulex* (top) and *D. magna* (middle and bottom). Before time = 1, all females experienced equal conditions in their natural environments. At time = 1 females were caught from natural populations and randomly assigned to starvation or well-fed treatment. Clutch sizes shown to the left of time = 1 are recorded from the wild caught females. With the help of age estimates of the eggs in their brood chambers (egg stage method, Threlkeld (1979)) the time of egg laying was calculated backwards. To the right of time = 1 are shown the clutches deposited into the brood chamber under alternated conditions. The arrows mark the first, the second and the third clutch of those females which had eggs in the last (fifth) egg stage when sampled from the ponds. The time axis is expressed in units of embryonic development periods (= duration of 1 adult instar) to allow comparison between experiments carried out at different temperatures. For further explanation see text.

Results

Figure 1 summarizes the results of this study. The abscissa represents the time at which a certain clutch was deposited into the brood chamber. Time = 1 on this axis represents the moment when the females were taken from their original populations, i.e. the moment of changing environmental conditions. The clutch sizes recorded in the brood pouches of females that came directly from the field, are shown to the left of time = 1. The oldest clutches were those in egg stage 5 (last developmental stage, arrows to the left of time = 1), freshly deposited eggs were those in egg stage 1 (those slightly left of time = 1).

During the time interval 1 to 2 the first clutch neonates of all females were released and the eggs of the second clutch were deposited into the brood pouch. Females which had eggs in their fifth stage when caught, released these offsprings the earliest. The size of their next clutch (the second one) is represented by the points (marked by arrows) first to the left of time = 1. These eggs were almost entirely nourished under field conditions and one would not expect that few hours of laboratory conditions had a strong influence on clutch size. Moving along the time axis to the right, the clutches being deposited into the brood chamber have been nourished under laboratory conditions for longer time periods. For example, the clutches shown at time = 1.5 were laid half a developmental time unit after the females had been brought to the laboratory.

Around time = 1.6 the two treatment groups diverged from one another. Females, which were well-fed in the laboratory, increased their clutch size, while the starved females reduced their clutch size. This effect was particularly clear in experiment 3 (Fig. 1). Clutches, which will be laid later than 0.6 developmental time units after an environmental change (here at time = 1), can be adjusted to increasing and decreasing feeding. There seems to be no difference between *Daphnia magna* and *D. pulex* in this respect. Clutches, which appear to the left of time = 2 (Fig. 1) are the third portions of eggs (second, laid under laboratory conditions). Eggs of these clutches experienced the laboratory conditions for more than one instar while being nourished in the ovaries. The points slightly to the right of time = 2 represent the third clutch recorded from the females which had been caught in the field with eggs in stage 5. (The first and the second clutches of these females are shown on Fig. 1 slightly to the right of time moments 0 (the most left points) and 1, respectively, and are marked with arrows).

In experiments 1 and 2 the increase of clutch size in the well-fed group did not show a simple switch to larger clutches, but slowly increased from time 1.6 to 2.3. We suggest that the clutch size cannot be adjusted on the basis of the new food conditions alone, but is also a function of the resource status of a female. Since all females came from rather poor feeding conditions, their resources were likely to be low at the moment the conditions improved. The continuous clutch size increase was not found for well-fed females in experiment 3. The low egg numbers in this experiment in comparison with the two other ones suggests that the well-fed females in experiment 3 were still food limited. The high temperature (25° C) and the subsequently high metabolic rate in this experiment possibly contributed to this effect.

Discussion

The presented experiments show that the number of eggs in a clutch which is currently being produced can be adjusted according to the changes of environmental conditions as late as 0.6 egg developmental unit (about 48 hours at 20° C) before the eggs are deposited into the brood chamber. This estimate is close to those obtained by Bradley et al. (1991a,b) in starvation experiments. Our experiments further demonstrate that the clutch size can be not only reduced, but also increased as a result of changing food conditions. Since the total production time of eggs is 2 adult instars (Makrushin 1981, Zaffagnini 1987) (about 6-7 days at 20° C), we speculate that more oocytes proliferate during clutch initiation than might be needed. At a later stage it is determined how many of these oocytes will become eggs. Those oocytes that did not develop further may be resorbed or used for nutrition of other eggs.

A study similar to our *Daphnia* study was done by Banta and Brown (1939). For the cladoceran *Moina* they found that the clutch size is determined 16.5 hours before egg laying, representing 0.3 egg development units. Both examples show a very high flexibility in response to changing environmental conditions. The adaptive advantage of this flexibility in the light of strongly fluctuating food conditions seems clear. The later the clutch size and by this the offspring size (see Bradley et al. 1991a for the relation between offspring size and number) is determined, the more precise will be the adjustment to the current environmental conditions. Whether any costs are involved in this strategy, is unclear, although it seems reasonable to assume that the resorption of eggs must have some costs and that

the further egg nutrition has moved on the higher these costs are. Thus, late clutch size determination reduces the chance of resulting with maladapted clutch and offspring sizes, but increases the costs of the excess eggs resorbing. Apart from possible constraints on egg production, we speculate that the timing of clutch determination evolved in such a manner that the costs of egg resorption (measured as fitness loss due to energetic costs) and the costs of maladjusted clutches compensate for each other.

Bradley et al. (1991a) proposed a new model of resource allocation into eggs. According to this model egg production is serial, i.e., eggs being provisioned one at a time. Bradley et al. reject the parallel egg provision model stating that "If eggs were provisioned at the same time we would predict no effect of starvation on the number of eggs produced, i.e., clutch size" (p. 328). We want to argue in favour of the parallel model, i.e., that all eggs of a clutch are provisioned simultaneously. We think that for each clutch many oocytes proliferate and start to grow. Later the actual clutch and egg sizes are determined about 0.5 to 0.6 developmental time units before the eggs are deposited into the brood pouch. The clutch size appears largely to be determined on the basis of current environmental conditions, but also current resource status of a female plays a role in this decision. We agree with Bradley et al. (1991a) that most resources for clutch production are laid down before the moment of clutch determination in the first half of each intermoult period. Thereafter the surplus oocytes are resorbed or serve to nutrition of other eggs (not nurse cells in the sense used by Zaffagnini (1987)). Thus, after clutch size is determined, the clutch mass (= offspring mass times clutch size) seems not to change any more (Bradley et al. 1991a).

Our parallel model easily explains the production of larger eggs when feeding conditions decrease during egg provision (Bradley et al. 1991 a). We speculate that the reduction of clutch size allows the resorption of more oocytes and by this more resource reallocation to the remaining eggs. Thus, at the moment of clutch determination the available resources for a clutch are distributed in a way to produce the most suited egg and clutch size. The more oocytes are resorbed, the larger eggs can be produced. To explain larger eggs after a period of starvation (Bradley et al. 1991a), the serial model requires a reallocation of energy from the mother to those eggs, for which provision was finished before the conditions decreased. This later on "topping up" appears unlikely to us.

The strongest support of the parallel model

with excess oocyte production, however, comes from cytological observations by Zaffagnini (1987). He showed that the "... use of the entire length of the ovary by the growing oocytes... allows simultaneous production of many eggs" (p. 249).

On the basis of parsimony and in agreement with the existing data we conclude that the production of eggs of each single clutch in *Daphnia* is parallel and that clutch size and egg size are adjusted by resorption of variable numbers

of oocytes, which are originally produced in excess.

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Планирование семьи у *Daphnia*: когда определяется число яиц в кладке?

Д. Эберт *, Л.Ю. Ямпольский **

* Institut für Zoologie, Universität Basel, Rheinsprung 9, CH-4051 Basel, Switzerland (present address: Department of Zoology, ABRG University of Oxford, South Parks Road, Oxford, OX1 3PS, UK
E-mail: EBERT@VAX.OXFORD.AC.UK)

** Институт общей генетики им. Н.И. Вавилова РАН, ул. Губкина, 3, Москва ГСП-1 В333, Россия

РЕЗЮМЕ. Общее время продукции яиц каждой кладки у дафний равно двум промежуткам между линьками (6-7 суток при 20° С). Ранее опубликованные данные позволяют предположить, что число яиц в кладке может быть сокращено во время их развития в яичниках в ответ на ухудшение пищевых условий. В экспериментах на *Daphnia magna* и *D. pulex* мы оценили минимальное время задержки, необходимое для того, чтобы увеличение или уменьшение концентрации пищи могло оказать влияние на число яиц в последующей кладке. Число яиц, откладываемых в яйцевую камеру уменьшалось, если самки испытывали нехватку пищи в течение последних 60 % периода между линьками, предшествующего моменту откладки яиц. То же время задержки было обнаружено для возрастания числа яиц в кладке в ответ на улучшение пищевых условий. Эти результаты показывают, что в начале периода развития яиц ооциты производятся в избытке. Окончательное число яиц в кладке определяется в соответствии с теми условиями питания, которые самка дафнии испытывает приблизительно в середине периода между линьками, предшествующего откладке яиц. Обсуждается механизм "настройки" числа яиц в кладке, служащий адаптацией к быстро меняющимся условиям среды.