
Maternal effects after sexual reproduction in *Daphnia magna*

Maarten Boersma, Hinnerk Boriss¹ and Suzanne E. Mitchell

Max-Planck-Institut für Limnologie, Postfach 165, D-24302 Plön, Germany

¹Present address: Department of Genetics and Ecology, Aarhus University, Ny Munkegade, Build. 540, DK-8000 Aarhus C, Denmark

Abstract. In this study we investigated the relationship between maternal size and size of ephippial hatchlings in *Daphnia magna*. We observed that larger females produce larger ephippia; these larger ephippia have a higher hatching probability, yield larger neonates, which in turn yield larger adults, producing more eggs. We interpret these observations as an explanation why older and larger females of *D. magna* continue to produce ephippia despite a higher predation risk.

Introduction

Most cladocerans are cyclic parthenogens, i.e. for most of the year they reproduce parthenogenetically, with sexual reproduction occurring only in certain limited periods of a growing season. This has led to a rather skewed interest towards parthenogenesis relative to the attention for sexual reproduction in these animals. Particularly for computations of the intrinsic rate of increase of the population, r , a quantity regularly used as a measure of fitness (Stearns, 1992), most researchers have taken into account parthenogenetic reproduction only. Only a few theoretical studies [e.g. (Taylor and Gabriel, 1993)] have incorporated both sexual and parthenogenetic reproduction. The lack of interest in sexual reproduction is mainly a result of the fact that many life history experiments are carried out with single individuals in jars and hence eggs have no opportunity to be fertilized. Additionally, hatching of sexual eggs is not immediate and as a result, it is difficult to assess the exact contribution of these sexual eggs to population growth. For a proper assessment of the relative contribution to population growth of instant parthenogenetic offspring versus delayed sexual offspring, a detailed knowledge of survival and future reproduction and hatching success of sexual eggs is needed. This information is difficult to obtain and hence, largely absent for natural systems.

Ephippial eggs have traditionally been studied in relation to survival under harsh conditions, such as extreme cold or drought [e.g. (Stross and Kangas, 1969)] and in relation to dispersal (Proctor, 1964; Proctor and Malone, 1965). Recently, the role these resting stages have, in both cladocerans and copepods, in relation to the existence of seed banks and to the origin of hybrids, has received a lot of attention (De Stasio, 1990; Taylor and Hebert, 1993; Schwenk and Spaak, 1995; Hairston and Cáceres, 1996; Cáceres, 1997; Gießler, 1997; Weider *et al.*, 1997), a fact illustrated by the two symposia and resulting proceedings on dormancy in crustaceans (Alekseev and Fryer, 1996; Brendonck *et al.*, 1998).

Most vertebrate predators of daphnids feed positively size selectively and when predation pressure is severe, only primiparous females manage to reproduce (Lampert, 1993). Females carrying ephippia face an especially severe predation risk as these animals are much more conspicuous as a result of the pigmentation

of the ephippium (Mellors, 1975). Very little is known about the age dependency of sexual reproduction. Some authors state that females do not normally produce sexual eggs very early in life (Wood and Banta, 1937; Lynch, 1989; Kleiven *et al.*, 1992; Spaak, 1995), but others (Zaffagnini, 1987; Pijanowska and Stolpe, 1996) have reported the opposite. In natural *Daphnia* populations, larger animals with ephippia are present (Mitchell, 1997) and the question arises as to why animals expose themselves to such a high predation risk when they could potentially produce the less conspicuous parthenogenetic eggs. Three explanations are possible: (i) the environmental conditions are so bad that a parthenogenetic brood will not survive to grow up and reproduce, so producing ephippia is the best strategy despite the high predation risks; (ii) ephippia are known to survive passage through fish and bird guts, and being conspicuous might be a good dispersal strategy (Proctor, 1964; Proctor and Malone, 1965; Mellors, 1975); (iii) a fitness advantage exists for hatchlings from ephippia produced by larger females, which compensates for the higher predation risk.

In this paper, we set out to test the third explanation as to why larger females still produce ephippia, despite the high predation risk. One possible advantage could be that larger females are capable of investing more in their offspring, which could lead to a higher fitness of animals born from these females. This dependence of neonate size on maternal size is well documented for parthenogenetic eggs (Glazier, 1992; Ebert, 1993; Boersma, 1995, 1997) but until very recently, no studies have investigated this relationship for sexual offspring [but see (Pfrender and Deng, 1998)]. An essential difference exists in the division of the total amount of available energy between individual offspring for sexual and asexual offspring. The ephippium structure generally determines a maximum brood size of two eggs. This implies that the normal trade-off between size and number of offspring, observed for the parthenogenetic mode of reproduction, is not present for the sexual phase. Hence, if animals have a high amount of available energy, this can lead to more energy per egg but not to a higher number of eggs. As under normal circumstances sexual eggs hatch in spring, with low predation pressures, it should be advantageous for hatchlings to be of a larger size, which is known to lead to a larger size at maturity with a higher number of eggs (Arbaciauskas and Gasiunaite, 1996; Arbaciauskas, 1998).

Method

We tested the questions raised above using two sets of ephippia in independent experiments. One set consisted of laboratory-crossed clones of *Daphnia magna*, with only a limited number of clones involved. The other set comprised *D. magna* ephippia collected from the field. The relationship between the size of the mother and the size of the ephippium produced by this animal was established by collecting field animals that were reproducing sexually and measuring the female body length and the length of the ephippium.

Ephippia were collected in February of 1998 from the sediment in the Rixdorfer Pferdetränke, a small pond close to Plön, Northern Germany (Mitchell, 1997). They were brought to the laboratory and stored in the dark at 5°C. Subsequently,

they were sorted and placed individually in microtitre plate wells filled with 225 μl of 0.45 μm filtered lake water, obtained from nearby lake Schöhsee. Hatching was carried out in March–April 1998 and initiated by placing the plates at natural light and temperature conditions (minimum day/night temperature 8°C/4°C; maximum temperature 11.5°C/9°C). The ehippia were checked daily, and all hatchlings were collected and measured before their first moult. The ehippia were measured afterwards to avoid accidental hatching due to the light and checked for the remaining number of unhatched eggs.

Laboratory ehippia were obtained from crosses between different clones of *D. magna*, using procedures described in the literature [e.g. (Wood and Banta, 1937)]. We carried out five different crosses between completely unrelated clones isolated from different locations in Europe. Crowded cultures of these clones were established and in a pairwise design, ~200 individuals per clone were added to 3 l of filtered lake water. All cultures were fed with 2.0 mg C of *Scenedesmus obliquus* l⁻¹ day⁻¹. The ehippia produced were hence a mixture of matings between and within clones. Ehippia were collected from the bottom of the culture vessels at regular intervals, measured, and placed in microtitre plate wells with filtered lake water. Subsequently, they were stored at 5°C in the dark for several months. Hatching was carried out as described above, and the hatchlings were subsequently cultured in 100 ml vessels and fed 1.5 mg l⁻¹ of *S. obliquus* daily. The experimental medium was changed every other day. Animals were measured again as they reached maturity and their clutch size was determined.

As an ehippium can contain two eggs, potentially two hatchlings can emerge from one ehippium. To avoid pseudo-replication, values of these two hatchlings from the same ehippium were averaged prior to the statistical analysis.

Results and discussion

Larger females produced larger ehippia (Figure 1a; Table I). This is to be expected as the ehippium is a part of the female's carapace. Hence, larger ehippia collected from the field and from the laboratory cultures were most likely produced by larger, older females.

We observed that 76.5% of the field-collected ehippia yielded at least one offspring, with an overall hatching success of 58.5% for field-collected ehippia assuming that every ehippium contains two eggs. The hatching rate for our laboratory crosses was lower. In this case, we observed a total of 133 animals

Table I. Regression coefficients and significance of relationships of maternal length with ehippial length and ehippial length with hatchling characteristics

	Intercept	Slope	<i>n</i>	<i>r</i> ²	<i>P</i>
Maternal length versus ehippium length	0.25	0.38	31	0.72	<0.001
Ehippial length versus length neonate (field)	0.43	0.27	90	0.46	<0.001
Ehippial length versus length neonate (lab)	0.59	0.11	93	0.32	<0.001
Ehippial length versus size at maturity (lab)	1.66	0.81	51	0.23	<0.001
Ehippial length versus number of eggs (lab)	-0.22	9.3	45	0.12	0.02

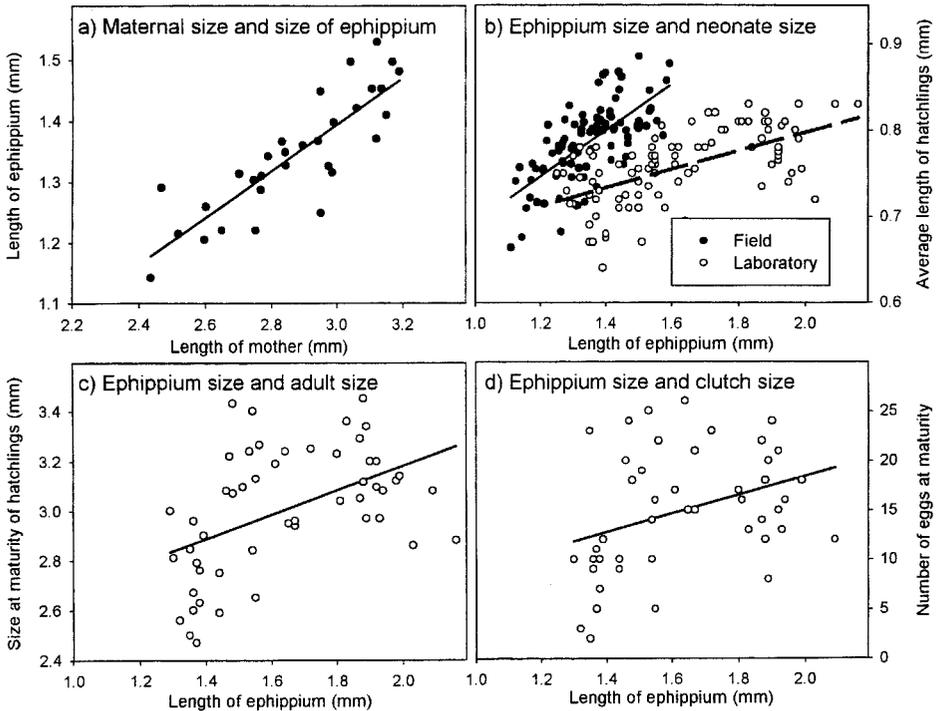


Fig. 1. Relationships between (a) length of the mother and size of her ephippium; (b) length of the ephippia and size of the neonates from the field-derived ephippia (closed symbols) and the laboratory crosses (open symbols); (c) length of laboratory-derived ephippia and size at maturity of the neonates hatched from the resting eggs in these ephippia; (d) length of laboratory-derived ephippia and the number of eggs of the first adult instar of animals hatched from these ephippia. Regression statistics are given in Table I.

hatching from 437 ephippia, which means a hatching rate of 15%. The frequency of empty ephippia was, however, higher for the laboratory crosses, yielding a 35% hatching rate after correction for these empty ephippia. De Meester (De Meester, 1993) observed that the hatching success of ephippia produced by intra-population crosses was higher than of those produced by clones taken from different populations. At the same time, within a population, the hatching success of ephippia produced by selfing was lower than the hatching success of those produced by clonal crosses. These observations could explain the relatively low hatching success for our laboratory crosses, as ephippia produced are a mixture of selfing and inter-population crosses.

Laboratory ephippia that yielded hatchlings were slightly larger than those which did not produce neonates, i.e. 1.62 mm (standard error 0.02) compared with 1.59 mm (S.E. 0.01), respectively, but this difference was not significant ($t_{434} = 1.01$; $P = 0.31$). For field-collected ephippia, the link between size of ephippia and hatching success was stronger. Ephippia from which at least one hatchling emerged were 1.35 mm (S.E. 0.01), whereas the ones that yielded no hatchlings

averaged 1.30 mm (S.E. 0.02). This difference in size was significantly different ($t_{117} = 2.03$; $P = 0.04$). A logit regression with size of the ephippium as the independent variable, and hatching success (0 or 1) as the dependent variable ($\chi^2_1 = 4.17$; $P = 0.04$), revealed that the hatching probability would be as low as 0.56 for the smallest ephippia we observed, and 0.91 for the largest ones. This is in contradiction to the results of Pfrender and Deng (Pfrender and Deng, 1998), who observed no differences in hatching success for differently sized ephippia. The difference is not caused by a difference in the frequency of empty ephippia for differently sized ones. In fact, we observed a non-significant tendency in the other direction for the laboratory dataset, i.e. larger ephippia have a higher probability of being empty than smaller ones.

In this study, we cannot separate genetic and environmental effects. Genetic differences in hatching success have been observed before [e.g. (De Meester and De Jager, 1993)] and maternal effects on hatching success have also been found (De Meester and De Jager, 1993; De Meester *et al.*, 1998). In particular, food availability during ephippia formation might be of importance for hatching success. Although we cannot exclude the possibility that larger individuals were simply better fed and hence had more resources to put into reproduction, it is not likely that this is the explanation of the patterns we found. In particular, all animals were cultured under similar conditions in the laboratory crosses and feeding differences between individuals are likely to have been small. However, as under food limitation different cohorts of *Daphnia* might be affected differently (Enserink *et al.*, 1996), this warrants further investigation.

Larger ephippia yielded larger neonates (Figure 1b) for both the laboratory crosses and for field collected animals (Table I). This means that as ephippium size is strongly positively correlated with size of the animal, larger mothers produced larger offspring, which were also larger at maturity (Table I; Figure 1c). Further, the correlation of ephippial length with size of the first brood of the hatchlings was also positive (Table I; Figure 1d). Hence, not only were the ephippial eggs produced by larger females and had a higher hatching probability, they also yielded neonates with a higher fitness under laboratory conditions. As ephippial eggs normally hatch in spring (Wolf and Carvalho, 1989) when the abundance of positively size-selective predators is typically low, this apparent fitness advantage of larger size of hatchlings from larger mothers is likely to occur under field conditions as well (Arbaciauskas and Gasiunaite, 1996; Arbaciauskas, 1998). This could explain why larger females continue to produce ephippial eggs, and might also explain why females which reproduce sexually continue to grow [see also (Taylor and Gabriel, 1993)], despite a maximum brood size of two sexual eggs independent of body size.

Acknowledgements

We thank Larry Weider, Luc De Meester, Karen Wiltshire and Winfried Lampert for fruitful discussions and comments on earlier versions of the manuscript.

References

- Alekseev, V.R. and Fryer, G. (eds) (1996) *Diapause in the Crustacea. Proceedings of the Symposium 'Diapause in Crustacea' (Hydrobiologia, 320)*.
- Arbaciauskas, K. (1998) Life-history traits of ephippial and parthenogenetically derived daphnids: indicators of different life-history strategies. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **52**, 339–358.
- Arbaciauskas, K. and Gasiunaite, Z.R. (1996) Growth and fecundity of *Daphnia* after diapause and their impact on the development of a population. *Hydrobiologia*, **320**, 209–222.
- Boersma, M. (1995) The allocation of resources to reproduction in *Daphnia galeata*: against the odds? *Ecology*, **76**, 1251–1261.
- Boersma, M. (1997) Offspring size and parental fitness in *Daphnia magna*. *Evol. Ecol.*, **11**, 439–450.
- Brendonck, L., De Meester, L. and Hairston, N.G. (eds) (1998) *Evolutionary and Ecological Aspects of Crustacean Diapause. Proceedings of the Symposium 'Diapause in Crustacea—with Invited Contributions on Non-crustacean Taxa' (Arch. Hydrobiol. Beih. Ergebn. Limnol., 52)*.
- Cáceres, C.E. (1997) Temporal variation, dormancy and coexistence—a field test of the storage effect. *Proc. Nat. Acad. Sci. USA*, **94**, 9171–9175.
- De Meester, L. (1993) Inbreeding and outbreeding depression in *Daphnia*. *Oecologia*, **96**, 80–84.
- De Meester, L. and De Jager, H. (1993) Hatching of *Daphnia* sexual eggs. I. Intraspecific differences in the hatching responses of *D. magna* eggs. *Freshwater Biol.*, **30**, 219–226.
- De Meester, L., Cousyn, C. and Vanoverbeke, J. (1998) Chemical interactions, maternal effects and the hatching of *Daphnia* diapausing eggs. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **52**, 263–272.
- De Stasio, B.T. (1990) The role of dormancy and emergence patterns in the dynamics of a freshwater zooplankton community. *Limnol. Oceanogr.*, **35**, 1079–1090.
- Ebert, D. (1993) The trade-off between offspring size and number in *Daphnia magna*: the influence of genetic, environmental and maternal effects. *Arch. Hydrobiol. Suppl.*, **90**, 453–473.
- Enserink, E.L., van der Hoeven, N., Smith, M., van der Klis, C.M. and van der Gaag, M.A. (1996) Competition between cohorts of juvenile *Daphnia magna*—a new experimental model. *Arch. Hydrobiol.*, **136**, 433–454.
- Gießler, S. (1997) Gene flow in the *Daphnia longispina* hybrid complex (Crustacea, Cladocera) inhabiting large lakes. *Heredity*, **79**, 231–241.
- Glazier, D.S. (1992) Effects of food, genotype, and maternal size and age on offspring investment in *Daphnia magna*. *Ecology*, **73**, 910–926.
- Hairston, N.G. and Cáceres, C.E. (1996) Distribution of crustacean diapause: micro- and macroevolutionary pattern and process. *Hydrobiologia*, **320**, 27–44.
- Kleiven, O.T., Larsson, P. and Hobæk, A. (1992) Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos*, **65**, 197–206.
- Lampert, W. (1993) Phenotypic plasticity of the size at first reproduction in *Daphnia*: the importance of maternal size. *Ecology*, **74**, 1455–1466.
- Lynch, M. (1989) The life history consequences of resource depression in *Daphnia pulex*. *Ecology*, **70**, 246–256.
- Mellors, W.K. (1975) Selective predation of ephippial *Daphnia* and the resistance of ephippial eggs to digestion. *Ecology*, **56**, 974–980.
- Mitchell, S.E. (1997) Clonal diversity and coexistence in *Daphnia magna* populations. PhD thesis. University of Hull, UK.
- Pfrender, M.E. and Deng, H.W. (1998) Environmental and genetic control of diapause termination in *Daphnia*. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **52**, 237–251.
- Pijanowska, J. and Stolpe, G. (1996) Summer diapause in *Daphnia* as a reaction to the presence of fish. *J. Plankton Res.*, **18**, 1407–1412.
- Proctor, V.W. (1964) Viability of crustacean eggs recovered from ducks. *Ecology*, **45**, 656–658.
- Proctor, V.W. and Malone, C. (1965) Further evidence of the passive dispersal of small aquatic organisms via the intestinal tract of birds. *Ecology*, **46**, 728–729.
- Schwenk, K. and Spaak, P. (1995) Evolutionary and ecological consequences of interspecific hybridization in cladocerans. *Experientia*, **51**, 465–481.
- Spaak, P. (1995) Sexual reproduction in *Daphnia*: Interspecific differences in a hybrid species complex. *Oecologia*, **104**, 501–507.
- Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Stross, R.G. and Kangas, D.A. (1969) The reproductive cycle of *Daphnia* in an arctic pool. *Ecology*, **50**, 457–460.
- Taylor, B.E. and Gabriel, W. (1993) Optimal adult growth of *Daphnia* in a seasonal environment. *Funct. Ecol.*, **7**, 513–521.

- Taylor,D.J. and Hebert,P.D.N. (1993) Habitat-dependent hybrid parentage and differential introgression between neighboringly sympatric *Daphnia* species. *Proc. Nat. Acad. Sci. USA*, **90**, 7079–7083.
- Weider,L.J., Lampert,W., Wessels,M., Colbourne,J.K. and Limburg,P. (1997) Long-term genetic shifts in a microcrustacean egg bank associated with anthropogenic changes in the Lake Constance ecosystem. *Proc. R. Soc. Lond. B, Biol. Sci.*, **264**, 1613–1618.
- Wolf,H.G. and Carvalho,G.R. (1989) Resting eggs of lake-*Daphnia*. II. *In situ* observations on the hatching of eggs and their contribution to population and community structure. *Freshwater Biol.*, **22**, 471–478.
- Wood,T.R. and Banta,A.M. (1937) Observations on procuring and hatching sexual eggs of *Daphnia longispina*. *Int. Revue Ges. Hydrobiol.*, **29**, 229–242.
- Zaffagnini,F. (1987) Reproduction in *Daphnia*. *Mém. Ist. Ital. Idrobiol.*, **45**, 245–284.

Received on December 5, 1998; accepted on August 19, 1999

