

Measuring the cost of reproduction

IV. Predation experiments with *Daphnia pulex*

Vasso Koufopanou and Graham Bell

Biology Department, McGill University, 1205 Ave Dr. Penfield, Montreal, Quebec, Canada H3A 1B1

Abstract. Gravid and barren *Daphnia pulex* were exposed to a variety of predators in laboratory aquaria. Small fish (guppies, sticklebacks and shiner fry) consistently preferred the gravid females, establishing the existence of a behavioural cost of reproduction. However, no such cost was associated with predation by more efficient visual predators (sunfish) or by nonvisual predators (hydras), and the excess of gravid females eaten by backswimmers was found to be attributable to their distribution in the water column. Moreover, the cost associated with predation by small fish was observed only when the *Daphnia* were presented against a light background, and was abolished when a dark background was substituted. In a further series of experiments with guppies we attempted to show that each egg added to the brood caused a decrease in survival; in two such experiments survival rate was related to body size but not to fecundity, while in a third the effect of body size did not appear, and a negative correlation between survival and fecundity could be demonstrated. Although these experiments unambiguously demonstrate a cost of reproduction they also illustrate the elusiveness of the phenomenon and emphasize the need to develop theories which specify the type and magnitude of costs generated by different ways of life.

Introduction

The reproductive cost hypothesis states that any increase in present reproduction should be accompanied by a decrease in future reproduction, since it is only then that reproduction can be optimized through natural selection (Williams 1966). Recently, there have been attempts to identify reproductive cost by measuring the correlations among life history parameters (Snell and King 1977; Rose and Charlesworth 1981; Bell 1983, 1984a, b), or by manipulating the amount of present reproduction by various means and measuring its effect on subsequent survival or fecundity (eg. Dean 1981; Partridge and Farquhar 1982; Tallamy and Denno 1982). It might be argued, however, that the physiological responses of animals raised under favourable conditions in the laboratory do not tell us much about processes in natural populations, where a slight degradation of crypsis or escape ability might be almost inevitably fatal. For example, it has been shown experimentally that preg-

nant female lizards were preyed on by snake predators more than males of comparable sizes (Shine 1980), and that brightly-colored male fish were preferred by predators to dull-colored males or females (Moodie 1970, cited by Semler 1971; Haas 1976). Such studies are only suggestive; in this paper the survival of gravid *Daphnia pulex* is compared directly to that of barren females of equal size in simple laboratory experiments.

General methods

All the predation experiments followed the same general pattern. Predators maintained in experimental aquaria were trained to begin foraging as soon as *Daphnia* were poured slowly out of a small petri dish. They were then allowed to feed until about half the prey had been eaten, as assessed by an observer seated at a distance of about half a metre from the front of the aquarium. All the remaining *Daphnia* were removed with an aquarium net, washed into a petri dish, and then counted and classified according to the presence or absence of eggs. Female *Daphnia* carrying asexual eggs (gravid females) were always tested against an equal number of females without eggs (barren females). Size was measured before and after predation in order to correct for any bias due to size-selective predation. The trials were kept fairly brief (from 0.5 to 60 min, according to the predator), so that the likelihood of the animals releasing their eggs or producing a new batch during the experiment was negligible. The numbers of prey available per individual predator in each replicate and the volume of the experimental chamber were adjusted to the size and feeding rate of different predators to allow normal foraging behavior and to ensure that satiation would not occur before the end of the experiment. Predators were fed *Daphnia* for at least one to two weeks prior to each experiment. A controlled daily feeding regime kept the predators moderately hungry before each experimental session throughout the whole experimental period.

Daphnia pulex originating from a single clone from a S. Ontario stock was used in all the experiments. Since reproduction in these animals is highly dependent upon food availability and the state of population growth, several cultures were maintained so that both reproductive and nonreproductive females of comparable sizes could be obtained at the same time. The cultures were fed with a suspension of green algae (*Ankistrodesmus*) and continuously aerated. The reproductive condition of the females could

Table 1. Comparison of survival rates between egg-bearing and barren female *Daphnia*. Predation experiments with small fish (guppies, sticklebacks, emerald shiner fry). The sample size in each comparison is shown in parenthesis. *N* gives the number of independent trials in each experiment. *P* = Probability that groups survive at equal rates (contingency chi-square tests)

Predator	Size	Background	Water depth	<i>N</i>	Survival		<i>P</i>
					Egg-bearing	Barren	
Guppy	Small immature	Light	16 cm	5	0.59 (136)	0.75 (136)	<0.01
		Light	16 cm	5	0.40 (136)	0.59 (136)	<0.01
	Medium immature	Light	3 cm	6	0.31 (125)	0.46 (125)	<0.025
		Dark	16 cm	11	0.49 (284)	0.42 (284)	n.s.
	Adult males	Light	16 cm	5	0.30 (136)	0.49 (136)	<0.01
Stickleback		Light	16 cm	6	0.38 (152)	0.52 (152)	<0.01
Shiner fry		Light	16 cm	8	0.61 (205)	0.68 (205)	n.s.

easily be assessed through the transparent carapace. To estimate size, live *Daphnia* were immobilized by reducing the water volume in a depression slide with a fine pipette, and their length (from base of dorsal spine to top of head) measured to the nearest hundredth of a mm using an ocular micrometer. Water was then immediately restored, taking care that no air was trapped inside the carapace, since this forces *Daphnia* to float on the water surface.

The results of the predation experiments, unless otherwise stated in the text, were analyzed as follows. All replicates of an experiment were pooled and tested with contingency chi-square against the null hypothesis that reproductive condition has no effect on survival of females. Pooling the results of individual trials of an experiment was considered necessary since the sample size of each trial was too small to be analyzed separately. Size effects were also tested on pooled results of experimental replicates with contingency chi-square tests under the null hypothesis that size has no effect on the survival of females. The legitimacy of pooling the results of different replicates was confirmed by performing homogeneity (contingency) chi-square tests. The significance level in all the tests performed was set at 5%.

Experiments with small fish

(A) *Qualitative effects.* Three different species of small planktivorous fish were used in this group of experiments: guppies (*Poecilia reticulata*), brook stickleback (*Culaea inconstans*) and emerald shiner fry (*Notropis atherinoides*). The guppies came from a stock population kept in a small pool at McGill University for several years while the stickleback and the minnows were collected from a roadside ditch and a small lake close to Montreal respectively. Three different size classes of guppies were tested separately: small immature fish (1.0–1.1 cm in standard length), medium immature fish (1.3–1.4 cm) and adult males (1.7–2.0 cm), in order to see if small differences in the size and capture efficiency of predators affect the occurrence and strength of reproductive cost. The sticklebacks and the emerald shiners used were 1.5–2.0 cm in standard length. Depending on the size of predator, groups of 4–10 fish were tested at a time, in a 6.5 l aquarium coated with light yellow paper on three sides and the bottom. This light background was used as a first step in the experiments since it seemed to contrast well with the darker greenish color of the eggs inside the carapace. A second experiment was carried out

with the medium-sized guppies using a darker, brown background in order to see if the nature of the background influenced the results. Prey density was kept constant in all experiments at 50 *Daphnia* per trial (7,600/m³). Special care was taken to restrict the size range of the prey as much as possible, and to make the size distributions of the different reproductive groups initially presented to the fish as nearly equal as possible, so that size would not be a confounding variable in each trial. The extreme range of body lengths of the *Daphnia* used in these experiments was 0.68 mm (1.64–2.32 mm), but the range of size in individual replicates of an experiment was less than 0.2 mm in almost all cases.

Table 1 summarizes the results of these predation experiments. All three species of small fish and all three size classes of guppies tested against the light background showed a preference for the gravid females. In all cases except for the shiner fry, the number of gravid females that survived predation was significantly smaller than the number of barren females. Results between replicates were homogeneous and size effects were not significant in any of the above cases. There was no significant difference in the results among different species of small fish nor among the three size classes of guppies tested. When guppies were tested against the dark background, however, no preference for the gravid females was shown. This suggests that the reproductive cost imposed by small fish is due to the increased visual contrast of gravid females against the background.

(B) *Quantitative effects.* To conclude this study an experiment was designed in order to look at the effect on survival of each additional egg carried in the brood-pouch, using medium-sized guppies in a 6.5 l aquarium against a light background. Fifty *Daphnia* were used in each of ten trials. After measuring the size of each animal the number of eggs it carried inside the brood pouch was estimated. If fewer than six eggs were present, all could be seen and counted directly. If more than five eggs were present, all the eggs that could be seen were counted, and to these the number of eggs that touched the upper surface of the carapace was added, this latter number being assumed to be equal to the number of eggs that were out of view. The accuracy of the estimation procedure was assessed from a sample of 75 individuals in which the number of eggs was estimated and then compared to the number counted after the eggs had been dissected out of the animals. The

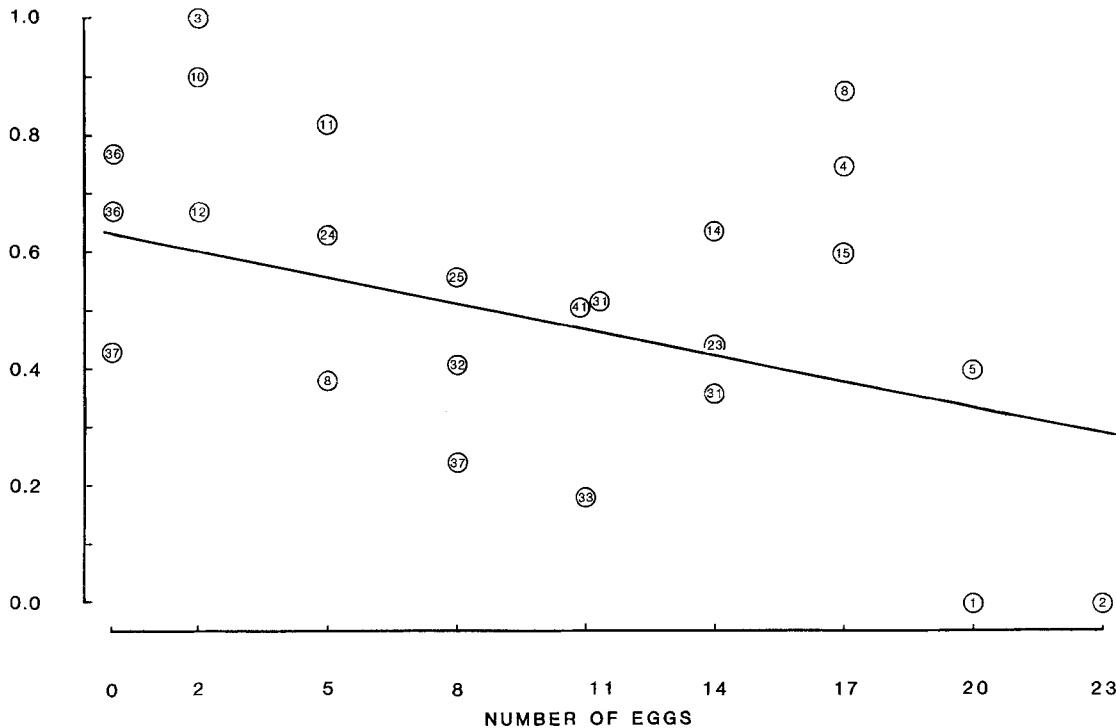


Fig. 1. The effect of fecundity on survival in a predation experiment using medium-sized immature guppies. Survival values were calculated for each cell corresponding to a given egg-number and size class, defined as the number remaining after predation in a given cell divided by the number in that cell initially presented to the fish. These values were then transformed with the arcsin square-root transformation commonly used to normalize proportions. Errors in estimation of egg-number resulted in misclassification of some animals and hence in survival values greater than one. Since the arcsin transformation cannot be performed on values greater than one, such cells were corrected by setting the survival equal to one, and redistributing the surplus animals in equal numbers to the two immediately neighbouring cells. In this way, all individual points were preserved for the analysis without altering the more general pattern of the results. This treatment affected only a very small proportion of the data (less than 5% of individuals). Differences in sample size between cells were taken into account in the analysis by weighting each point by the number of animals in that cell initially presented to the fish. Each point on the scatter diagram represents the mean from one trial, with numbers indicating the sample size of each observation. The best-fit least squares regression line is shown (weighted by the sample size in each point): $y = -0.01487x + 0.91478$, $r^2 = 0.15$, $P = 0.03$ (one-tailed F test). This differs slightly from the equation given in the text, which refers to the mean values of pooled replicates.

relationship between estimated and counted egg-number was:

$$\text{Estimate} = (1.115) \text{Count} - 0.457 \quad (r^2 = 0.87)$$

The procedure slightly overestimated the actual numbers but the regression of count on estimate is linear and the scatter of points restricted. Frequencies of individuals in each size and egg-number category before and after predation were compared in order to obtain a survival value for a given size and fecundity.

The small sample size in this experiment (50 animals per replicate) produced a large variance among trials. The experiment (Experiment 1) was therefore repeated with a sample size of 150 animals per trial in larger aquaria, maintaining a density of *Daphnia* similar to that of the first experiment, and using 21 instead of 7 medium-sized guppies in order to keep the number of prey available per predator constant. Two different experiments were performed, each consisting of three replicates. In the first (Experiment 2), the experimental chamber was open above, while in the second (Experiment 3) the chamber was covered with cardboard so that light could enter only through the sides. In the latter case *Daphnia* were more equally distributed throughout the water column.

A fairly large variation in egg-number between animals of similar size is required to assess the effect of fecundity on survival. In *Daphnia* and many other animals however, fecundity increases with body size. In these experiments we tried to loosen this correlation by manipulating the food density of *Daphnia* cultures and by restricting the size range of animals used. It was not possible, however, to eliminate the effect of size altogether, and both egg-number and size effects must be considered together in the following discussion.

The results of the three experiments (1, 2, and 3) which tested the effect of fecundity on survival were analyzed by regression (see legend to Fig. 1). Stepwise regression was performed in order to see if either egg-number or size were significant variables in predicting the observed pattern of survival values. In both the first and second experiments, only size was entered as a significant variable in the models, the effect of size being positive in both cases. The predictive equations were:

Experiment 1:

$$\text{survival} = (0.310) \text{size} - 0.987$$

$$(r^2 = 0.07, p < 0.05, df = 63)$$

Table 2. Regression coefficients b ($\times 10^3$) of survival on fecundity (egg-number) for separate size classes of prey (\pm standard error of estimate). P gives the significance of the regression

Size class (mm)	Survival					
	Experiment 1		Experiment 2		Experiment 3	
	$b \pm SE$	P	$b \pm SE$	P	$b \pm SE$	P
1.90			-56.92 ± 18.62	0.04	-28.97 ± 24.49	0.30
1.98			3.28 ± 11.69	0.79	-26.08 ± 15.51	0.14
2.06			0.76 ± 15.71	0.96	-23.82 ± 9.61	0.06
2.14			4.56 ± 14.35	0.76	-8.82 ± 17.41	0.64
2.22	-6.15 ± 6.70	0.38	18.91 ± 10.68	0.14	10.04 ± 15.06	0.53
2.30	-7.31 ± 9.86	0.48				
2.38	-9.37 ± 9.26	0.34				
2.40	0.02 ± 10.47	0.99				

Experiment 2:

survival = (0.400) size - 1.320
($r^2 = 0.22$, $p < 0.001$, $df = 69$)

In the third experiment only egg-number was entered, with a negative coefficient, and size did not improve the predictive power significantly. The equation obtained for this experiment was:

Experiment 3:

survival = (-0.012) egg-number + 0.908
($r^2 = 0.07$, $p < 0.05$, $df = 34$)

Regressions were also run by size, in order to look at the effect of egg-number on survival in each size class separately. The coefficients of the regressions for each size class together with their significance are presented in Table 2. Most of the higher coefficients appear with a negative sign, and in the first and third experiments all coefficients except those for the highest size class are negative. The individual regressions, however, are not statistically significant, perhaps due to the small sample size involved in each regression. The overall effects of body size and fecundity on survival, for each of the three experiments are summarized in Table 3.

In conclusion, it seems that increased fecundity does have a weak negative effect on survival, though a significant result was demonstrated only in Experiment 3. This may be due to the larger sample size in each replicate, the absence of any strong effect of size and the better distribution of *Daphnia* throughout the entire water column which made the different types more equally available to the fish. Figure 1 shows the overall effect of egg-number on survival in the three trials of Experiment 3. Despite the large scatter in the data, there is a fair consistency among the three replicates and a significant negative relation ($P = 0.03$ for

Table 3. The effects of egg-number and of size on survival of *Daphnia* in predation experiments with guppies. Total results for experiments 1, 2, 3. The sample size in each observation is shown in parenthesis

	Survival		
	Experiment 1	Experiment 2	Experiment 3
Effect of egg-number ^a			
0	0.44 (46)	0.45 (122)	0.62 (109)
2	0.63 (19)	0.47 (15)	0.80 (25)
5	0.56 (32)	0.43 (67)	0.63 (43)
8	0.55 (40)	0.45 (91)	0.38 (94)
11	0.58 (57)	0.56 (70)	0.41 (105)
14	0.56 (64)	0.64 (78)	0.44 (68)
17	0.59 (70)	0.70 (46)	0.70 (27)
20	0.44 (66)	0.48 (25)	0.63 (8)
23	0.37 (59)	0.36 (14)	
26	0.59 (34)		
29	0.83 (18)		
32	0.30 (23)		
Effect of body size ^b			
1.90		0.37 (57)	0.47 (75)
1.98		0.40 (82)	0.60 (104)
2.06		0.43 (91)	0.52 (117)
2.14	0.30 (20)	0.55 (112)	0.54 (86)
2.22	0.45 (151)	0.54 (97)	0.45 (97)
2.30	0.49 (106)	0.83 (35)	
2.38	0.59 (158)	0.53 (19)	
2.46	0.63 (80)	0.79 (14)	

^a Egg-number category

^b Size class in mm

one-tailed test). There was no significant deviation from linearity.

Experiments with other predators

Experiments were also performed with a larger fish predator of *Daphnia*, the sunfish *Lepomis gibbosus*. Young individuals of 4.0–4.7 cm in standard length were tested, one at a time, in a 90 l aquarium against a light background and at two densities – low (10–20 *Daphnia*/trial or 100–200/m³) and high (200 *Daphnia*/trial or 2,200/m³).

We also used ephippial females (in which the brood pouch has darkened walls and together with the two eggs it contains is cast off as a single propagule) against non-ephippial females (bearing neither eggs nor an ephippium) in a separate series of trials, to confirm that the sunfish were capable of visual discrimination between alternative prey. Although a significant preference was detected for ephippial compared to non-ephippial females at both low and high densities, there was no preference for the gravid over barren females (Table 4).

A decreased survival of gravid females was observed when backswimmers (*Notonecta*) were used as predators, in an experimental set-up similar to that of the small fish series (Table 5). At the same time, however, it was observed that more gravid females were swimming at the top 2 cm layer of the aquarium, where the *Notonecta* were foraging, and this could explain our result. Since the reproductive cost associated with the small fish predators might also

Table 4. Comparison of survival rates between reproductive and nonreproductive female *Daphnia*. Predation experiments with pumpkinseed sunfish. The sample size in each comparison is shown in parenthesis. The number of independent trials performed in each density is given by *N*. Probability that different groups survive at equal rates (chi-square contingency tests) is given by *P*

Density	<i>N</i>	Survival			<i>P</i>
		Dark Ehippial	Light Ehippial	Non Ehippial	
a. Ehippial females					
Low	14	0.37 (70)	0.39 (70)	0.57 (70)	<0.025
High	5	0.35 (340)	0.43 (340)	0.62 (340)	<0.001
b. Egg-bearing females					
		Egg-bearing	Barren		
Low 1	12	0.48 (60)	0.50 (60)		n.s.
Low 2	9	0.49 (90)	0.33 (90)		<0.05
High	6	0.40 (600)	0.44 (600)		n.s.

Table 5. Comparison of survival rates between egg-bearing and barren females. Predation experiments with invertebrate predators (brown hydra, backswimmers). Sample sizes are shown in parentheses. *N* gives the number of independent trials performed in each case. *P* is probability that both groups survive at equal rates (contingency chisquare test)

Predator	<i>N</i>	Survival		<i>P</i>
		Egg-bearing	Barren	
Brown hydra	8	0.47 (150)	0.50 (150)	n.s.
Back-swimmer	13	0.46 (216)	0.59 (216)	<0.01

be due to an unequal distribution of *Daphnia* between the different water layers, we performed an experiment with guppies using a water column reduced to 3 cm. Such an arrangement would not permit the segregation of gravid and barren *Daphnia* between different water layers. The results of this experiment conformed to the ones already described in the previous section (see Table 1), suggesting that the observed reproductive cost was due to a preference for the egg-bearing females rather than to a nonrandom distribution of prey and predators. Experiments were also performed using brown hydra (*Hydra pseudooligactis*), as an example of a predator whose foraging depends entirely on nonvisual cues. No reproductive cost was observed, suggesting that eggs, which may weigh as much as the entire body weight of a nonreproductive female (Green 1956), do not in this case reduce the escape ability of gravid females.

General conclusions

It is not easy to extrapolate the results of controlled laboratory experiments to more natural situations. Our findings should be regarded as descriptions of what has been observed in particular well-defined situations, rather than as

instances of an invariable general rule. Although a reproductive cost can be found, measured and quantified on certain occasions, it may be absent on others. In these experiments, the occurrence of the reproductive cost was influenced by a variety of factors, including the size or species of predator, the cues upon which predation is based, the background against which the *Daphnia* are displayed, the distribution of *Daphnia* in the water column, the relative availability of different prey types, and the size of the female. Such factors are well known to affect predation patterns in nature. Thus, cost in *Daphnia* appears not to be a constant effect of reproduction on the animal, but rather an effect whose occurrence and strength varies with the conditions under which it is being measured. In some cases, such as when *Daphnia* are preyed on by small fish against a light background, reproductive cost may be substantial, the risk of gravid females being increased by 7–19% compared to equal-sized barren females. In many other cases it is apparently zero.

This conclusion points to the inadequacy of simple optimality models which have assumed that reproductive cost is an invariant consequence of reproduction (Gadgil and Bossert 1970; Schaffer 1974a; Bell 1980) and emphasizes the need to incorporate cost functions that can vary in space and time. Having obtained a theory of the life history in terms of the cost function, we now require a theory of the cost function, which will predict the existence, magnitude and shape of reproductive cost curves under specified conditions.

Our second conclusion is that the effect of size on life history parameters may be stronger than the antagonistic effects among life history parameters, and thus be a much more important determinant of the life history than has been assumed by simple optimality models at the intraspecific level (see also Bell 1983). In *Daphnia* and many other animals, fecundity is directly related to body size, and survival under predation is strongly affected by body size. It may be that life history parameters interact though their effects on body size rather than directly. In this study, a significant negative effect of increased fecundity on survival was obtained in only one out of three experiments, indicating that cost is not an all-or-none effect due to the presence or absence of eggs in the brood pouch, but a continuous effect which increases with increasing fecundity. However, the fact that cost was not measurable in the other two experiments points to the subtlety of the effect and the ease with which it can be overridden by other effects such as that of body size. Furthermore, in these experiments, only about a half to a third of the range of adult body size was enough to induce significant effects in two of the three experiments, while the whole range of fecundities was used to obtain an effect similar in magnitude in the remaining experiment.

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