WILEY

Nordic Society Oikos

The hidden cost of reproduction: reduced food intake caused by spatial constraints in the body cavity

Stephen C. Weeks, Dept of Biology, Univ. of Akron, Akron, OH 44325-3908, USA

Investment in reproduction is thought to draw energetic resources away from other investments, namely growth and energy storage. Those interested in documenting this negative relationship often assume that a fixed pool of resources in excess of those needed for maintenance are divided up among growth, storage and reproduction, and thus increased investment in one of these "surplus" compartments reduces the potential for investment in the other two compartments. However, in a series of experiments I document a negative relationship between investment in reproduction and the amount of food consumed caused by a volumetric constraint of the body cavity. This negative relationship indicates that reproductive investment actually reduces the total pool of resources available to reproductive individuals. Considering this volumetric constraint may prove important to those interested in documenting trade-offs in life history traits, in that negative correlations between reproduction and growth or fat storage can be the indirect result of reduced overall energy budgets rather than the direct result of partitioning a fixed energy pool.

In many life-history theories, assimilated energy is assumed to be partitioned into three compartments: maintenance, growth, and reproduction (Law 1979, Michod 1979, Sibley and Calow 1986, van Noordwijk and de Jong 1986). A few models also add a fourth compartment: energy storage (Schaffer et al. 1982, Kozlowski 1991). Energy devoted to basic metabolic functions (the maintenance component) is often assumed to be diverted from the energy budget first, and the remaining energy, or "surplus power" (Ware 1980), is then partitioned into growth, reproduction, and storage. Since metabolic energy is often assumed to be independent of the other three components, diversion to one of the remaining components requires a reduced investment (often termed a "trade-off") in one or both of the other two components (Sibley and Calow 1986). The most well-studied of these trade-offs is that due to the investment in reproduction and the energetic costs that reproduction exacts from growth and storage. This diversion of energy can reduce future survival or reproduction, and thus investment in current reproduction can be negatively correlated with future fitness. Such a trade-off is termed the "cost of reproduction", and has been measured in a number of taxa (Bell and Koufopanou 1985, Reznick 1985, Roff 1992, Stearns 1992).

A common assumption of these life-history models is that the resource stream is independent of the reproductive condition of the individual. For instance, allocation to reproduction is assumed to not affect resource acquisition. This assumption is important to formulation of the predicted trade-offs between the three surplus compartments, since any modification of energy intake or maintenance metabolism could cause either a positive or negative correlation between compartments that is independent of the predicted trade-offs of allocation proposed in the models (Tuomi et al. 1983, Roff 1992). In a number of semelparous (e.g., salmon or octopus) and several iteroparous organisms (e.g., mouth brooding fishes and pythons), this assumption is clearly violated, since the onset of reproduction is concomitant with a cessation of food intake. Thus, in these species we see arrested growth and dramatic reductions of lipid reserves due, in part, to a lack of energy input (Madsen and Shine 1993).

However, in many iteroparous organisms it is often assumed that observed negative correlations between energy compartments represent trade-offs in investment (for review see Bell and Koufopanou 1985, Reznick 1985). It is the purpose of this communication to show that the size of the body cavity in an iteroparous species, the eastern mosquitofish (*Gambusia holbrooki* Girard), physically constrains the maximum volume of food that can be ingested by gravid females, such that investment in reproduction and food ingested are negatively correlated. This type of physical constraint might be common among animals, and could be considered an additional, "indirect" cost of reproduction. Thus, negative correlations between reproduction and growth or storage, which have been interpreted as indicative of

a direct cost of reproduction, may actually be due, wholly or in part, to the indirect effect of reproduction reducing the total amount of food that can be ingested. The implications of this relationship to empirical and theoretical life-history studies are discussed.

Materials and methods

The eastern mosquitofish (*Gambusia holbrooki*, hereafter "mosquitofish") is a common fish species of lakes, ponds, marshes, and riverine backwaters of the Atlantic and Gulf coasts of the United States. Mosquitofish grow to 2–5 cm standard length and probably live one year or less in most habitats. Mosquitofish are internally fertilized and bear live young, producing several broods per year, each up to several dozen offspring. *Gambusia* are lecithotrophic (Reznick 1981, Meffe 1986), meaning that all the energy (in the form of yolk) for the developing embryos is deposited before fertilization. Therefore, though the embryos enlarge throughout their 21–30 d developmental period (which is externally obvious by the dramatic swelling of the female's body cavity), they lose 30–40% of their initial dry mass (Reznick 1981, Meffe 1986).

Three separate analyses were conducted to test for a negative correlation between reproductive investment and food intake: two laboratory experiments and a field comparison. In the first laboratory experiment, 65 gravid mosquitofish, collected from Risher Pond on the Savannah River Site in South Carolina (see Meffe [1991] for site descriptions), were used to test for a negative correlation between mass of reproductive tissues and mass of the intestinal tract. All females were fed as much frozen brine shrimp as they would eat in three feeding periods, separated by 30 min each. At the end of the third feeding, the females were anesthetized in MS-222 and preserved in 5% buffered formalin. Anesthetized fish did not regurgitate during formalin preservation. These fish were later dissected to extract their ovaries and intestines. Because unfertilized eggs and the early embryonic stages of lecithotrophic fish are more dense than the later-stage embryos (Reznick 1981, Meffe 1986, Reznick and Miles 1989), dry mass is an unreliable measure of volume for the reproductive tissues. Therefore, wet masses of both tissues were quantified to test for the predicted trade-off.

A field comparison was conducted in a similar fashion to the first laboratory study to determine whether trade-offs existed in naturally feeding mosquitofish. A total of 171 gravid females was collected from Risher Pond. Anesthesia and preservation were conducted in the field as outlined above. The other measures were the same as those in the laboratory study above.

The final laboratory experiment compared the total number of frozen brine shrimp that gravid females in different stages of gestation could ingest in three feeding periods. Gravid females from each of two ponds (Risher Pond and Pond C, see Meffe 1991) were collected in the field and placed into separate 18.5-l aquaria. The fish were acclimated to the laboratory feeding protocol by feeding them frozen brine shrimp three times a day (separated by 30 min each) for at least one week before measurements were recorded. In the first comparison, the numbers of brine shrimp consumed were recorded at 3, 12, and 24 d after the beginning of a gestational cycle in 14 females from Risher Pond, to observe the maximal feeding rate in these females during the ~30 d of the developmental period of their offspring. In the second comparison, 10 Pond C females were examined 3, 12, 24, and 34 d after the beginning of a gestational cycle, to observe if the feeding rates were consistent with Risher Pond females, and to determine if the females would once again consume at a higher rate at the beginning of the following gestational cycle (day 34). The last measure was three days after each female gave birth to a second brood of offspring, and thus is a second measure of maximal food intake at the beginning of a gestational cycle. This period was, on average, 34 d (range 32–38). Only intact brine shrimp of approximately equivalent sizes were used in these comparisons.

In the first laboratory experiment and the field comparison, the predicted trade-off between reproductive volume and intestine volume was calculated using a multiple regression of standard length and ovarian wet mass on wet mass of the intestine. This method accounts for differences in size among the females in the calculation of the correlation of reproduction and ingested food. To normalize data distributions, the natural log of wet mass of the intestine was used as the dependent variable.

In the second laboratory experiment, the number of brine shrimp consumed in the feeding trials was summed for each day, and this sum was used as the dependent variable in a univariate repeated measures analysis of variance using females as blocks (Snedecor and Cochran 1980, Hicks 1982). This univariate repeated measures test is more powerful than the commonly used multivariate test (Snedecor and Cochran 1980), but assumes that "every pair of subplot times has the same correlation, p" (Box 1950), which can be tested using a "sphericity test" (Anderson 1958, SAS 1985). Both data sets met the assumption of sphericity, indicating that the univariate test was warranted and did not require adjustments to the degrees of freedom (Anderson 1958, SAS 1985). The independent variable was days since last clutch. The comparisons were separated by field collection site because of the difference in the days compared (see above). Four of the ten females from Pond C were not measured 34 d after their first clutch. However, there was no difference in average size between those measured and those not measured at this

346

point. A posteriori multiple comparisons were made using the Ryan-Einot-Gabriel-Welsch multiple range test (SAS 1985, Day and Quinn 1989).

Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed for the two laboratory studies.

Results and discussion

There was a clear negative correlation between ovary mass and mass of the intestine in mosquitofish from Risher Pond, after removing the effects of body size (Table 1). This negative correlation was more dramatic in the laboratory (Table 1A), where the fish were fed the maximum they would eat in a 1.5-h time span. It could be argued that mosquitofish normally do not eat at a maximal rate, and therefore that the laboratory estimates did not well reflect natural feeding rates experienced in the field. Therefore, a field collection was made from Risher Pond to observe the relationship in naturally-fed females. The negative relationship was not as strong in the field (Table 1B), but it was still significant, suggesting that gravid females ingest food at a sufficiently high rate to cause a constraint when the females are in the later stages of pregnancy.

There are a number of reasons the comparisons of ovary mass to mass of the intestine is not expected to be a completely accurate reflection of the volumetric constraint proposed. First, measurements of wet mass are inaccurate due to evaporative losses during measurement. Second, since the dry mass of the ovaries are actually declining while the embryos are growing volumetrically, there is a tendency to underestimate the true volumetric differences using a mass measure. Finally, in the field measures, there is no way to control for differences in density of food consumed, which would tend to add variance to the estimates of differences in intestine volume. Nevertheless, the use of wet mass as a surrogate of volume in the current study was sensitive enough to detect a negative correlation between ovary mass and mass of the intestine, suggesting that females with larger estimated volumes of developing embryos in their body cavities were physically constrained to con-

Table 1. Multiple regression of standard length and wet mass of ovaries (g) on ln [wet mass of intestine (g)]. A) Relationship calculated from fish fed in the laboratory (N = 65). B) Relationship calculated from field-collected fish (N = 171).

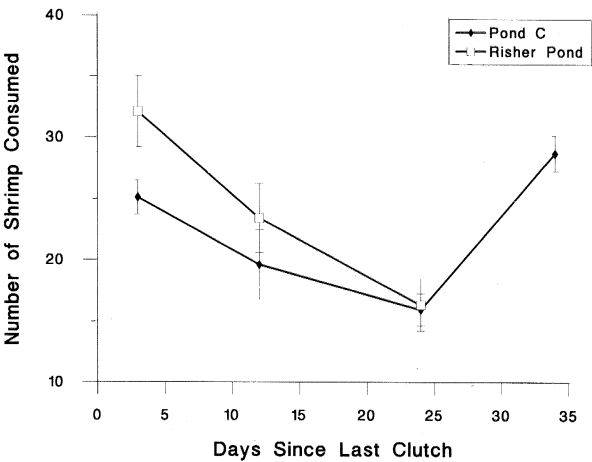| Variable	Slope	Standard error	P-value
A) Risher Pond (lab)			
Standard length	0.112	0.017	0.0001
Ovary mass	−1.860	0.648	0.0056
B) Risher Pond (field)			
Standard length	0.082	0.006	0.0001
Ovary mass	−1.157	0.481	0.0172

Fig. 1. Changes in the number of brine shrimp ingested with days since last clutch. Error bars portray one standard error of the mean.

sume less food than females with lesser volumes devoted to reproduction.

A more precise estimate of a feeding constraint on gravid females was used in the second laboratory experiment. In this case, the actual number of prey items consumed in a 1.5-h time span was compared using individual females measured at different stages during the development of their offspring. In the Risher Pond females, there was a significant decline ($F_{2,25} = 12.71$; $P < 0.001$) in the number of prey consumed during the developmental period (usually 27–35 d) of a single clutch (Fig. 1). Comparing mean prey consumed across days revealed that all three days were significantly different at the $P < 0.05$ level. Clearly, as the embryos developed and grew in volume, the total number of prey consumed decreased in these females.

Since the number of prey consumed was not independent of an individual's age, one could argue that the decline of prey ingested was merely a reflection of some process correlated with aging, or with time spent in the laboratory. Therefore, a second test was initiated with females from Pond C. The design was the same as in the Risher Pond test, except that one further measure of prey ingested was taken three days after a second laboratory brood was born (Fig. 1). Differences among days was highly significant ($F_{3,23} = 12.25$; $P < 0.0001$), with the pattern during the first 24 d mirroring that found in the Risher Pond females. However, the last measure clearly reflects a rebound in prey ingested after giving birth to the second brood. This decline followed by a rebound in prey consumption is clearly consistent with the notion that the reduced prey consumption in gravid females was caused by the increase in the volume of the reproductive tissues, which reduced maximum intestinal volume, rather than being a reflection of aging or time spent in the laboratory. Note that the lower number of brine shrimp consumed in the Pond C trials was due to

the smaller size of the Pond C females, which were an average of 5 mm smaller in standard length than the Risher Pond females.

Taken together, these three studies make a strong case for a physical constraint on food intake during investment in reproduction. The body cavity in *Gambusia* females visibly swells during reproduction, but this extension appears to be insufficient to allow the same level of feeding as when the females are not reproductive. The current study has not linked actual energetic intake differences among gravid and non-gravid females in the field. Gravid females might change feeding strategies (e.g., consume higher-energy foods) or become more efficient at digesting a smaller volume of food. However, assuming gravid females do not change feeding strategies or increase their efficiency of absorption, females investing in reproduction must either rely on energy stores, decrease or eliminate growth, or forage for longer periods to compensate for the reduced food ingested during reproduction. Relying on energy stores or reducing growth because of reduced food intake could partially explain the observed negative correlations between current and future reproduction that have been reported in a number of studies (reviewed in Bell and Koufopanou 1985, Roff 1992, Stearns 1992). Increased foraging time could lead to an increased likelihood of predation, especially if reproductive females are also less effective at avoiding predation (Shine 1980).

The observation that the physical allocation of body mass to reproduction alters life-history characters and behavior is not novel. Allocation to reproduction may have a negative effect on energy acquisition in reptiles, not because of a spatial constraint in the body cavity, as suggested herein, but because reduced locomotory ability of gravid females may reduce predatory efficiency (Shine 1980). Also, allocation to reproduction has been associated with increased risk of predation, both due to reduced locomotory ability (Shine 1980, 1988) and thermoregulatory behavior (Shine 1980) in reptiles, and in zooplankton due to reduced locomotory ability and increased visibility to predators (Mellors 1975, Hairston et al. 1983, Winfield and Townsend 1983, Koufopanou and Bell 1984). A physical constraint of the body cavity on reproductive allocation has also been suggested for hermit crabs occupying differently-sized mollusc shells (Bertness 1981a, b), for salamanders (Kaplan and Salthe, 1979) and for lizards (Vitt and Congdon, 1978). Finally in vertebrates, the upper limit of offspring size has been shown to be functionally constrained by the size of the pelvic girdle (Luetenegger 1979, Congdon and Gibbons 1987, Sinervo and Licht 1991).

These previous studies notwithstanding, the current results are the first to reveal a negative correlation between reproductive volume and maximal food intake, presumably caused by a spatial constraint imposed by the maximum volume of the body cavity in gravid females. Since these observations are correlational in nature, the direct cause of the reduced food intake cannot be proven in this study. It is possible that behavioral modifications in gravid females (such as a shift in foraging strategies) may cause the reduction in feeding rate (Madsen and Shine, 1993). However, observations from the current study are not consistent with this behavioral-shift hypothesis. The mosquitofish showed no signs of refusing food at any point in the feeding trials until they could not physically consume another brine shrimp. In fact, several females with half-consumed brine shrimp visible in their throats were observed near the end of each feeding trial for all 4 time treatments. These observations clearly suggest that physical limitation of the stomach required the fish to end consumption rather than reduced foraging activity. Therefore, although space limitation in the body cavity is the likely cause of the observed negative correlation of food and reproductive volume, future studies of behavioral changes in gravid mosquitofish should be conducted to estimate the contribution of changes in foraging strategies to reduced food acquisition.

A negative correlation between reproduction and food acquisition has ramifications to both empiricists and theoreticians interested in life-history evolution. First, trade-offs measured between reproduction and growth or storage may not be solely due to allocation decisions between these three energy compartments, but might rather be due, in part, to the indirect effect of a reduced overall energy budget in reproducing animals (Tuomi et al. 1983). Thus, empiricists interested in comparing breeding and non-breeding animals must pay close attention to the overall amount of food actually ingested in their experimental organisms. Also, measures of the costs of reproduction might be different among different levels of feeding, or by feeding in different ways. For example, a trade-off between growth and reproduction might be most noticeable at higher food levels because of a greater constraint on feeding when maximal feeding rate is possible. However, this effect might be tempered by the commonly reported positive association of growth and reproduction when food is plentiful (Bell and Koufopanou 1985, Reznick 1985). The second example relates to the mode of feeding in different studies. A feeding regime where food is supplemented in one large batch and where the uneaten portion dissipates over time, might be more prone to causing a volumetric constraint on feeding relative to the same amount of food being supplied in smaller amounts over a longer period of time.

A negative correlation between food acquisition and investment in reproduction may also be important to life-history models of energy allocation strategies. Many life-history models assume that a given amount of surplus energy, E, is allocated to growth, reproduction, and storage, and these models have no recursive

348

elements that allow modification of E with different investment strategies (Law 1979, Michod 1979, Schaffer et al. 1982, Sibley and Calow 1986, van Noordwijk and de Jong 1986, Kozlowski 1991). It is unclear how a negative relationship between allocation to reproduction and food acquisition would affect the predictions of these models, but it is possible that optimal allocation decisions might be significantly altered by the incorporation of this negative relationship in these models.

The current results suggest that greater care is needed in tests for trade-offs between reproduction and other life-history measures. Both empiricists and theoreticians should consider the possibilities of the effect of reproduction on food aquisition in their systems or models so that indirect effects are not mistaken for direct effects.

References

Anderson, T. W. 1958. An introduction to multivariate statistical analysis. – Wiley, New York.

Bell, G. and Koufopanou, V. 1985. The cost of reproduction. – Oxford Surv. Evol. Biol. 3: 83–131.

Bertness, M. D. 1981a. The influence of shell-type on hermit crab growth rate and clutch size (Decopoda: Anomura). – Crustaceana 40: 197–205.

– 1981b. Conflicting advantages in resource utilization: the hermit crab housing dilemma. – Am. Nat. 118: 432–437.

Box, G. E. P. 1950. Problems in the analysis of growth and wear curves. – Biometrics 6: 362–389.

Congdon, J. D. and Gibbons, J. W. 1987. Morphological constraint on egg size – a challenge to optimal egg size theory. – Proc. Natl. Acad. Sci. USA 84: 4145–4147.

Day, R. W. and Quinn, G. P. 1989. Comparisons of treatments after an analysis of variance in ecology. – Ecol. Monogr. 59: 433–463.

Hairston, N. G., Walton, W. E. and Li, K. T. 1983. The causes and consequences of sex-specific mortality in a freshwater copepod. – Limnol. Oceanogr. 28: 935–947.

Hicks, C. R. 1982. Fundamental concepts in the design of experiments. – Holt, Rinehart and Winston, New York.

Kaplan, R. H. and Salthe, S. N. 1979. The allometry of reproduction: an empirical view in salamanders. – Am. Nat. 113: 671–689.

Koufopanou, V. and Bell, G. 1984. Measuring cost of reproduction. IV. Predation experiments with *Daphnia pulex*. – Oecologia 64: 81–86.

Kozlowski, J. 1991. Optimal energy allocation models – an alternative to the concepts of reproductive effort and cost of reproduction. – Acta Oecol. 12: 11–33.

Law, R. 1979. Optimal life histories under age-specific predation. – Am. Nat. 114: 399–417.

Luetenegger, W. 1979. Evolution of litter size in primates. – Am. Nat. 114: 525–531.

Meffe, G. K. 1986. Cannibalism, food availability, and reproduction in mosquitofish: a critique. – Am. Nat. 127: 897–901.

– 1991. Life history changes in eastern mosquitofish (*Gambusia holbrooki*) induced by thermal elevation. – Can. J. Fish. Aquat. Sci. 48: 60–66.

Mellors, W. K. 1975. Selective predation on ephippial *Daphnia* and the resistance of ephippial eggs to digestion. – Ecology 56: 974–980.

Michod, R. E. 1979. Evolution of life histories in response to age-specific mortality factors. – Am. Nat. 113: 531–550.

van Noordwijk, A.J. and de Jong, G, 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. – Am. Nat. 128: 137–142.

Reznick, D. N. 1981. "Grandfather effects": the genetics of interpopulation differences in offspring size in the mosquitofish. – Evolution 35: 941–953.

– 1985. Costs of reproduction: an evaluation of the empirical evidence. – Oikos 44: 257–267.

– and Miles, D. B. 1989. Review of life history patterns in Poeciliid fishes. – In: Meffe, G. K. and Snelson, F. F. (eds), Ecology and evolution of livebearing fishes (Poeciliidae). Prentice-Hall, Englewood Cliffs, NJ, pp. 125–148.

Roff, D. A. 1992. The evolution of life histories. Theory and analysis. – Chapman and Hall, New York.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5 ed. – Cary, NC.

Schaffer, W. M., Inouye, R. S. and Whittam, T. S. 1982. Energy allocation by an annual plant when the effects of seasonality on growth and reproduction are decoupled. – Am. Nat. 120: 787–815.

Shine, R. 1980. "Costs" of reproduction in reptiles. – Oecologia 46: 92–100.

– 1988. Constraints on reproductive investment: a comparison between aquatic and terrestrial snakes. – Evolution 42: 17–27.

Sibley, R. M. and Calow, P. 1986. Physiological ecology of animals. – Blackwell, Oxford.

Sinervo, B. and Licht, P. 1991. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. – Science 252: 1300–1302.

Snedecor, G. W. and Cochran, W. G. 1980. Statistical methods. – Iowa State Univ. Press, Ames, IA.

Stearns, S. C. 1992. The evolution of life histories. – Oxford Univ. Press, Oxford.

Tuomi, J., Hakala, T. and Haukioja, E. 1983. Alternative concepts of reproductive effort, costs of reproduction, and selection in life-history evolution. – Am. Zool. 23: 25–34.

Vitt, L. J. and Congdon, J. D. 1978. Body shape, reproductive effort, and relative clutch mass in lizards: Resolution of a paradox. – Am. Nat. 112: 595–608.

Ware, D. M. 1980. Bioenergetics of stock and recruitment. – Can. J. Fish. Aquat. Sci. 37: 1012–1024.

Winfield, I. J.and Townsend, C. R. 1983. The cost of copepod reproduction: increased susceptibility to fish predation. – Oecologia 60: 406–411.