Reference: *Biol. Bull.* **212:** 143–150. (April 2007)

How Does Metabolic Rate Scale With Egg Size? An Experimental Test With Sea Urchin Embryos

AMY L. MORAN[1,*] AND JONATHAN D. ALLEN[2]

[1]*Department of Biological Sciences, 132 Long Hall, Clemson University, Clemson, South Carolina 29631; and* [2]*Department of Biology, 6500 College Station, Bowdoin College, Brunswick, Maine 04011*

Abstract. The consequences of changes in egg size for the development of marine invertebrates have been the subject of much theoretical and experimental work. Models that explore larval developmental modes in the context of maternal investment per offspring are often couched in an energetic framework, but the relationships between egg size and the energetics of larval development are poorly understood. We used blastomere separations to examine how experimental reductions in egg size affected (1) larval metabolic rate and (2) larval resistance to starvation. We found that separating blastomeres at the 2- and 4-cell stage resulted in average reductions of 50% and 75%, respectively, in larval metabolic rate. This suggests that, in an experimental context, mass-specific metabolic rate does not change with egg size. We also found that a 50% reduction in egg volume did not reduce the resistance of larvae to starvation when particulate food was withheld. This suggests that the material supplied to larvae in the egg is used primarily for construction of the larval body, rather than as a buffer against starvation or as a means of reducing reliance on exogenous fuel to sustain maintenance metabolism.

Introduction

Egg size has traditionally been viewed as a critical factor in the evolution of complex life histories of marine invertebrates. Egg size is linked to fundamental traits such as developmental mode and length of larval development (Thorson, 1950; Vance, 1973a, b; Strathmann, 1985; Hadfield and Miller, 1987; Sinervo and McEdward, 1988; Wray and Raff, 1991), larval form (McEdward, 1986; Strathmann, 2000), size at metamorphosis (Strathmann, 1985; Emlet *et al.,* 1987), juvenile growth and survival (Marshall *et al.,* 2003), resistance to starvation (Anger, 1995; Bridges and Heppell, 1996), and fertilization success (Levitan, 2000, 2006). Because of the importance of this life-history character, many models have been created to identify the selection processes that influence its evolution.

Two of the more influential theories addressing egg-size evolution are the point of no return concept (PNR) (Hjort, 1914), and the fecundity-time model (Vance, 1973a). The first of these, the PNR concept, postulated the presence of a critical period in early larval development that is determined by depletion of energy reserves in the egg. Larvae that do not feed by the end of this period cannot subsequently recover even if food becomes available, and the length of the critical period is set largely by egg energy; thus, larger eggs enhance starvation resistance (Hjort, 1914; Blaxter and Hempel, 1963). Numerous studies support the presence of a critical period in the early development of fish (Blaxter and Hempel, 1963; May, 1973), crustaceans (Anger and Dawirs, 1981; Anger, 1995), and molluscs (His and Seaman, 1992; Laing, 1995; but see Moran and Manahan, 2004). The presence of a critical period likely affects year-to-year variability in recruitment in many taxa due to the timing of spawning relative to resource availability; organisms with a short critical period must tie their spawning closely to phytoplankton blooms to ensure success (Cushing, 1990; Starr *et al.,* 1990).

A second highly influential model is the fecundity-time hypothesis of Vance (1973a), which postulated a trade-off between the fecundity benefit of producing numerous, small eggs and the cost of increased time to metamorphosis for species developing from small eggs. In the Vance model and the many more recent modeling studies it has inspired

Received 8 October 2006; accepted 17 January 2007.

* To whom correspondence should be addressed. E-mail: moran@clemson.edu

Abbreviations: MSMR, mass-specific metabolic rate; PNR, point of no return concept.

(*e.g.,* Podolsky and Strathmann, 1996; McEdward, 1997; Levitan, 2000; Miner *et al.,* 2005; and others reviewed in Strathmann, 1985; Havenhand, 1993), a larger egg is energetically more costly for the parent to produce, but shortens the duration of planktonic development by reducing the larva's dependence on exogenous food.

Both the PNR concept and the fecundity-time model are framed in part as energetics arguments. One energetics assumption made by both models is that large eggs contain more energy than small eggs; this assumption is largely borne out across species (*e.g.,* Emlet *et al.,* 1987; Jaeckle, 1995), although not always within species (McEdward and Carson, 1987; McEdward and Coulter, 1987). A second assumption, which is sometimes explicit (*e.g.,* Anger and Dawirs, 1981; Anger, 1995) but more often implicit, is that the mass-specific metabolic rates (MSMR) of embryos and larvae scale in a particular way with egg size. The PNR concept and Vance's (1973b) discussion of optimal planktotrophic egg size under food-limited conditions each implicitly assume that MSMR decreases with increasing egg size. If this were not the case, under the PNR concept an evolutionary increase in egg size would not enhance starvation resistance: tissues would use energy at the same rate regardless of larval size, and all else being equal, large and small larvae would reach the point of no return at the same time. An extensive body of literature shows a broad pattern across many taxa in which MSMR does in fact decrease with increasing size; as a general pattern, log MSMR scales with the log of mass to the power of 0.75 (the so-called 3/4 power scaling law; Schmidt-Nielsen, 1984; West *et al.,* 1997). However, there is little agreement about the physiological or physical constraints underlying this relationship, and very little is known about the scaling of egg size and larval metabolic rate in marine invertebrates.

In Vance's fecundity-time model (1973a, b) and in recent models it has inspired (*e.g.,* McEdward, 1997; McEdward and Miner, 2006; Miner *et al.,* 2005), the increased amount of maternal investment in larger eggs is generally considered to function as energy that fuels larval development; large eggs contain a greater supply of endogenous energy, which larvae use as fuel to reduce dependence on exogenous sources. Although egg energy is an important parameter in these models, it is clearly relevant to consider not only the raw amount of energy available in the egg, but also the *rate* at which that energy is consumed by the larva (*e.g.,* Marsh and Manahan, 1999). In particular, to better understand how egg size is related to larval duration and development rate, the relationship between MSMR and size must be explored. For example, if MSMR of embryos and larvae decreases with increasing egg size, then under the fecundity-time model larvae will receive greater-than-proportional energetic returns on maternal investment as egg size increases. Not only will starvation resistance increase with egg size, but if maternal investment in larger eggs functions as fuel for development, then a reduction in the MSMR of larger eggs would allow larvae to develop proportionally farther along their trajectory without exogenous food sources. Thus, for both the PNR concept and the fecundity-time model, an understanding of the relationship between egg size and embryonic and larval MSMR is fundamental to predicting the potential response of egg size to selection and the strength and direction of selection for egg size under different environmental regimes.

In this study, we used the technique of blastomere separation to experimentally reduce the egg size of two species of echinoid echinoderms; subsequently, we measured both the starvation resistance of larvae produced from full- and half-sized eggs and the metabolic rates of larvae produced from full-, half-, and quarter-sized eggs. Blastomere separation is a simple embryological manipulation that provides a powerful means of testing the causal relationships between parental investment per offspring and the fitness of those offspring (Bernardo, 1991; McEdward, 1996). The regulative nature of echinoderm development allows experimental manipulations of embryos during early cleavage stages to produce sibling offspring with variable levels of parental investment (Driesch, 1892; Horstadius, 1973) that can complete development to metamorphosis and survive to sexual maturity (Cameron *et al.,* 1996). Previous studies on echinoid echinoderms have focused on the consequences of changes in egg size for larval size and shape, time to metamorphosis, and size at metamorphosis (Sinervo and McEdward, 1988; Hart, 1995; Allen *et al.,* 2006). However, no studies have used this technique to examine a potential mechanism underlying these changes in larval size and growth rates, namely, the relationship between egg size and metabolic rate.

In our study, we used blastomere separation to address two questions. First, how does a change in egg size affect larval metabolic rate? Second, do embryos from smaller eggs exhaust endogenous reserves and starve sooner than embryos from larger eggs? These experiments test the hypothesis that larger eggs provide an energetic buffer against starvation, and address the question of how larval and embryonic metabolic rate scales with egg size.

Materials and Methods

Spawning

Adult individuals of *Mellita quinquiesperforata* (Leske, 1778) and *Arbacia punctulata* (Lamarck, 1816) were obtained from sand bars and floating docks near Beaufort, North Carolina, in June 2003 and maintained in a recirculating seawater system at 22 °C with a practical salinity (*S*) of 35. Spawning was induced through intracoelomic injection of 0.53 mol l^{-1} KCl (Strathmann, 1987). Eggs were collected by inverting spawning females over glass beakers of artificial seawater (ASW; Instant Ocean, Aquarium Sys-

tems Inc., Mentor, Ohio). Concentrated sperm were collected from spawning males by mouth pipette and were stored on ice until use. Eggs were washed once in filtered seawater prior to the addition of a dilute suspension of sperm. Eggs and sperm were gently stirred until >90% of eggs were fertilized. Fertilization percentage was estimated by examining 50 eggs under a compound microscope. Eggs were scored as fertilized if the fertilization envelope (FE) was obvious surrounding the egg.

Blastomere separations

Arbacia punctulata. Blastomeres were isolated at the 2-cell stage according to the method of Harvey (1940). Within 5 min of fertilization, eggs were shaken vigorously for 1 min to remove the FE and then allowed to settle. Embryos developed undisturbed in ASW at 21 °C for about 1 h until they had completed first cleavage. Embryos were then placed in hypertonic ($S = 60$) ASW for 10 min to separate the two blastomeres. Isolated blastomeres (half-size embryos) and unseparated embryos (whole-size embryos whose envelopes had not been removed but that had been shaken and were treated with hypertonic seawater) were then transferred to ASW ($S = 32$) and placed in an environmental chamber at 21 °C where they were allowed to continue normal development.

Mellita quinquiesperforata. Zygotes were poured once through a 100-μm Nitex mesh 5 min post-fertilization to remove the FE. About 50% of FEs were removed by this treatment. Zygotes were then bathed in a solution of calcium-free seawater (CaFSW; recipe in Strathmann, 1987) for 30 min at 21 °C, with regular changes of the CaFSW every 10 min. When the embryos reached the first cell division, they were once again poured through a 100-μm Nitex mesh to isolate the two blastomeres. Isolated blastomeres (half-size embryos) and embryos that retained the fertilization envelope (whole-size embryos) were then returned to normal ASW and continued to develop at 21 °C. To generate quarter-size embryos, zygotes whose FEs had been removed were allowed to develop through the first cell division in normal ASW and were then bathed in CaFSW as above. At the time of the second cell division, the embryos were poured through a 100-μm Nitex mesh to isolate the four blastomeres and were then returned to ASW for the remainder of development.

Starvation experiments

Because all spawns contained some abnormally developing larvae, we excluded abnormal larvae from starvation experiments by allowing both whole- and half-sized embryos to develop at concentrations of 10 larvae ml^{-1} for 2 days at 21 °C before we selected embryos for the starvation experiment. After the 2-day period, larvae were examined live under a dissecting microscope (Olympus SZX9). Larvae that were normal in appearance (*i.e.,* with symmetrical arm development, actively beating cilia, directional movement, and fully formed guts) were sorted from abnormal larvae (asymmetrical, lacking arms or gut, swimming in a nondirectional manner, or otherwise imperfect). Single larvae from the normal pool were then haphazardly chosen and pipetted into individual wells of 12-well cell culture plates (Falcon no. 353043), for a total of 60 whole-sized and 60 half-sized larvae. To eliminate possible plate effects or location-within-plate effects, whole- and half-sized larvae were placed in alternating wells in each plate. Each well contained 5 ml of 0.22-μm filtered ASW made up to $S = 32$. Larvae were transferred to fresh filtered ASW in new, cleaned wells every other day.

Respiration

Respiration rates of larvae were measured using the method of Marsh and Manahan (1999), a commonly used procedure that is relatively simple, highly repeatable, and produces results that are equivalent to those of other methods for measuring larval oxygen uptake. Larvae were suspended in freshly filtered ASW (0.22 μm) in small respiration chambers of known volume (~500–700 μl). Different numbers of individuals, ranging from 3 to 150, were added to each chamber to test for effects of larval density on respiration (we saw no such effects in our experiments; Fig. 1). Seven replicate chambers were used for each measurement of the respiration rate of whole, halved, or quartered larvae. Larvae were incubated in the respiration chambers at 22 °C for 4–6 h. After this interval, 250-μl subsamples

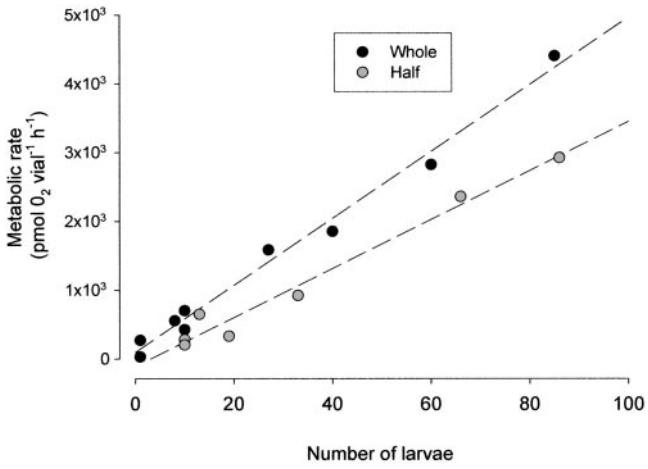

Figure 1. Oxygen utilization of different numbers of whole- and half-sized larvae of *Mellita quinquiesperforata.* Data are also shown in Table 1, Experiment M2. The per-larva metabolic rate is calculated as the slope of the line of per-vial oxygen utilization rate plotted against number of larvae per vial. Linear regression equations are $y = 48.7x + 95$, $r^2 = 0.99$ (whole larvae); and $y = 35.6x - 111.1$, $r^2 = 0.97$ (half-sized larvae).

from each chamber were taken with a temperature-equilibrated gas-tight syringe. Oxygen tension was measured in each sample with a Strathkelvin polarographic oxygen sensor (model 1302). Larvae in each chamber were then counted, and oxygen consumption per animal was calculated as the slope of the regression line of oxygen consumed per hour against number of larvae in each chamber. The standard error of the slope around each regression line yielded the standard error of each estimate.

For *Mellita,* we measured the respiration of offspring from four spawns, each from a different pair of parents. For the first spawn (Spawn M1, 26 July 2003), respiration rates of whole- and half-sized larvae were measured 48 h after fertilization. For the second spawn (M2, 24 Aug. 2003), respiration was measured for 24-h-old whole- and half-sized larvae. For the third spawn (M3, 30 Aug.–1 Sept.), respiration rates were measured on whole-, half-, and quarter-sized larvae at 24 h and again at 48 h after fertilization. For the fourth spawn (M4, 15–17 Sept.), respiration rates were measured on whole- and half-sized larvae at 18, 24, and 36 h post-fertilization.

One respiration run was performed on whole- and half-sized larvae of *Arbacia* (Spawn A1) when larvae were 5 d old.

Results

Respiration

Respiration rates for all spawns are given in Table 1. Summarized over the whole table, the respiration rates of half-sized larvae ranged between 26% and 73% of the respiration rates of whole-sized larvae from the same date and spawn. On average, half-sized larvae had respiration rates equivalent to 50.6% ± 17.5% (SD) of the respiration rate of whole-sized larvae (averaged from all 8 paired comparisons between whole- and half-sized larvae from Table 1). Respiration rates of quarter-sized larvae were measured on only two occasions and were 35% and 14% of the rate of whole-sized larvae from the same respiration runs (average = 24.5%). Respiration rates over time of *Mellita* larvae from experiments M3 and M4, including whole-, half-, and quarter-sized larvae, are shown in Figure 2.

Starvation experiments

All *Arbacia* larvae were dead by day 45 of the starvation experiment, and larval mortality was nearly constant for both groups throughout the 45-day period (Fig. 3). Mortality rates were very similar between the whole- and half-sized larvae: 2.32 ± 0.08 percent day^{-1} for whole-sized larvae and 2.21 ± 0.14 percent day^{-1} for half-sized larvae. These percentages were equivalent to mortality rates of 1.39 larvae day^{-1} and 1.32 larvae day^{-1} for the two groups, respectively. Survivorship was slightly higher for half-sized than for whole-sized larvae, but this pattern was not statistically significant (Kaplan-Meier survival analysis comparing distributions of probability of death-at-time for each individual

Table 1

Summary of respiration experiments with whole, half-sized, and quarter-sized larvae of Mellita quinquiesperforata *and* Arbacia punctulata

Species	Experiment	Date	Treatment, age	Respiration rate ± SE (pmol O_2 larva^{-1} h^{-1})	Percent of whole
Mellita	M1	26 July	Whole, 48 h	90.1 ± 6.6	—
			Half, 48 h	41.3 ± 1.3	46%
	M2	24 Aug.	Whole, 24 h	48.7 ± 2.1	—
			Half, 24 h	35.6 ± 2.6	73%
	M3	30 Aug.	Whole, 24 h	90.3 ± 5.0	—
			Half, 24 h	62.0 ± 7.6	69%
			Quarter, 24 h	31.4 ± 14.0	35%
		1 Sept.	Whole, 48 h	58.8 ± 5.7	—
			Half, 48 h	23.2 ± 2.7	39%
			Quarter, 48 h	8.2 ± 0.9	14%
	M4	16 Sept. a	Whole, 18 h	30.7 ± 4.1	—
			Half, 18 h	7.9 ± 6.4	26%
		16 Sept. b	Whole, 24 h	30.9 ± 2.3	—
			Half, 24 h	15.2 ± 4.0	49%
		17 Sept.	Whole, 36 h	36.7 ± 4.2	—
			Half, 36 h	12.9 ± 1.7	35%
Arbacia	A1	27 Mar.	Whole, 5 d	10.8 ± 1.7	—
			Half, 5 d	7.3 ± 0.7	68%
Grand Mean			Whole		—
			Half		50.6%
			Quarter		24.5%

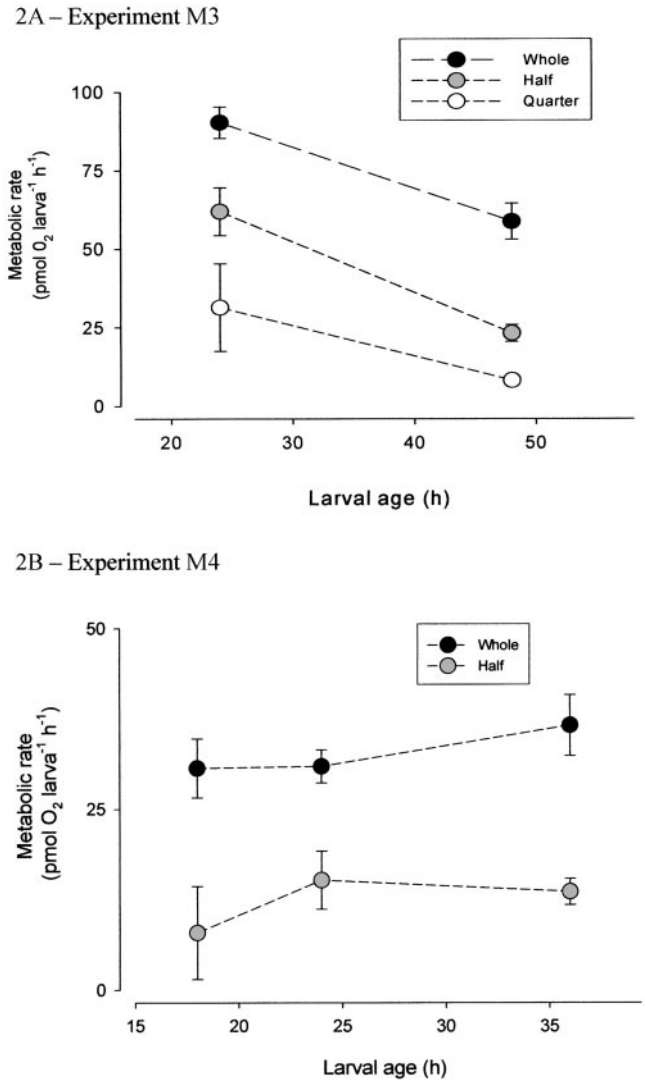

Figure 2. Metabolic rates of larvae of *Mellita quinquiesperforata* from whole, half-, and quarter-sized larvae (A) and from whole and half-sized larvae (B) from two separate spawns using different parents. Error bars are generated from the standard error of the slope as calculated in Fig. 1.

larva, grouped by half- and whole-sized larvae: Mantel chi-square statistic = 0.386, df = 1, $P = 0.535$ (Systat statistical package ver. 11, 2004)).

Discussion

We found no difference in mortality rates between whole- and half-sized larvae when we reduced the size of eggs by 50% through blastomere separation and raised larvae without food. Thus, egg size did not affect how long larvae could survive in the absence of particulate food sources, and large eggs did not provide a buffer against mortality in this system. This conclusion is not consistent with Hjort's (1914) concept that a point of no return in larval development is set by the amount of endogenous energy available in the egg to offset a poor larval feeding environment. Our results are, however, consistent with the many other studies on larvae of soft-bodied marine invertebrates (reviewed in Moran and Manahan, 2004) that suggest that these larvae are highly resistant to starvation due to their ability to use dissolved organic material (DOM) or other non-microalgal food sources to fuel maintenance metabolism.

All larvae in this study, from both whole and halved eggs, survived much longer than would have been expected if they relied on endogenous reserves alone. To determine the length of time over which endogenous reserves could theoretically sustain larvae of *Arbacia punctulata,* we calculated the hourly energy use of whole-sized larvae by converting per-larva oxygen utilization (Table 1) into energy using the oxyenthalpic equivalent of protein and lipid oxidation at 484 kJ mol^{-1} O_2. This yielded an energy utilization rate of 5.2 μJ h^{-1} larva^{-1}. We then obtained values for energetic content of eggs of *A. punctulata* from Bolton *et al.* (2000); these values ranged from a low of 257 to a high of 532 μJ egg^{-1} that could potentially be used to fuel metabolic requirements. By dividing the total amount of energy available in the egg by the hourly energy expenditure of larvae, we estimated that larvae of *A. punctulata* should completely exhaust their endogenous reserves somewhere between 49 and 103 h, or about 2 and 4 days, after fertilization (for larvae from eggs with the lowest and highest energetic contents, respectively).

Assuming that half-sized eggs had half the energetic content of whole eggs and that metabolic rates were also reduced by 50%, which was very close to the average reduction seen in our experiments, larvae from half-sized eggs would have the same predicted time to exhaustion of endogenous reserves (2–4 days after fertilization) as larvae

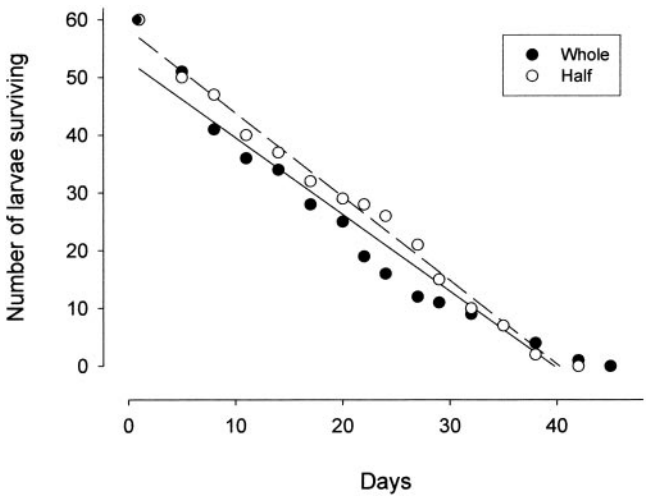

Figure 3. Survival of whole and half-sized larvae of *Arbacia punctulata* maintained in individual cell-culture wells without particulate food. Mortality rates are given in the text. Solid line = whole larvae; dotted line = half-sized larvae.

from nonreduced eggs. Using our measured hourly energy utilization rates for half-sized *A. punctulata* larvae (the species used in starvation experiments, Table 1), which were 68% of rates of whole-sized larvae, or 3.5 uJ h^{-1} $larva^{-1}$, the predicted time to exhaustion of endogenous reserves was shorter than for larvae from whole eggs, ranging from 36.7 to 76 h (1.5 to 3.2 days) (calculations as above, assuming energetic content of halved eggs was 50% that of whole eggs).

These calculations indicate that larvae of *A. punctulata* survived well beyond the theoretical point of complete exhaustion of egg energy. If we use the highest estimates of egg-energy value from Bolton *et al.* (2000), the longest duration to complete exhaustion was slightly over 4 days. In our experiments, 85% of starved larvae, either whole- or half-sized, survived to 5 days of age. Even after 35 days, 12% of larvae were still alive (Fig. 3), and some larvae survived to day 42, 10 times longer than our 4-day estimate based on energy budgets. Thus, one possibility is that these larvae did not sustain their metabolism entirely on endogenous reserves, but also utilized energy from exogenous sources such as DOM. Soft-bodied marine larvae take and up and utilize DOM from seawater (Jaeckle and Manahan, 1989; Manahan, 1990; Welborn and Manahan, 1990; Manahan and Wright, 1991) and may rely on DOM in the absence of particulate food; starved larvae deprived of particulate food are known to continue maintenance metabolism long past the theoretical point of complete utilization of endogenous reserves (Moran and Manahan, 2004). Our larvae did not have access to unrealistically high levels of DOM or bacteria; to avoid unnaturally high levels of either, we reared larvae individually (to prevent the possibility of live larvae utilizing DOM from dead culturemates) and with regular water changes. Although it is difficult to assess what fraction of total DOM represents energy that larvae can utilize, overall DOM levels in Instant Ocean are low and similar to concentrations in tropical ocean waters (Atkinson and Bingman, 1997). Likewise, larvae reared in filtered seawater had comparable survival rates to larvae reared in Instant Ocean (Moran, unpubl. data). Thus, our data support the idea that eggs, embryos, and larvae of soft-bodied marine organisms are not a closed energy system and are not analogous energetically to closed systems such as the eggs of reptiles and birds (*e.g.,* Mitchell, 2001).

The degree of reduction in metabolic rate with size varied between experiments, but when averaged across experiments, half- and quarter-sized embryos had metabolic rates that were about 50% and 25% of the rates of their whole-sized siblings. The variation among experiments may be due to the fact that embryos from reduced eggs develop more slowly than embryos from whole eggs (Marcus, 1979; Sinervo and McEdward, 1988; Allen, 2005); thus, at a given age, an embryo from a half-sized egg is at an earlier stage of development than a full-sized embryo, and a quarter-sized embryo would be earlier still. In invertebrate embryos of other species, metabolic rate increases from the newly fertilized embryo to a fully formed larva, and then declines if larvae are not fed (*e.g.,* Marsh and Manahan, 1999; Moran and Manahan, 2004). If larvae of *Mellita* and *Arbacia* show the same pattern, it is possible that controlling for both age and stage of development (rather than age alone) might yield more consistent results.

We assumed that the mass of larvae from half- and quarter-sized embryos was 50% and 25% of the mass of whole eggs, respectively, and we feel this is a reasonable assumption; blastomere separations at the 2- and 4-cell stage yield embryos with 50% and 25% the number of cells, respectively (Dan-Sohkawa and Satoh, 1978; Takahashi and Okazaki, 1979), and we see no *a priori* reason to expect proportionately different mass losses between whole and reduced larvae in early development. Because half- and quarter-sized embryos have cell numbers reduced by the same proportions, many factors that affect metabolic rate, such as mitochondrial density, biochemical composition, and metabolic density, likely remained the same across embryo sizes. Thus, these factors may have governed mass-specific metabolic rates (MSMR) in our experiments rather than parameters such as surface-area-to-volume ratios or fractal geometry of distribution networks that scale allometrically with size (West *et al.,* 1997). Regardless of mechanism, our experimental changes to egg size were not consistent with a size-dependent change in MSMR, and under equal conditions and without feeding, a large embryo would be expected to exhaust its energy reserves just as soon as a small embryo.

Our data come from experimental manipulations of egg size, and therefore may not reflect the evolutionary relationships between egg size and MSMR in nature. An evolutionary increase in egg size might be accompanied by an increase in the proportion of metabolically inactive tissue (*e.g.,* lipid) in the egg, as is seen between planktotrophic and lecithotrophic congeneric sea urchins (Hoegh-Guldberg and Emlet, 1997). This would likely lead to an inverse relationship between egg size and MSMR when interspecific comparisons are made across developmental modes. However, there is little evidence that the proportional representation of metabolically active tissue varies with size either among (Jaeckle, 1995) or within (Guisande and Harris, 1995) planktotrophic species, suggesting that within this feeding mode, increased egg size may generally not function to enhance resistance to starvation in nature.

Instead, as has been shown through both experimental (Sinervo and McEdward, 1988) and comparative (McEdward, 1986; Herrera *et al.,* 1996) work with echinoids, larger eggs make larvae that are larger and more developmentally advanced at the onset of feeding. In addition, interspecific comparisons of larval metabolic rate at the onset of feeding reveal a positive correlation between egg

size, larval size, and maximal metabolic activity (McEdward, 1986). Because larger larvae have bigger feeding structures, they can feed at a higher rate than smaller larvae (Hart and Strathmann, 1994; Hart, 1995), and because egg size is independent of size at metamorphosis in echinoids (Emlet *et al.,* 1987), in a given food environment larvae of species that produce large eggs can reach metamorphosis more rapidly than species that produce small eggs (Sinervo and McEdward, 1988; Herrera *et al.,* 1996; Levitan, 2000). Thus, our metabolic data are consistent with the idea that within planktotrophic species, very little of the material in the eggs is used to fuel metabolism once early morphogenesis is complete (Sewell, 2005); rather, regardless of egg size, the majority of materials in the egg are building blocks (proteins, *etc.*) for construction of the larval body.

Acknowledgments

We thank A. Dachino, C. Shields, and L. Streble for assistance in rearing larvae, and J. McAlister, P. Marko, and R. Podolsky for helpful discussion. This work was supported by NSF grant OCE- 0603755 to A. Moran and NSF Doctoral Dissertation Improvement Grant EEP-0308799 to J. Allen and R. Podolsky.

Literature Cited

Allen, J. D. 2005. Life-history evolution and the costs of small egg size in echinoderms. Ph.D dissertation, University of North Carolina, Chapel Hill. 150 pp.

Allen, J. D., C. Zakas, and R. D. Podolsky. 2006. Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development. *J. Exp. Mar. Biol. Ecol.* **331:** 186–197.

Anger, K. 1995. Starvation resistance in larvae of a semiterrestrial crab, *Sesarma curacaoense* (Decapoda, Grapsidae). *J. Exp. Mar. Biol. Ecol.* **187:** 161–174.

Anger, K., and R. R. Dawirs. 1981. Influence of starvation on the larval development of *Hyas araneus* (Decapoda, Majidae). *Helgol. Wiss. Meeresunters.* **34:** 287–311.

Atkinson, M. J., and C. Bingman. 1997. Elemental composition of commercial seasalts. *J. Aquaric. Aquat. Sci.* **8:** 39–43.

Bernardo, J. 1991. Manipulating egg size to study maternal effects on offspring traits. *Trends Ecol. Evol.* **6:** 1–2.

Blaxter, J. H. S., and G. Hempel. 1963. The influence of egg size on herring larvae. *J. Cons. Cons. Int. Explor. Mer* **28:** 211–240.

Bolton, T. F., F. I. M. Thomas, and C. N. Leonard. 2000. Maternal energy investment in eggs and jelly coats surrounding eggs of the echinoid *Arbacia punctulata. Biol. Bull.* **199:** 1–5.

Bridges, T. S., and S. Heppell. 1996. Fitness consequences of maternal effects in *Streblospio benedicti* (Annelida: Polychaeta). *Am. Zool.* **36:** 132–146.

Cameron, R. A., P. S. Leahy, and E. H. Davidson. 1996. Twins raised from separated blastomeres develop into sexually mature *Strongylocentrotus purpuratus. Dev. Biol.* **178:** 514–519.

Cushing, D. H. 1990. Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Adv. Mar. Biol.* **26:** 249–293.

Dan-Sohkawa, M., and N. Satoh. 1978. Studies on dwarf larvae developed from isolated blastomeres of the starfish, *Asterina pectinifera. J. Embryol. Exp. Morphol.* **46:** 171–185.

Driesch, H. 1892. Entwicklungmechanische Studien. I. Der Werth der beiden ersten Furchungszellen in der Echinodermentwicklung. Experimentelle Erzeugen von Theil- und Doppelbildung. *Z. Wiss. Zool.* **53:** 160–178; 183–184.

Emlet, R. B., L. R. McEdward, and R. R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. Pp. 55–136 in *Echinoderm Studies,* M. J. Jangoux and J. M. Lawrence, eds. Balkema, Rotterdam.

Guisande, C., and R. Harris. 1995. Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus. Limnol. Oceanogr.* **40:** 476–482.

Hadfield, M. G., and S. E. Miller. 1987. On developmental patterns of opisthobranchs. *Am. Malacol. Bull.* **5:** 197–214.

Hart, M. W. 1995. What are the costs of small egg size for a marine invertebrate with feeding planktonic larvae? *Am. Nat.* **146:** 415–426.

Hart, M. W., and R. R. Strathmann. 1994. Functional consequences of phenotypic plasticity in echinoid larvae. *Biol. Bull.* **186:** 291–299.

Harvey, E. B. 1940. A new method of producing twins, triplets and quadruplets in *Arbacia punctulata,* and their development. *Biol. Bull.* **78:** 202–216.

Havenhand, J. N. 1993. Egg to juvenile period, generation time, and the evolution of larval type in marine invertebrates. *Mar. Ecol. Prog. Ser.* **97:** 247–260.

Herrera, J. C., S. K. McWeeney, and L. R. McEdward. 1996. Diversity of energetic strategies among echinoid larvae and the transition from feeding to nonfeeding development *Oceanol. Acta* **19**(3–4): 313–321.

His, E., and M. N. L. Seaman. 1992. Effects of temporary starvation on the survival, and on subsequent feeding and growth, of oyster (*Crassostrea gigas*) larvae. *Mar. Biol.* **114:** 277–279.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer* **20:** 1–227.

Hoegh-Guldberg, O., and R. B. Emlet. 1997. Energy use during the development of a lecithotrophic and a planktotrophic echinoid. *Biol. Bull.* **192:** 27–40.

Horstadius, S. 1973. *Experimental Embryology of Echinoderms.* Clarendon Press, Oxford.

Jaeckle, W. B. 1995. Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. Pp. 49–77 in *Ecology of Marine Invertebrate Larvae,* L. R. McEdward, ed. CRC Press, New York.

Jaeckle, W. B., and D. T. Manahan. 1989. Feeding by a "nonfeeding" larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod *Haliotis rufescens. Mar. Biol.* **103:** 87–94.

Laing, I. 1995. Effect of food supply on oyster spatfall. *Aquaculture* **131:** 315–324.

Levitan, D. R. 2000. Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. *Am. Nat.* **156:** 175–192.

Levitan, D. R. 2006. The relationship between egg size and fertilization success in broadcast-spawning marine invertebrates. *Integr. Comp. Biol.* **46:** 298–311.

Manahan, D. T. 1990. Adaptations by invertebrate larvae for nutrient acquisition from seawater. *Am. Zool.* **30:** 147–160.

Manahan, D. T., and S. H. Wright. 1991. Uptake of dissolved organic matter from seawater by marine invertebrates: the Grover C. Stephens era. *Am. Zool.* **31:** A3–A3.

Marcus, N. H. 1979. Developmental aberrations associated with twinning in laboratory-reared sea urchins. *Dev. Biol.* **70:** 274–277.

Marsh, A. G., and D. T. Manahan. 1999. A method for accurate measurements of the respiration rates of marine invertebrate embryos and larvae. *Mar. Ecol. Prog. Ser.* **184:** 1–10.

Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size

affects the post-metamorphic performance of a colonial marine invertebrate. *Ecology* **84:** 3131–3137.

May, R. C. 1973. Larval mortality in marine fishes and the critical period concept. Pp. 3–20 in *The Early Life History of Fish,* H.S. Blaxter, ed. Springer-Verlag, Berlin.

McEdward, L. R. 1986. Comparative morphometrics of echinoderm larvae. I. Some relationships between egg size and initial larval form in echinoids. *J. Exp. Mar. Biol. Ecol.* **96:** 251–265.

McEdward, L. R. 1996. Experimental manipulation of parental investment in echinoid echinoderms. *Am. Zool.* **36:** 169–179.

McEdward, L. R. 1997. Reproductive strategies of marine benthic invertebrates revisited: facultative feeding by planktotrophic larvae. *Am. Nat.* **150:** 48–72.

McEdward, L. R., and S. F. Carson. 1987. Variation in egg organic content and its relationship with egg size in the starfish *Solaster stimpsoni. Mar. Ecol. Prog. Ser.* **37:** 159–169.

McEdward, L. R., and L. K. Coulter. 1987. Egg volume and energetic content are not correlated among sibling offspring of starfish: implications for life history theory. *Evolution* **41:** 914–917.

McEdward, L. R., and B. G. Miner. 2006. Estimation and interpretation of egg provisioning in marine invertebrates. *Integr. Comp. Biol.* **46:** 224–232.

Miner, B. G., L. A. McEdward, and L. R. McEdward. 2005. The relationship between egg size and the duration of the facultative feeding period in marine invertebrate larvae. *J. Exp. Mar. Biol. Ecol.* **321:** 135–144.

Mitchell, N. J. 2001. The energetics of endotrophic development in the frog *Geocrinia vitellina* (Anura: Myobatrachinae). *Physiol. Biochem. Zool.* **74:** 832–842.

Moran, A. L., and D. T. Manahan. 2004. Physiological recovery from prolonged 'starvation' in larvae of the Pacific oyster *Crassostrea gigas. J. Exp. Mar. Biol. Ecol.* **306:** 17–36.

Podolsky, R. D., and R. R. Strathmann. 1996. Evolution of egg size in free-spawners: consequences of the fertilization-fecundity trade-off. *Am. Nat.* **148:** 160–173.

Schmidt-Nielsen, K. 1984. *Scaling: Why Is Animal Size So Important?* Cambridge University Press, Cambridge.

Sewell, M. A. 2005. Utilization of lipids during early development of the sea urchin *Evechinus chloroticus. Mar. Ecol. Prog. Ser.* **304:** 133–142.

Sinervo, B., and L. R. McEdward. 1988. Developmental consequences of an evolutionary change in egg size: an experimental test. *Evolution* **42:** 885–899.

Starr, M., J. H. Himmelman, and J. C. Therriault. 1990. Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science* **247:** 1071–1074.

Strathmann, M. F. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast.* University of Washington Press, Seattle.

Strathmann, R. R. 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Annu. Rev. Ecol. Syst.* **16:** 339–361.

Strathmann, R. R. 2000. Functional design in the evolution of embryos and larvae. *Semin. Cell Dev. Biol.* **11:** 395–402.

Takahashi, M. M., and K. Okazaki. 1979. Total cell number and number of the primary mesenchyme cells in whole, 1/2 and 1/4 larvae of *Clypeaster japonicus. Dev. Growth Differ.* **21:** 553–566.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev. Camb. Philos. Soc.* **25:** 1–45.

Vance, R. R. 1973a. On reproductive strategies in marine bottom invertebrates. *Am. Nat.* **107:** 339–352.

Vance, R. R. 1973b. More on reproductive strategies in marine bottom invertebrates. *Am. Nat.* **107:** 352–361.

Welborn, J. R., and D. T. Manahan. 1990. Direct measurements of sugar uptake from seawater into molluscan larvae. *Mar. Ecol. Prog. Ser.* **65:** 233–239.

West, G. B., J. H. Brown, and B. J. Enquist. 1997. A general model for the origin of allometric scaling laws in biology. *Science* **276:** 122–126.

Wray, G. A., and R. A. Raff. 1991. The evolution of developmental strategy in marine invertebrates. *Trends Ecol. Evol.* **6:** 45–50.