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Scaling of Gelatinous Clutches: Effects of Siblings' Competition for Oxygen on Clutch Size and Parental Investment per Offspring

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ABSTRACT: Theories on the evolution of clutch size are primarily influenced by examples from terrestrial animals, yet most animal phyla occur exclusively in water. Oxygen has a lower diffusion coefficient and lower solubility in water than in air, and siblings in aquatic clutches often compete for oxygen. Mitigating this competition could affect allocation of resources to offspring. Gelatinous clutches are common in aquatic habitats and have evolved multiple times in many phyla. We hypothesized that spacing of embryos by gel enhances delivery of oxygen but that gel is organically costly. A model of diffusion predicts that clutch thickness should scale inversely with the square root of embryo concentration, indicating a need to reduce embryo concentration (and increase gel volume) disproportionately with increasing clutch thickness. For embryos in artificial clutches constructed with agarose gel, development was faster in clutches with more gel per embryo, as predicted. For natural gelatinous clutches of gastropods, thick clutches had disproportionately larger volumes of gel and disproportionately more organic material invested in gel relative to embryos. Thus, for aquatic gelatinous clutches, requirements for oxygen supply can affect trade-offs involving clutch thickness and parental investment per offspring: resources are diverted to gel, and the proportion diverted increases with clutch thickness.

Keywords: allometry, clutch, oxygen diffusion, gel, egg mass, sibling competition.

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Constraints on clutch size and on parental investment per offspring are a major focus of life-history theory (Roff 1992; Stearns 1992). Assumptions and predictions have been primarily influenced by terrestrial examples, but assumptions that appear to be plausible in one environment may not apply in another. Constraints on aquatic clutches are expected to differ from those on terrestrial clutches. Differences between water and air in oxygen solubility, oxygen diffusion coefficient (10^4 lower in water), and viscosity (Strathmann 1990; Morris and Bridges 1994) combine to limit the delivery of oxygen into a mass of embryos in water. Oxygen limitation can lead to longer development times or smaller size at hatching (Strathmann and Strathmann 1995). Thus, oxygen limitation can decrease survival, either by increasing the length of period when embryos are exposed to hazards or by reducing the quality of hatchlings. In this study, we focused on the effects of oxygen limitation on clutch size and parental investment per offspring.

Aquatic clutches include two extreme types, and in both types oxygen supply into a mass of embryos is more restricted than in air (Crisp 1959; Giorgi and Congleton 1984; Strathmann and Chaffee 1984; Hess 1991; Seymour and Roberts 1991; Booth 1995; Strathmann and Strathmann 1995; Cohen and Strathmann 1996). Those with large eggs (>1 mm in diameter) have large interstitial pores that allow flow of oxygenated water to the embryos. In masses with small eggs (about 0.1 mm in diameter), embryos are commonly embedded in a gelatinous matrix and oxygen reaches embryos by diffusion. Some gelatinous clutches include pores with interstitial flow, as in some amphibians (Seymour and Bradford 1995), but the majority do not, and oxygen is supplied to central embryos by diffusion. Supply of oxygen, rather than elimination of wastes, limits development rates within these masses of aggregated embryos (Strathmann and Strathmann 1995).

Oxygen limitation suggests two hypotheses for gelatinous clutches. (1) The addition of gel permits a larger

clutch of embryos by increasing the diffusive supply of oxygen to embryos deep within the clutch. (2) The use of organic material for gel is a costly diversion of materials that could be allocated to other uses. In combination, these hypotheses predict that the difference between investment in embryonic material alone and total investment per offspring, which includes the extraembryonic material, is greater for thicker clutches. (In this article, a *clutch* is defined as a group of embryos with its gel or other accessory structures, and the terms *clutch* and *egg mass* are interchangeable.)

To generate quantitative testable hypotheses, we first developed a simple model that predicted an upper limit for egg mass thickness under ideal conditions for diffusion and, more generally, that predicted a scaling relationship between egg mass thickness and requirements for spacing embryos within the gel. We then tested the qualitative predictions of the model with artificial gelatinous masses and the quantitative predictions with measurements from natural gelatinous egg masses. Finally, we used natural masses to determine whether the spacing requirements of thicker masses result in a greater organic investment in gel.

Methods

Model

The model corrected erroneous assumptions and the resulting erroneous predictions of an earlier model of supply and consumption of oxygen within gelatinous masses (Strathmann and Chaffee 1984; see appendix). The model also included additional clutch shapes and compared diffusive supply in well-mixed water and in still water surrounding the clutch. The model was deliberately simple, with masses described by only three parameters, so that the relationships among the parameters of interest would be clear. These three parameters (clutch shape, clutch thickness, and concentration of embryos) describe conspicuous differences among clutches that are easily measured.

Assumptions and Implications of the Model. We modeled diffusion through gelatinous masses to determine (1) a maximum egg mass thickness ($2R_{max}$) at which diffusion could supply sufficient oxygen throughout the mass for a given concentration of embryos and (2) an exponent of scaling between egg mass thickness and concentration of embryos. The equations resulted from balancing supply of oxygen against a rate of consumption. Rate of consumption was treated as uniform throughout the mass, rather than localized in discrete embryos. Maximum thickness ($2R_{max}$) is defined such that oxygen concentration declines to zero at the center of the mass. We considered three different shapes: a sphere, an infinite cylinder, and an infinite sheet. These shapes approximate three common shapes of egg masses: globose spheroids, strings, and ribbons. For spheres and cylinders, R_{max} is the radius. For sheets, R_{max} is half the distance through the sheet.

We modeled two extreme cases of oxygen supply: those of supply in well-mixed water and those in still water. We were particularly interested in the first case, which assumes no boundary layer, because it represents the most favorable condition of flow for oxygen supply to the surface of the egg mass. The solution for well-mixed water yields an upper limit for egg mass thickness allowed by diffusion for a given concentration of embryos. We modeled the second case (still water) only for the sphere, which provides an analytical solution that is unavailable for the other shapes. The diffusion model with no boundary layer is equivalent to others derived elsewhere for infinite sheets and cylinders (Hill 1929; Powell 1989). The derivations for a sphere with and without a boundary layer are given in detail in the appendix.

For the case of no boundary layer, the solution for diffusion through an egg mass at steady state is

$$R_{max} = \sqrt{\frac{FDC_R}{(NM/V)}} = \sqrt{\frac{FDC_R}{S}}, \qquad (1)$$

where R_{max} is 1/2 the maximum egg mass thickness allowed by diffusion, F is the shape factor (6 for a sphere, 4 for an infinite cylinder, and 2 for an infinite sheet; see appendix), D is the diffusion coefficient for oxygen in gel, C_R is the concentration of oxygen at the surface of the egg mass, and S is the oxygen consumption rate per unit volume of the mass ($S = NM/V$, where N is the number of embryos, M is the oxygen consumption of an embryo, and V is the volume of the egg mass). The term N/V, within the term S, represents the concentration of embryos in the mass. Concentration of oxygen at the surface of the egg mass (C_R) is assumed to be constant. The three properties of the egg mass that we vary are clutch thickness (R_{max}), clutch shape (F), and concentration of embryos (N/V). All other terms are treated as constants. This simple model examines design constraints that could affect trade-offs for gelatinous clutches. Adding more parameters could make the model more realistic, but that would obscure relationships among three parameters that vary conspicuously among a great variety of gelatinous clutches. In the discussion of natural clutches, we shall return to features of egg masses and environments that depart from the model's assumptions.

In the simple scaling model, maximum thickness of the mass ($2R_{max}$) is sensitive to the concentration of em-

bryos (N/V). The inverse relationship between R_{max} and $\sqrt{N/V}$ suggests that lowering the concentration of embryos in the mass (by spacing embryos apart with extra-embryonic material) would allow thicker egg masses. The scaling of R_{max} to the $-1/2$ power of N/V indicates that a disproportionate reduction in the concentration of embryos is required for an increase in egg mass thickness and would require a disproportionate increase in extra-embryonic material to space embryos apart in the egg mass. In other words, the important prediction of the model is that increasing the clutch size (and thereby clutch thickness) would require a disproportionate increase in the amount of gel per embryo and therefore may entail a disproportionately increased cost in organic materials.

In still water, where the boundary layer is thickest, an external gradient in oxygen concentration develops around the egg mass as it consumes oxygen. Oxygen must diffuse through the external gradient as well as through the egg mass. This case is analogous to that of a mass composed of two materials with two diffusivities, D_W of the oxygen in the boundary layer and D_M of oxygen in the mass. For a spherical mass with a boundary layer we obtain

$$R_{max} = \sqrt{\frac{6D_M C_\infty}{S\left(1 + 2\frac{D_M}{D_W}\right)}}. \quad (2)$$

Because $(1 + 2D_M/D_W)$ is greater than 1, R_{max} is smaller for a given embryo concentration in still water than in well-mixed water. However, the exponent of the scaling relationship between R_{max} and S remains the same. The two models (eqq. [1] and [2]) place bounds on thickness of the mass allowed by diffusion. Egg mass thickness ($2R_{max}$) can be greatest in well-mixed water and is smallest in still water:

$$\sqrt{\frac{6D_M C_\infty}{S\left(1 + 2\frac{D_M}{D_W}\right)}} < R_{max} < \sqrt{\frac{6D_M C_\infty}{S}}. \quad (3)$$

In intermediate cases of flowing water, R_{max} would depend on oxygen delivery to the mass, which in turn would depend on boundary layer thickness.

Testing the Model's Predictions. We first compared thicknesses of natural egg masses ($2R$) (see section titled "Natural Masses," below) to maximum thicknesses ($2R_{max}$) predicted by the model (eq. [1]). We plotted estimates of egg mass radius or half thickness (R) and egg mass shape/oxygen consumption per mass volume (F/S) for natural masses to determine the fit of our data to the predicted relationship. For these comparisons we used published estimates of M, C_R, and D (see the section "Values for Constants," below). We then tested for the scaling relationship between embryo concentration and clutch thickness. We grouped the constants D and C_R into a single term ($1/a$) to obtain the allometric equation

$$F/S = aR_{max}^b \quad (4)$$

and tested whether our estimates for F/S and R of natural masses scaled to the exponent (b) of 2 predicted by the model.

Values for Constants (M, C_R, *and* D). Metabolic rate (M) was estimated by converting egg volumes to dry organic weight (DOW) and then converting DOW to estimates of oxygen consumption. The assumed dry organic weight (DOW) per volume of an egg was 0.36×10^{-6} µg µm^{-3}, an estimate for *Tritonia diomedea,* for which we were able to obtain measurements. This estimate is lower than those expected from measurements of organic carbon from *Haminaea callidegenita* (Gibson and Chia 1991) and from a polychaete (Bridges 1993) but greater than estimates for echinoderms (Jaeckle 1995). The assumed oxygen consumption per DOW in the egg was 10^{-6} µL O_2 s^{-1} µg^{-1}, a value near the middle of estimates for larvae (Crisp 1976; Hoegh-Guldberg and Manahan 1995). Our estimates of oxygen consumption per embryo ranged from about 10^{-7} to 2.5×10^{-6} µL O_2 s^{-1}.

The assumed ambient concentration of oxygen (C_R) was 5 µL O_2 cm^{-3}, near the value for air-saturated seawater at 20°C (Horne 1969). The assumed diffusion coefficient for oxygen in gel (D) was 1.5×10^{-5} cm^2 s^{-1}, which is 75% of the diffusion coefficient for oxygen in water. Estimated effects of gel masses on the O_2 diffusion coefficient in water are close to this assumption: 70% and 100% for entire egg masses of the gastropod *Melanochlamys diomedea* (Cohen and Strathmann 1996), 75% for frog egg mass gel (Burggren 1985), and about 70% for a 2% agar gel (Sato and Toda 1983).

Experimental Gelatinous Masses

Artificial masses were constructed from live zygotes of the tropical sea urchin *Tripneustes gratilla* (Linnaeus) and low melting point agarose gel (Strathmann and Strathmann 1989). Two parts of 2% agarose in seawater at 35°C were added to one part seawater with a suspension of fertilized eggs at 22°- 25°C. This mixture was poured into cylindrical molds and cooled to 22°- 25°C. The resulting gelled mass was freed from the mold into seawater. Initial counts of embryos and molding of masses

were completed within 75 min after fertilization, before first cleavage.

Treatments used masses of different sizes but similar cylindrical shape. Three sizes were used with two replicates each. The masses had wet weights of about 2.6, 6.2, and 12.0 g with diameters of 16, 22, and 29 mm and heights of 12.5, 15.5, and 18 mm, respectively. The artificial masses did not test exact scaling relationships relative to the model; they tested whether spacing with gel is a sufficient means of alleviating crowding and maintaining development rates. Thus, it was sufficient that diffusion distances of larger masses were greater in all directions than those of smaller masses. Variation in the ratio of height to diameter and diameters greater than heights did not affect the qualitative outcome of the experiment. Each mass contained approximately 2×10^6 embryos.

The temperature during development in artificial masses was near that of the sea (24°- 25°C). Each mass was supported on a plastic mesh and aerated vigorously by an air stone placed below the mesh. The experiment was terminated when embryos at the periphery of the egg masses developed into early unhatched blastulae at 9 h after fertilization. Embryos near the surface of the masses developed normally and at the same rate as free embryos in water until the blastula stage, when the agarose began to interfere with expansion of the embryos.

Development was halted by cooling masses to near 0°C. The gel cylinders were cut at half the height of the cylinder, and the chilled embryos were videotaped at intervals spaced 1.3 mm along the radius. Developmental stages of 20 embryos were recorded at each interval along the transect.

Gel could affect supply of oxygen to embryos in two ways. The simple diffusion model assumes that supply equals consumption, and the gel improves diffusive supply of oxygen by spacing embryos. Under nonequilibrium conditions, however, oxygen could be supplied to embryos for brief intervals out of the reservoir of oxygen dissolved in the gel. A preliminary experiment showed that the initial oxygen reservoir was sufficient to sustain normal development rates of central embryos in thick (28 mm) artificial gel masses with embryos at low concentrations (10^4 embryos mL^{-1}) during 3.5 h in hypoxic (nitrogen-bubbled) water. Therefore, results of previous experiments (Strathmann and Strathmann 1989) may have been influenced by the larger initial reservoir of oxygen in larger artificial masses. To examine effects of gel on supply by diffusion, rather than the nonequilibrium effect of an initial oxygen reservoir, we ran experiments with a higher concentration of embryos and for 8 h, a time sufficient for embryos to deplete the initial reservoir of oxygen dissolved in the gel. Even at the lowest concentration of embryos, each embryo had a reservoir of less than 0.3×10^{-4} μL O_2, and sea urchin embryos consume more than 10^{-4} μL O_2 h^{-1} at these temperatures (Yanagisawa 1975).

Natural Egg Masses

Types of Natural Masses. The study included seven gastropod mollusks: a prosobranch, *Lacuna* spp.; three nudibranch opisthobranchs, *Archidoris montereyensis* (Cooper), *Melibe leonina* (Gould), and *T. diomedea* Bergh; and three cephalaspidean opisthobranchs, *Haminaea vesicula* Gould, *H. callidegenita* Gibson and Chia, and *M. diomedea* (Bergh). In all but *H. callidegenita,* the species hatch as feeding planktonic larvae. In *H. callidegenita,* both larvae and metamorphosed juveniles hatch from each mass (Gibson and Chia 1991). The similar masses of two *Lacuna* species, *L. vincta* and *L. variegata,* were not distinguished. Comparisons were among species. Intraspecific variation was small.

Adults were collected in 1992 and 1993 in the San Juan Archipelago, Washington, or other sites in this region of the northeast Pacific. Adults laid egg masses when maintained in laboratory sea tables at 11°C with their preferred food and substratum.

The masses included a range of shapes and sizes (figs. 1- 3) and were classified as globose spheroids, strings, and ribbons. Masses of *Lacuna* spp. are initially string-shaped (toroidal) when laid but swell into small globose masses during development. In all these masses, embryos are contained within fluid-filled capsules (figs. 1- 3). The number of embryos per capsule varies among species. In figures 1*b, d,* 2*b, d,* and 3*d,* each capsule surrounds one or occasionally two dark embryos. In figures 1*f* and 3*b,* each capsule surrounds many embryos. In most masses, the capsules are embedded in gel, and there is an outer "rind" of gel in which there are no embryos. In the string-shaped mass of *T. diomedea* (fig. 3*a, b*), large capsules filled with embryos are packed within a gelatinous tube. The strings of *T. diomedea* are at an extreme in a continuum from masses with no gel between embryos to those with progressively larger amounts of gel between encapsulated embryos.

Measurements of Natural Masses. Freshly deposited masses (zygote stage) were measured with calipers. The shortest axis was defined as the thickness. This dimension was the width of the globose spheroids at the middle of the long axis, the diameter of the circular cross section of strings, and the thickness of ribbons.

To determine the concentration of embryos, we counted the number of capsules within a sample of known wet weight and multiplied by the mean number of embryos per capsule for that mass. We blotted whole masses with an absorbent tissue (Kimwipe) before

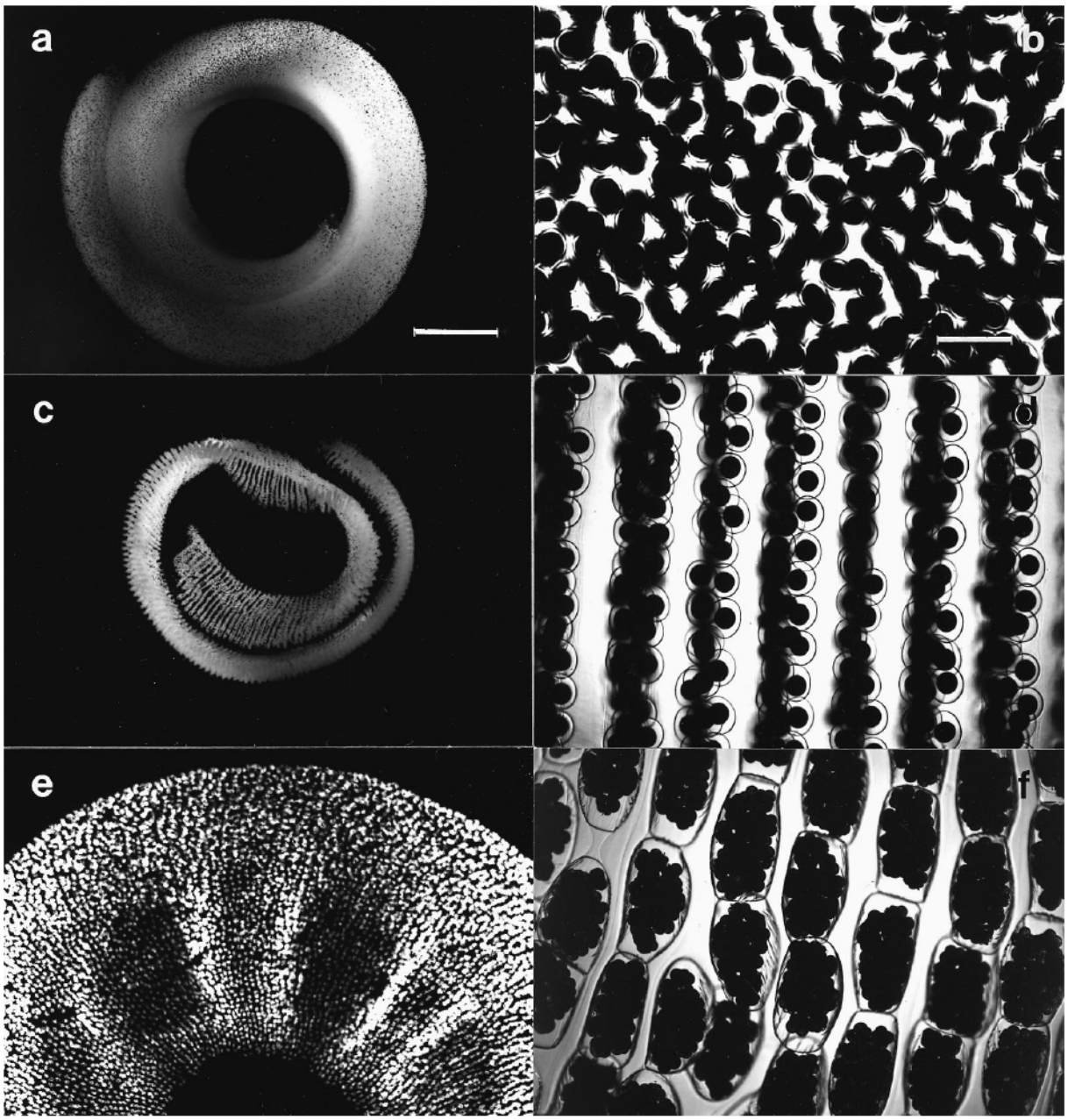

Figure 1: Ribbon-shaped masses. *a, b, Archidoris montereyensis. c, d, Haminaea vesicula. e, f, Melibe leonina.* Scale bar for *a, c, e* = 5 mm, for *b, d, f* = 300 μm. Whole masses are on the left. Close-up views of arrangements of embryos and capsules are on the right.

weighing. Because embryos were not distributed homogeneously within masses but occurred at lower densities at the tail ends, we cut subsamples from the middle part, slicing perpendicular to the long axis. We teased apart weighed subsamples with fine forceps to remove capsules for counting.

We used wet weight to estimate egg-mass volume because densities of masses were close to water (about 1 g cm^{-3}) and did not differ significantly among species (Kruskal-Wallis, df = 6, $T = 3.06$, $P > .3$). The power of this test was 0.94 (performed for the ANOVA case at $\alpha = 0.05$; Zar 1984) against the alternative hypothesis that densities of masses differ.

Because egg masses swell as development proceeds, masses with embryos at early stages should have the highest concentrations of embryos. Early stage masses were used for most comparisons so that estimates of organic content of gel would not be inflated by the fouling

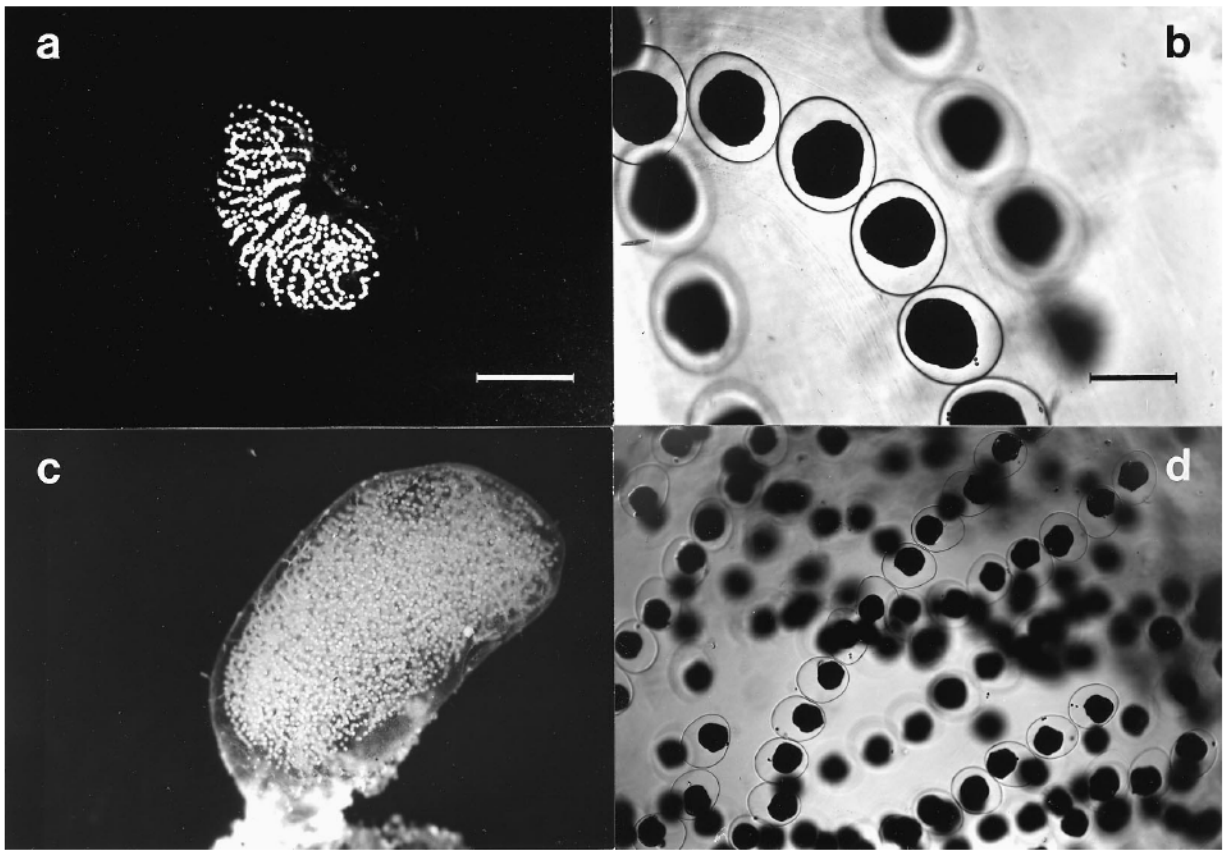

Figure 2: Globose masses. *a, b, Haminaea callidegenita. c, d, Melanochlamys diomedea.* Scale bar for *a, c* = 5 mm, for *b, d,* = 300 µm. Whole masses are on the left. Close-up views of arrangements of embryos and capsules are on the right.

organisms on older masses (see below). We determined the magnitude of swelling for three kinds of masses. For *H. vesicula* and *M. diomedea,* we estimated embryo concentration at later stages by counting larvae that hatched from preweighed subsamples at late stages (with unhatched embryos also counted and included) and compared these concentrations with those for other masses at early stages. For *Lacuna* spp. we weighed the same egg masses at early and late stages.

Because correlation coefficients for regressions exceeded 0.99, we used ordinary least squares regressions and *t*-tests of slopes to compare observed and predicted relationships for concentration of embryos and thickness of masses. With such high correlation coefficients, the ordinary least squares regressions are nearly the same as the appropriate reduced major axis regressions (LaBarbera 1989).

Organic Content of Gel. Dry organic weight (DOW), calculated as dry weight minus ash weight, was the measure of organic material per embryo. These estimates included gel, capsules, and capsule fluid as well as material within embryos. Because older masses become fouled by diatoms and other microflora, only masses with zygote-stage embryos were used. For estimates of dry weight, samples with a wet weight of 0.1- 0.5 g were dried in an oven on preburned aluminum boats at 60°C for 48 h. For ash weights, dry samples were burned in a muffle furnace at 500°C for 12 h. These times were sufficient to dry and burn samples to constant mass.

The gel that surrounds and separates the encapsulated embryos is a mucopolysaccharide (Kress and Schmekel 1992). Attempts to obtain a separate estimate for organic content of this gel alone were successful for three species, each by a different method. For *T. diomedea* and *H. callidegenita,* we were able to separate encapsulated embryos from the gel. For *T. diomedea,* DOW of encapsulated embryos and of gel was determined by weighing each separately. For *H. callidegenita,* DOW of gel per embryo was determined by subtracting DOW of encapsulated embryos from DOW of the mass. For *H. vesicula,* DOW was determined for gel alone, by allowing the sacoglossan *Olea hansineensis* to prey on the embryos, leaving the gel intact (Crane 1971). The DOW of embryos with capsules was then determined by subtraction.

Although dry weight minus ash weight has been widely

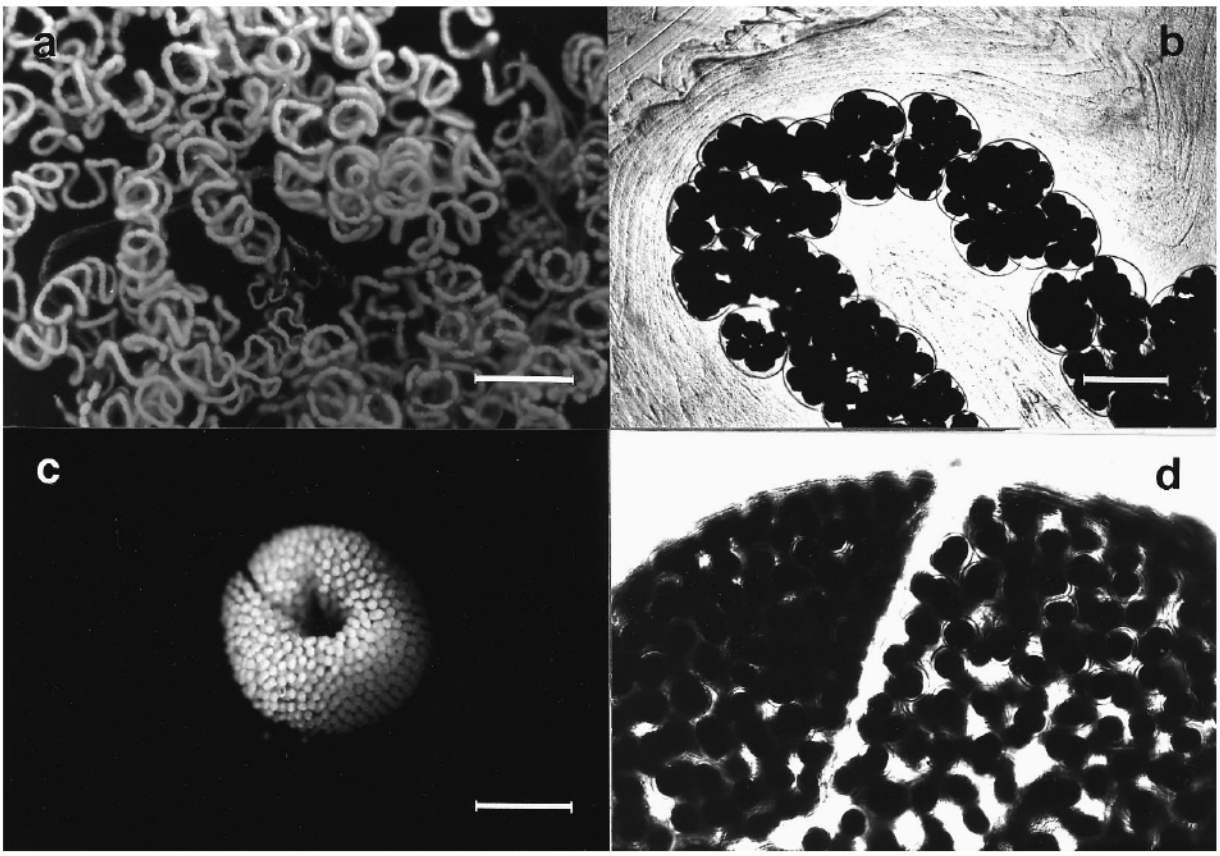

Figure 3: String-shaped masses. *a, b, Tritonia diomedea. c, d, Lacuna* spp. Scale bar for $a = 10$ mm, for $c = 1$ mm, for $b, d = 300$ µm. Whole masses are on the left. Close-up views of arrangements of embryos and capsules are on the right.

used to estimate DOW, water of hydration retained in drying but lost in ashing may be included in the estimate of DOW, with 14% of the weight of salts from seawater lost upon ashing (Moreno 1996). The comparison among species of total DOW per embryo would not be affected by a 14% correction for water of hydration. However, because of the large volume of sea water in gel, we did examine the effect of subtracting a 14% correction on the estimates of DOW in gel alone.

Results

Experimental Gelatinous Masses

In artificial masses of constant embryo number but variable gel volume, embryos in larger masses developed to the blastula stage at a greater distance from the edge of the mass (fig. 4), and a greater number of embryos reached the blastula stage throughout the mass. Mean percentage of embryos reaching the blastula stage (along the radial transect) was 12% for small masses, 20% for medium masses, and 25% for large masses. At the center of large masses, the most advanced embryos had only reached the four-cell stage. Retarded development at the center of even the least crowded masses confirmed that the initial oxygen reservoir was depleted and that development to advanced stages required diffusion into the mass.

Natural Egg Masses

Measurements of Natural Masses. Of the three egg mass shapes, the globose were thickest, with shortest axes of 5- 7 mm, and the ribbons and strings were thinnest, with shortest axes of 1- 2 mm (table 1). The data formed two clusters in which the thick masses differed greatly from the thin masses. As predicted, concentration of embryos, measured as total volume of embryos per wet weight of mass, decreased as mass thickness increased (fig. 5, data from tables 1- 3; Spearman rank correlation −0.929).

Embryo concentrations were lower at later developmental stages than at the zygote stage. Such a decrease is expected because of the expansion of capsules (Kress 1971) or gel. Compared with masses at the zygote stage (table 3), embryo concentrations decreased by 46%

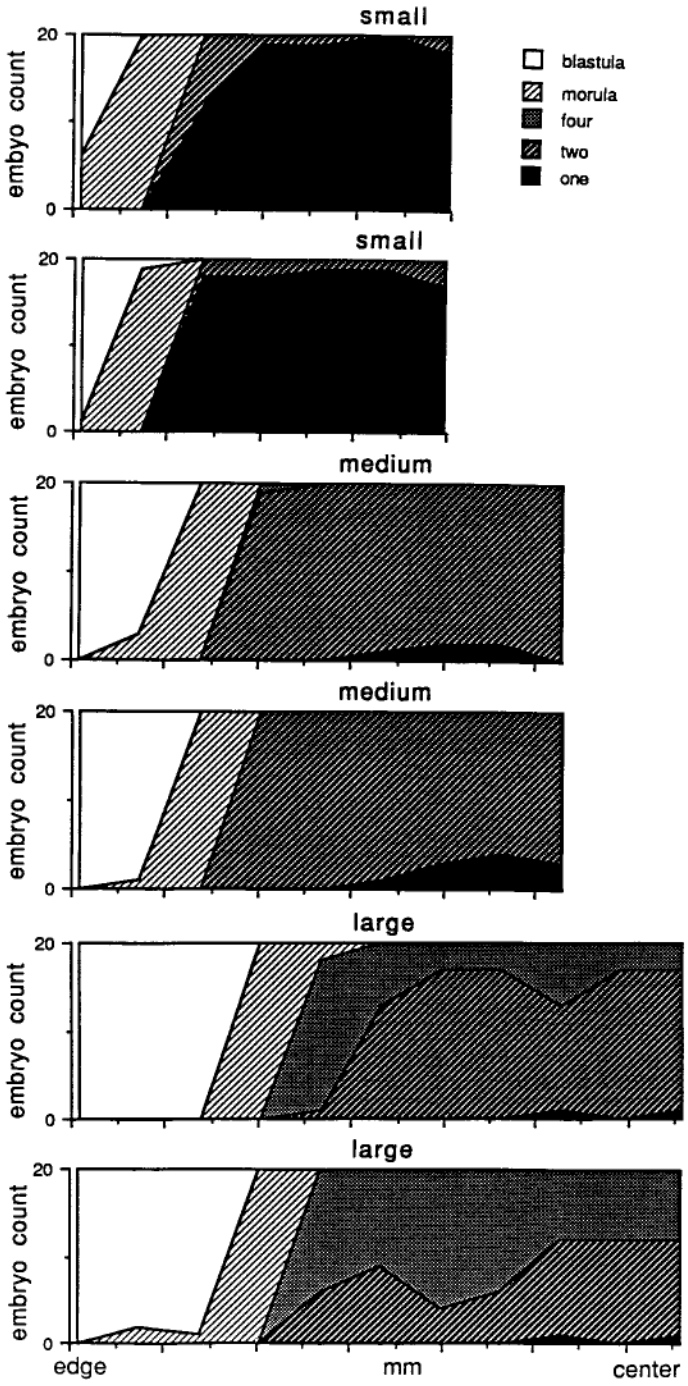

Figure 4: Results from artificial egg masses of differing volumes but with the same number of embryos. Shading shows the number of embryos that reached stages of development along radii of cylindrical masses, from the surface to the center. Stages were one-cell embryos to blastula (key at upper right); 20 embryos were sampled at 1.3-mm intervals. Each graph is for a different mass with two replicates at each size. The *X*-axis is distance from the edge (left) to the center (right) in millimeters, with longer axes for cylinders with greater radii.

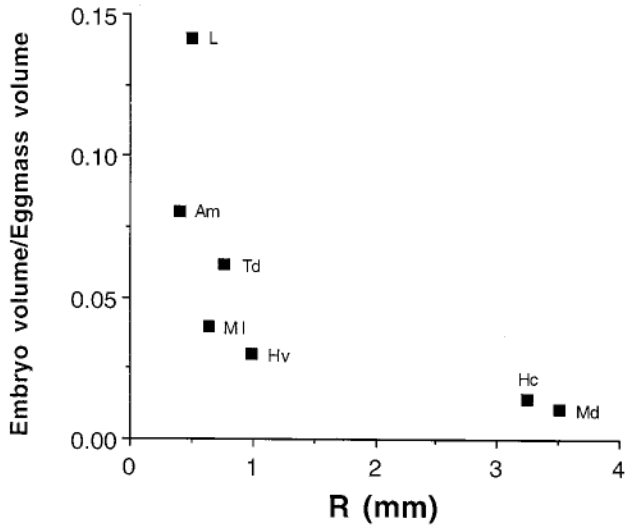

Figure 5: The relationship between concentration of embryos (by volume) and half the mass thickness (R) for gelatinous clutches of seven gastropod species. Species are *Archidoris montereyensis* (*Am*), *Lacuna* spp. (*L*), *Melibe leonina* (*Ml*), *Tritonia diomedea* (*Td*), *Haminaea vesicula* (*Hv*), *Haminaea callidegenita* (*Hc*), and *Melanochlamys diomedea* (*Md*).

(down to 70,000 ± 20,000 g^{-1}, $n = 11$) for *Haminaea vesicula* masses at the trochophore or veliger stages, and by 37% (down to 23,000 ± 2,000 g^{-1}, $n = 8$) for *Melanochlamys diomedea* masses at the veliger stage. Masses of *Lacuna* spp. increased in wet weight by a factor of 3.0 (SD = 0.8, $n = 5$) from early to late stages, reducing the concentration of late stage embryos by 67%. Thus the concentrations of embryos in table 3 are the estimated maxima for those during development and are those occurring when rates of oxygen consumption are low.

Table 2: Dimensions of embryos and capsules (zygote stage)

Species	*Embryos per capsule*	*Diameter of zygote (µm)*	*Volume of embryo (10^6 µm^3)*
Archidoris montereyensis	1- 2	84 ± 2 (2)	.31
Lacuna spp.	1	100 ± 2 (3)	.52
Melibe leonina	18 ± 2 (5)	80 ± 1 (3)	.27
Tritonia diomedea	17 ± 2 (5)	85 ± 2 (3)	.32
Haminaea vesicula	1	75 ± 3 (4)	.22
Haminaea callidegenita	1	236 ± 2 (3)	6.9
Melanochlamys diomedea	1	83 ± 3 (3)	.30

Note: Values are means ± SE, with n (in parentheses) = number of egg masses sampled. Ten embryos and capsules were measured for each mass. Volumes of embryos were calculated as spheres.

Testing the Model's Predictions. Thicknesses (R) of natural egg masses were close to the values predicted by the model (R_{max}) for well-mixed water (table 1). The fit of the model (eq. [1]) to estimates of R and F/S for natural masses was close (fig. 6), suggesting that the values used for the constants (M, D, and C_R) were appropriate. The observed scaling relationship for R with F/S (exponent b in eq. [4]) was 1.4 (fig. 7), significantly less than the predicted exponent of 2 but also significantly greater than 1 (t-tests, $P < .01$).

Organic Content of Gel. Thick masses appear to have a greater organic investment in extraembryonic material, indicating a cost of thicker masses. The organic content (DOW) of egg mass material allocated per unit volume of embryo was greater for the thicker masses (fig. 8).

For the three masses for which gel and embryos were

Table 1: Shapes, sizes, and predicted maximum radii or half thicknesses for gelatinous masses (zygote stage)

Species	*Shape*	F	*Wet weight of whole mass (g)*	*Radius or half thickness* (R) *(mm)*	*Predicted radius or half thickness* (R_{max}) *Still water (mm)*	*Well mixed (mm)*
Archidoris montereyensis	Ribbon	2	1.8 ± .5 (3)	.4 ± .1 (5)	×××	.71
Lacuna spp.	Torus	4	.0041 ± .0007 (4)	.51 ± .02 (5)	×××	.77
Melibe leonina	Ribbon	2	.45 ± .07 (7)	.64 ± .06 (5)	×××	1.01
Tritonia diomedea	String	4	9 ± 4 (4)	.76 ± .04 (5)*	×××	1.17
Haminaea vesicula	Ribbon	2	.43 ± .07 (8)	.99 ± .08 (5)	×××	1.21
Haminaea callidegenita	Globose	6	.25 ± .05 (6)	3.2 ± .3 (6)	1.9	2.9
Melanochlamys diomedea	Globose	6	.48 ± .07 (4)	3.5 ± .1 (5)	2.1	3.4

Note: Values are mean ± SE; numbers in parentheses are number of egg masses. The predicted thickness was from the diffusion model for different shapes, counts of embryos per wet weight, and oxygen consumption estimated from embyro size, as described in the text; F is the diffusion model's factor for shape of mass. Predictions for still water are unavailable for cylinders and sheets.

* R without the gel rind is .41 ± .05 (3).

Table 3: Concentration of embryos and organic content of egg masses at the zygote stage

Species	*Number of embryos per wet weight of mass* (μg^{-1})	*DOW of mass per wet weight of mass*	*DOW of mass per embryo* (μg)	*DOW of mass per embryo volume* ($g\ cm^{-3}$)
Archidoris montereyensis	.26 ± .02 (5)	.073 ± .002 (5)	.29 ± .02	.94
Lacuna spp.	.27 ± .02 (5)	.14 ± .01 (5)	.55 ± .05	1.06
Melibe leonina	.15 ± .02 (5)	.028 ± .002 (5)	.19 ± .02	.70
Tritonia diomedea	.19 ± .03 (5)	.034 ± .002 (5)	.19 ± .02	.59
Haminaea vesicula	.13 ± .01 (5)	.025 ± .001 (5)	.19 ± .02	.86
Haminaea callidegenita	.0021 ± .0001 (6)	.024 ± .002 (6)	11.6 ± .7	1.68
Melanochlamys diomedea	.037 ± .005 (5)	.0141 ± .0002 (5)	.41 ± .05	1.37

Note: Values are means ± SE; numbers in parentheses are number of masses. Dry organic weight (DOW) is estimated as dry weight minus ash weight. Wet weight or dry weight of a mass includes gel, capsules, and embryos.

separated, organic investment solely in gel per embryo or per embryo volume was greatest in the thickest mass (*Haminaea callidegenita;* table 4). Organic density of gel varied among these species by a factor of two. The DOW as a percent of wet weight was 1.2% for *Tritonia diomedea,* 0.74% for *H. vesicula,* and 1.4% for *H. callidegenita.* With a 14% correction for water of hydration of salts, the estimates are 1.1%, 0.6%, and 1.2%, respectively.

Discussion

This study focused on the effects of a physical factor- oxygen limitation- on clutch size and reproductive allocation. Results indicate that, because of constraints on oxygen diffusion in water, an allometric increase in organic investment is required for gelatinous clutches to increase in thickness. The model of diffusion predicted that for adequate supply by diffusion the concentration of embryos in an egg mass must decrease disproportionately with increasing egg mass thickness (eq. [1]). Artificial and natural masses confirmed the qualitative predictions of the model. The artificial masses demonstrated that the

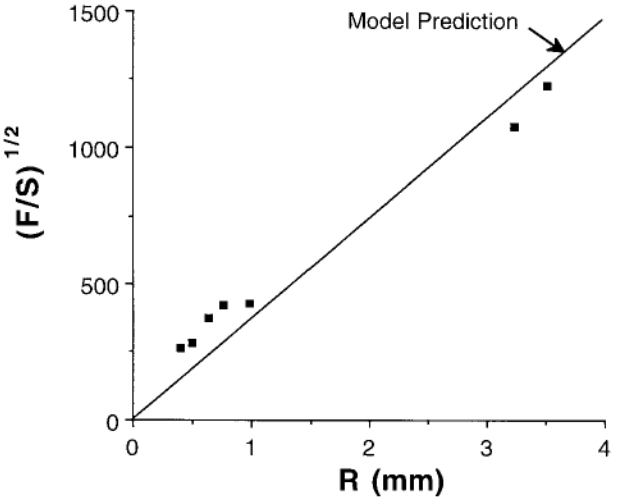

Figure 6: The fit of observed egg masses to predictions of the model. The line is the relationship between $(F/S)^{1/2}$ and R predicted by equation (1). Data points are estimates for gelatinous clutches of seven gastropod species; F is a shape factor for masses (2, 4, or 6); S is the estimated oxygen consumption per mass volume in $\mu L\ O_2\ s^{-1}\ mm^{-3}$; R is half the thickness of the mass in millimeters.

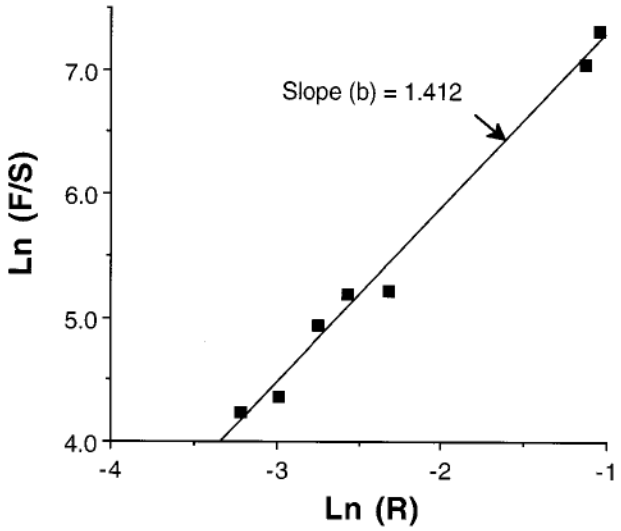

Figure 7: An estimate of scaling relationships from observed masses. The regression line for R versus F/S from natural masses is $\ln(F/S) = 8.712 + 1.412 \ln(R)$ ($n = 7$, $r = 0.995$, SE of the constant = 0.155, SE of the exponent = 0.064). The variables R, F, and S are estimated from gelatinous clutches of seven gastropod species (*points*). These variables (R, F, and S) are defined as in figure 6 except R is in centimeters. The slope of the line is the exponent (b) of R from equation (4). In contrast, the predicted value of b from the diffusion model is 2.

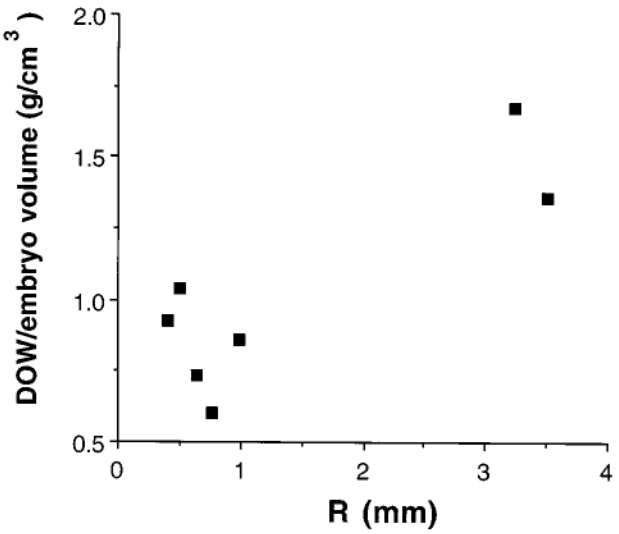

Figure 8: Parental investment per offspring volume (as dry organic weight per volume of egg) versus mass thickness (R) for gelatinous clutches of seven gastropod species. The dry organic weight is for all components of the egg mass: gel, capsules, and embryos. The radius (R) is half the thickness of the clutch.

addition of gel allows more rapid development for the same number of embryos within a mass (fig. 4). Among natural masses, thicker masses had disproportionately lower concentrations of embryos (fig. 5) and a greater volume of gel per embryo. The values for maximum thickness predicted by the model (R_{max} in eq. [1]) and measured from natural masses (fig 6; table 1) were close. As values for diffusion coefficient (D) and ambient oxygen concentration (C_R) could not get much larger, this result suggests that natural masses may be near the upper limit in thickness allowed by diffusion.

The scaling exponent for thickness (R) in relation to concentration of embryos (contained within F/S) was 1.4 for natural masses (fig. 7), lower than the value of 2 predicted by the model (exponent b in eq. [4]). A lower value for natural masses indicates that the rate of increase in gel volume is lower than expected, but a value greater than 1 indicates that gel volume still increased disproportionately for a corresponding increase in egg mass thickness. Differences in shape of the mass (F) do not affect the scaling exponent and only change the predicted value for R_{max} by a factor of $\sqrt{2}$ or $\sqrt{3}$. Also, a thick boundary layer does not affect the scaling exponent, though it does lower the predicted R_{max} for a given concentration of embryos (eq. [2]).

Although the addition of gel in natural masses should improve the supply of oxygen to embryos, this gel represented a substantial investment in extraembryonic organic material (fig. 8). The organic content of gel made up 30%- 58% of the organic content of egg masses (table 4). With a 14% correction for water of hydration of salts in gel, the gel still made up 25%- 54% of the organic content of egg masses.

Consequences of the Gel Matrix for Life-History Trade-Offs

For aquatic clutches of small eggs, a gelatinous matrix surrounding embryos enhances the diffusive supply of oxygen, but a lower concentration of embryos is achieved by a larger volume of gel and hence a larger amount of extraembryonic organic material. The costs and benefits of adding gel suggest that there may be an optimal amount of gel for an egg mass with a given shape and number of embryos. More gel could increase survival of embryos because of decreased oxygen demand per volume of egg mass, but the allocation of organic matter to gel could reduce fecundity. The cost in organic material of increasing the amount of gel would increase in proportion to gel volume, while the benefit to fitness would increase until a diffusive supply of oxygen provided adequate levels for development and survival of embryos. The optimal amount of gel for an egg mass would be that at which the difference between benefits and costs is greatest.

For gelatinous clutches, the relationship between costs and benefits of gel implies constraints on clutches and

Table 4: Organic content of encapsulated embryos and gel

Species	*DOW of an encapsulated embryo (μg)*	*DOW of gel per embryo (μg)*	*DOW of gel per embryo volume (g cm^{-3})*	*% of total DOW of mass that is gel*
Tritonia diomedea	.116 ± .004 (3)	.065 ± .009 (3)	.22	36
Haminaea vesicula	.136	.057 (1)	.26	30
Haminaea callidegenita	4.87 ± .24 (3)	6.73	.98	58

Note: Values are means ± SE; numbers in parentheses are number of masses. Dry organic weight (DOW) is estimated as dry weight minus ash weight. Total dry weight of a mass includes gel, capsules, and embryos.

parental investment that differ from constraints for other clutches. Gelatinous aquatic clutches differ from those in air in that parental investment per offspring depends on size and shape of the clutch. Gelatinous clutches also differ from aquatic clutches whose oxygen is supplied by interstitial flow between embryos. With interstitial flow, larger eggs (and therefore greater parental investment per offspring) can increase supply of oxygen into thick egg masses because the larger channels between embryos offer less resistance to flow, but in that case the greater investment per offspring is mostly in the embryo rather than in a matrix for spacing embryos (Hess 1991; Strathmann 1995).

For a given shape, larger gelatinous clutches are thicker, and the disproportionate increase in parental investment per offspring with increasing clutch thickness differs from the common assumption that investment per offspring is not influenced by total investment in a clutch (Winkler and Wallin 1987). This assumption is reasonable for free spawners and for many aggregated clutches but not for gelatinous clutches supplied by diffusion.

Gelatinous egg masses are common, have evolved many times, and have evolved in numerous phyla. They are reported from species of anthozoans (Hand and Uhlinger 1992), nemerteans (Schmidt 1934; Riser 1974), nematodes (Hyman 1951; Mackintosh 1960; Maggenti 1962), chaetognaths (Alvariño 1990), gastropods of diverse orders (Hurst 1967; Pechenik 1979; Geraerts and Joose 1984), cephalopods (Arnold 1984), at least eight families of polychaetes (Chapman 1965; McEuen et al. 1983; Wilson 1986; Strathmann 1987; Bhaud and Grémare 1988; Sato and Osanni 1996), insects (Hinton 1981), amphibians (Burggren 1985), and fish (Newsome and Tompkins 1985; Erickson and Pikitch 1993). In contrast, clutches of eggs of birds or other terrestrial vertebrates, which have been the focus of studies of the evolution of clutch sizes for 50 yr (Lack 1947), are composed of unusually large and few eggs and have a single evolutionary origin.

Because of the emphasis in the literature on sizes of terrestrial clutches, effects of competition among siblings for oxygen have not been addressed in theories on the evolution of clutch size or parental investment per offspring (Roff 1992; Stearns 1992). The constraints from diffusive supply of oxygen fall into the general category of competition among siblings for a resource (Parker and Begon 1986), but competition for oxygen has distinctive features. Competition for oxygen becomes more severe as offspring are stacked more deeply (in thicker clutches) rather than simply as the number in a clutch increases. Also, a different type of parental investment is required to offset the constraint. Often, competition for food occurs after hatching and is predicted to result in larger eggs that will develop into larger hatchlings, presumed to have a competitive advantage in feeding. In contrast, competition for oxygen occurs before hatching and results in greater parental investment in extraembryonic structures that are abandoned when offspring leave the mass.

Extraembryonic Organic Material as a Cost of Separating Embryos

Dry organic weight (DOW) of extraembryonic material was greater in thicker masses. Thus, increasing the volume of material that spaces embryos apart requires more organic material per embryo. Our result agreed with a previous comparison of two nudibranch species, in which a clutch with a greater energy content per embryo volume had a larger amount of gel matrix per embryo (Todd 1979). Our estimates of DOW as 0.6%- 1.2% of the wet weight of gel for the opisthobranchs *T. diomedea, H. vesicula,* and *H. callidegenita* were similar to the estimate of 0.5%- 1.5% for the gel in the clutch of the polychaete *Scoloplos armiger* (Chapman 1965).

For *H. callidegenita,* the estimate of organic content of gel depended on measurements of embryo organic content (table 4) because organic content of gel was obtained by subtraction. Our estimate of organic density of *H. callidegenita* embryos (0.71 mg mm^{-3}) was three times greater than the organic carbon per volume estimated for this species by wet oxidation (0.22 mg carbon mm^{-3}) (Gibson and Chia 1991). Although we expected our estimate of DOW of an embryo per egg volume to be only about twice the organic carbon per volume, our estimates were still close to expected values. If our estimate of DOW of embryos was high, then we have underestimated the DOW in gel for the thickest mass and have erred conservatively.

A large investment in extraembryonic materials is also found in clutches of embryos enclosed in fluid-filled purse-like capsules, an alternative means of retention and protection (Rawlings 1994, 1996). For the prosobranch gastropod *Conus,* 20%- 50% of the organic matter in a clutch was in the thick capsule walls (Perron 1981). Extraembryonic material constituted an estimated 45% of the biomass deposited as spawn by the prosobranch *Nucella lamellosa* (Stickle 1973). The cost of a gel matrix (table 3) is similar to the cost of purse-like capsules.

Advantages of Thick Clutches

If thicker clutches entail a greater cost in extraembryonic materials, why should clutches be thick? A thicker clutch requires a greater volume of gel to space embryos and consequently a greater investment in extraembryonic ma-

terials, while a thin clutch can provide adequate diffusion with less gel.

Depositing embryos in aggregated clutches may aid in protection from predators or environmental hazards or retention at favorable sites (Strathmann 1985; Pechenik 1986; Rumrill 1990), and thicker clutches may be more effective for protection or attachment. Embryos on the underside of the gelatinous ribbon of *Archidoris montereyensis* had greater survival following a day of solar radiation (Biermann et al. 1992), suggesting a protective function of gel. A thick mass can protect embryos against brief changes in temperature, oxygen, salinity, or other materials in external water by slowing exchange between the mass and the surrounding fluid (Pechenik 1983; Woods and DeSilets 1997; also see "Methods").

One alternative to spacing embryos within a clutch would be to divide them among smaller clutches. However, costs of finding numerous suitable sites may preclude this strategy. Availability of attachment sites can limit deposition of clutches (Pechenik 1978; Brenchley 1981; De Martini 1991). Butterflies that laid their eggs in batches rather than singly realized greater fecundity, suggesting a trade-off between search time for appropriate deposition sites and sibling competition (Courtney 1984). For some aquatic animals, investment in extraembryonic gel may be less costly than travel to suitable deposition sites or than lower reproductive output because of scarce sites. For aquatic animals an extreme method of separating embryos is to release eggs or zygotes individually into the plankton, but that option entirely eliminates advantages of aggregation of offspring for retention or protection.

Departures from the Model

The model examined the implications of a single constraint: diffusive supply of oxygen. The objective for the model was not to simulate accurately diffusion and consumption in a mass but, under simple design constraints, to predict an approximate upperbound to egg mass thickness ($2R_{max}$) at which oxygen would be limiting and to predict a scaling relationship between clutch thickness and the required spacing by gel.

Some features of natural egg masses deviate from the assumed design constraints and may explain departures from the model's predictions. First, as oxygen is depleted at the center of a mass, central embryos may reduce consumption and lag in development. Peripheral hatching in some masses may then allow central embryos to resume development as oxygen supply increases (Booth 1995; Strathmann and Strathmann 1995), permitting clutches to be thicker than predicted.

Second, many masses have gelatinous material that does not space embryos, such as an outer layer of stiffer gel (a rind) or gel that attaches masses to the substratum (such as a tether). Because these structures do not increase the space between embryos, they do not aid supply of oxygen but do add to the investment per offspring. Such investments may affect the scaling relationship in natural masses by imposing disproportionate costs of materials for small masses (Todd 1987) and may have caused the scaling exponent in natural masses to be less than the predicted value of 2.

Third, embryos are not uniformly distributed within a mass and are not equally spaced in all dimensions. The number of embryos per capsule had no discernible effect on observed trends, but the distribution of capsules in the gel may have affected the relationship between mass thickness and embryo concentration. Embryos in a string could be spaced by elongating the clutch only in the long axis of the cylinder, which would require less gel than with the assumed spacing in all dimensions. Similarly, a ribbon could be expanded with gel in two dimensions. Such spacing is evident in some of the ribbons that we sampled (fig. 1) and could decrease the scaling exponent for mass thickness relative to embryo concentration. Oxygen consumption is not homogeneous when embryos are aggregated within masses.

Fourth, capsules and gel swell during development (Kress 1971; Seymour and Roberts 1991), and oxygen consumption increases during development (Yanagisawa 1975). Swelling may enhance supply of oxygen by diffusion (Strathmann and Chaffee 1984), but the reduction in concentration of embryos due to swelling does not match the increase in metabolic rate during development (Strathmann and Strathmann 1995; Cohen and Strathmann 1996). Although we may have overestimated oxygen consumption for the measured early-stage masses, later-stage masses nevertheless approach the limits of oxygen supply that are assumed in the model.

Fifth, enhanced oxygen delivery from embryonic stirring of capsule fluid has been suggested (Burggren 1985; Hunter and Vogel 1986), but its role is uncertain (Seymour and Roberts 1991). Even if effective, stirring does not entirely compensate for greater metabolic rates at later stages (Strathmann and Strathmann 1995; Cohen and Strathmann 1996).

Sixth, we assumed an isometric scaling of metabolism with embryo volume. A review of data shows approximately isometric scaling of metabolic rate with larval body mass (Hoegh-Guldberg and Manahan 1995), although some larger embryos contain nutrient stores that are metabolically inert (Hoegh-Guldberg and Emlet 1997). Using an allometric scaling to account for possibly greater nutrient stores in larger eggs would have improved the fit of observed and predicted scaling relationships between R and F/S (exponent b in eq. [4]). Thus, an isometric scaling was a conservative assumption.

Seventh, attached microflora can either increase oxygen concentration through photosynthesis to exceed air saturation or consume oxygen (Bachmann et al. 1986; Pinder and Friet 1994; Cohen and Strathmann 1996). Such microflora may create a diel fluctuation in the concentration of oxygen at the surface of a mass and could either increase or decrease the maximum thickness. The effect of a boundary layer can be less than that of a fouling microflora.

Eighth, a thick boundary layer could reduce oxygen supply to an egg mass (eq. [3]), but motion of water around an egg mass is complicated by movements of the mass. Some gelatinous egg masses oscillate in flowing water. Oscillation may enhance delivery of oxygen to a surface (Koehl and Alberte 1988), but at high current speeds the layers of ribbon can be pushed against each other, resulting in an increase in the effective thickness of the mass and reduction of flow past surfaces (Biermann et al. 1992). Finally, values that were constant in the model vary among egg masses and environments.

Despite the model's simplifications, it predicted a general relationship between spacing of embryos and egg mass thickness similar to that observed for natural masses. The model and observations on both experimental and natural egg masses indicate that siblings' competition for oxygen constrains aggregation of eggs into clutches.

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APPENDIX

Derivation of Equations

The general expression for diffusion into a mass is

$$\frac{dC}{dt} = D\nabla^2 C - S, \tag{A1}$$

where the change in oxygen concentration C over time t is equal to the diffusion coefficient D of oxygen (L^2T^{-1}) times the Laplacian squared (sum of the spatial second derivatives) of concentration minus the oxygen sink S (table A1). Oxygen sink (S) is the oxygen consumption rate per unit of egg-mass volume (moles $O_2\,L^{-3}T^{-1}$); $S = NM/V$, where N is the number of embryos, M is the oxygen consumption per embryo (moles $O_2\,T^{-1}$), and V is volume of the egg mass. The term N/V represents the concentration of embryos in the mass. Use of a single value for the diffusion coefficient (D) ignores possible differences in diffusion in the gel, capsules, or embryos, and S is assumed to be uniform throughout the mass.

At steady state the equations are no longer time dependent and may be expressed as follows for a sphere (Crank 1975; Carslaw and Jaeger 1978):

$$D\left[\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dC}{dr}\right)\right] = S; \tag{A2}$$

for an infinite cylinder:

$$D\left[\frac{1}{r}\frac{d}{dr}\left(r\frac{dC}{dr}\right)\right] = S; \tag{A3}$$

and for an infinite sheet:

$$D\frac{d^2C}{dr^2} = S, \tag{A4}$$

where r is the radius of a sphere or cylinder or half the thickness of a sheet.

R_{max} *for a Sphere in Well-Mixed Water*

Integrating the diffusion equation (A2) for the sphere gives

$$r^2\frac{dC}{dr} = \frac{Sr^3}{3D} + K_1. \tag{A5}$$

At the center of the mass, $r = 0$, and $dC/dr = 0$ by symmetry. Thus, $K_1 = 0$. Integrating again,

$$C = \frac{Sr^2}{6D} + K_2. \tag{A6}$$

To obtain a value for K_2 we use the equation for the surface of the sphere, at $r = R$, where the concentration of oxygen at the surface of the sphere (C_R) is known,

$$C_R = \frac{SR^2}{6D} + K_2. \tag{A7}$$

Rearranging equation (A7) with respect to K_2 and substituting this expression for K_2 in equation (A6) gives the general equation

Table A1: Definitions of symbols

Symbol	*Definition*	*Dimensions*
C_M	Concentration of O_2 in the egg mass	mol L^{-3}
C_R	Concentration of O_2 at the surface of the egg mass	mol L^{-3}
C_W	Concentration of O_2 in the boundary layer around the egg mass	mol L^{-3}
C_∞	Concentration of O_2 in the surrounding water	mol L^{-3}
D	Diffusion coefficient	L^2T^{-1}
D_M	Diffusion of coefficient of the egg mass	L^2T^{-1}
D_W	Diffusion coefficient of water around the egg mass	L^2T^{-1}
F	Shape factor for masses	×××
H	O_2 solubility in sea water/O_2 solubility in gel	×××
$K_1 \sim K_4$	Constants of integration	×××
M	O_2 consumption of an embryo	mol T^{-1}
N	Number of embryos in an egg mass	×××
r	Variable distance along the radius (sphere or cylinder) or half thickness (sheet)	L
R	Radius of a sphere or infinite cylinder or half the thickness of a sheet	L
R_{max}	Maximum R allowed by diffusion	L
S	Egg mass metabolic sink of O_2 (mol O_2/egg mass volume/time)	mol $L^{-3}T^{-1}$
V	Volume of egg mass	L^3

Note: L = length, T = time, M = mass, mol = amount of oxygen.

$$C = C_R + \frac{Sr^2}{6D}\left[1 - \left(\frac{R}{r}\right)^2\right]. \tag{A8}$$

For the concentration of oxygen C to equal 0 at the center of the mass where $r = 0$, S must take on the value $6DC_R/R^2$. Thus,

$$R_{max} = \sqrt{\frac{FDC_R}{S}}. \tag{A9}$$

The variable F is the shape factor and is 6 for a sphere. Performing analogous calculations (integrating [A3] and [A4]) for other shapes yields identical solutions except that for an infinite cylinder $F = 4$ and for an infinite sheet $F = 2$; R_{max} is the maximum radius (sphere or cylinder) or half thickness (sheet) allowed by diffusion; and C_R is equal to the ambient oxygen concentration because the model assumes no boundary layer. Volume of the egg mass (V in the S term) and R_{max}^2 do not cancel each other because R_{max} is dependent on the specific boundary conditions, while N/V is a property of the egg mass. This canceling was an error in a previous model (Strathmann and Chaffee 1984).

R_{max} *for a Sphere in Still Water*

At the surface of the egg mass R, the relationship between C_M, the concentration of oxygen inside the mass, and C_W, the concentration of oxygen in the water outside the mass, is

$$C_W(R) = HC_M(R), \tag{A10}$$

where H accounts for the differences in solubility for oxygen in the gel relative to that in water. Within a spherical mass $0 < r < R$,

$$D_M\left[\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dC_M}{dr}\right)\right] = S. \tag{A11}$$

In the boundary layer outside the mass, $R < r < \infty$,

$$D_W\left[\frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{dC_W}{dr}\right)\right] = 0. \tag{A12}$$

Integrating the diffusion equation for a spherical mass (A11), we obtain

$$r^2\frac{dC_M}{dr} = \frac{Sr^3}{3D_M} + K_1. \tag{A13}$$

At the center of the mass, $r = 0$, and $dC/dr = 0$ by symmetry. Thus, $K_1 = 0$. Integrating again,

$$C_M = \frac{Sr^2}{6D_M} + K_2. \tag{A14}$$

Integrating the diffusion equation for the water outside the mass (A12), we obtain

$$\frac{dC_W}{dr} = \frac{K_3}{r^2}. \tag{A15}$$

Integrating again,

$$C_W = -\frac{K_3}{r} + K_4. \tag{A16}$$

At $r = \infty$, $C_W = C_\infty$. Thus $K_4 = C_\infty$. To obtain K_3 one assumes a balanced oxygen flux at the surface of the mass (R),

$$D_M \frac{dC_M}{dr}(R) = D_W \frac{dC_W}{dr}(R). \quad \text{(A17)}$$

Substituting equation (A13) on the left, and substituting equation (A15) on the right, we obtain

$$K_3 = \frac{SR^3}{3D_W}. \quad \text{(A18)}$$

A value for K_2 comes from the relationship in equation (A10),

$$C_W(R) = -\frac{SR^3}{3D_W}\frac{1}{R} + C_\infty = H\left(\frac{SR^2}{6D_M} + K_2\right). \quad \text{(A19)}$$

Rearranging this equation gives a value for K_2,

$$K_2 = -\frac{SR^2}{3}\left(\frac{1}{HD_W} + \frac{1}{2D_M}\right) + \frac{C_\infty}{H}, \quad \text{(A20)}$$

that can be substituted into equation (A14). Thus, the solutions are

$$C_M(r) = \frac{Sr^2}{6D_M} - \frac{SR^2}{3}\left(\frac{1}{HD_W} + \frac{1}{2D_M}\right) + \frac{C_\infty}{H} \quad \text{(A21)}$$

and

$$C_W(r) = -\frac{SR^3}{3D_W}\frac{1}{r} + C_\infty. \quad \text{(A22)}$$

If we assume that the solubilities of oxygen in water and gel are the same ($H = 1$), then at R, $C_W = C_M$, and

$$C_M(R) = C_W(R) = -\frac{SR^2}{3D_W} + C_\infty. \quad \text{(A23)}$$

A value of 1 for H is probably a good approximation because the apparent drop in oxygen concentration across the surface of *Melanochlamys diomedea* egg masses was 0%- 8.3% of air saturation, and this difference could be in part from stirring sensitivity of the electrodes (Cohen and Strathmann 1996). Substituting equation (A23) for C_R in equation (A9), where D equals D_M, and rearranging the terms with respect to R_{max} yields

$$R_{max} = \sqrt{\frac{6D_M C_\infty}{S\left(1 + 2\frac{D_M}{D_W}\right)}}. \quad \text{(A24)}$$

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