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Embryos at the Edge of Tolerance: Effects of Environment and Structure of Egg Masses on Supply of Oxygen to Embryos

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Abstract. Oxygen concentrations in gelatinous egg masses of two species of opisthobranch gastropods were examined with microelectrodes. Embryos in central positions are near the limit of the oxygen supply required for development. This limit is approached despite a diffusion constant for oxygen in masses that is close to that in water. Closed-chamber respirometry shows that oxygen is consumed by masses in the dark but generated in the light. Internal oxygen concentrations were greater in bright than in dim light. Thus photosynthesis and respiration of microorganisms associated with the masses affects the supply of oxygen to embryos within the mass. This effect of light was confirmed for egg masses of a polychaete. These observations, together with other published observations on the effects of hypoxia on development, indicate that the developmental rates of embryos in egg masses may depend on algal photosynthesis and metabolism. Flow around the masses also affects delivery of oxygen to embryos, but masses in dim light are at the limit of adequate supply even in a strong flow with a very thin boundary layer. Because the central embryos are near the limit for adequate supply of oxygen by diffusion, their development rate thus depends on light, abundance of photosynthetic and heterotrophic microorganisms, flow, and oxygen concentration in the surrounding water.

Introduction

Supply of oxygen limits the rates of development of crowded embryos with limited diffusional exchange (Strathmann and Strathmann, 1995). Retarded development of internal embryos suggests that egg masses of diverse taxa approach the limits of adequate diffusional exchange (Chaffee and Strathmann, 1984; Giorgi and Congleton, 1984; Lucas and Crisp, 1987; Seymour and Roberts, 1991). Such limits could affect the size and form of clutches and the adequacy of sites for deposition of egg masses. Models of supply of oxygen by diffusion suggest that many egg masses are near the limits of thickness of mass and concentration of embryos (Crisp, 1959; Strathmann and Chaffee, 1984; C. E. Lee and R. R. Strathmann, in prep.). Adequate gas exchange for embryos might even influence evolution of alternative modes of development, with dispersal of embryos in the plankton being a simple means of ventilating large clutches of embryos (Strathmann and Strathmann, 1995).

Other environmental factors also impose functional constraints on an egg mass. Functional requirements for the retention and protection of embryos may explain many features of masses. The constraints associated with supply of oxygen and demand for oxygen nevertheless set limits on the structure of clutches. Understanding the physiological limits on egg masses is one step toward understanding the diversity within these limits.

The study described here had two objectives. The first was to test assumptions of models for limits on aggregations of embryos that are supplied with oxygen by diffusion. These assumptions include (1) diffusion constants close to that for oxygen in water, (2) oxygen gradients that approach an oxygen concentration of zero at the center of masses of embryos, and (3) rates of oxygen consumption close to those for hatched larvae. The models predict limits on the structure of clutches: the size and form of

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the mass and the concentration of embryos. Are the assumptions approximately correct?

The second objective was to examine the effects of fouling on the supply of oxygen to embryos. Many egg masses are fouled by unicellular algae and other microorganisms. Algal photosynthesis in light and respiration in darkness can have a profound effect on the supply of oxygen to the embryos of some amphibians (Bachmann *et al.*, 1986). Scaling models for the thickness of clutches and concentrations of embryos in clutches have considered only the respiration of the embryos (Strathmann and Chaffee, 1984; Seymour and Bradford, 1995). If the thin films of fouling microorganisms that are common on egg masses have large effects on oxygen supply, then scaling of egg masses is sensitive to fouling and light intensities as well as to such internal constraints as the concentration of embryos, oxygen consumption by embryos, and the diffusion constant for oxygen.

Materials and Methods

Egg masses

Egg masses of the cephalaspidean opisthobranch gastropods *Melanochlamys diomedea* (Bergh) and *Haminaea vesicula* Gould were used for most observations. Some observations were confirmed with egg masses of the nereid polychaete *Nereis vexillosa* Grube. The egg masses were either collected from False Bay and Argyle Lagoon, San Juan Island, Washington state, or deposited in the laboratory by adult cephalaspideans collected from those locations. Before use, masses were maintained in continuous flow aquaria or in aerated beakers at 12° to 15°C, which was 1°–2°C above the temperature in tidally mixed channels.

The egg masses of *M. diomedea* and *H. vesicula* are described by Hurst (1967) under the names of *Aglaja diomedea* and *Haminoea virescens* and by Strathmann (1987). The egg masses of *M. diomedea* are elongated spheroids often about 1 cm or less through the short axes and greater than 1 cm in the long axis, with a tether at one end that extends into the sediment. The egg masses of *H. vesicula* are thin ribbons about 2 mm thick and 8 mm broad; they are attached at one edge to aquatic plants, shells from dead bivalves, driftwood, or other surfaces. The egg masses of both species are often found intertidally in pools on sand flats, where temperatures at low tide can reach 25°C, or in shallow lagoons. These masses are at times within a few centimeters of the water's surface, exposed to full sunlight.

As in other opisthobranchs, the embryos are in small fluid-filled capsules embedded in a gelatinous matrix. In *H. vesicula* and *M. diomedea* each capsule contains one embryo or occasionally two, with sufficient room for the embryos to rotate once they develop cilia.

The egg masses of both species were sufficiently regular to be measured to the nearest 0.5 mm with calipers. The thinner egg mass of *H. vesicula* has a higher concentration of embryos, about 70,000 g^{-1} in contrast to about 23,000 g^{-1} for *M. diomedea* (C. E. Lee and R. R. Strathmann, in prep.). Eggs of the two species are similar in size, with reported diameters of 75 and 90 μm for *H. vesicula* and 83 and 98 μm for *M. diomedea* (C. E. Lee, pers. com.; Strathmann, 1987).

The egg masses of *Nereis vexillosa* are described by Johnson (1943). They are irregular, several centimeters across, but divided by lobes and channels. Because of the irregular positions and sizes of lobes and channels, minimum diffusion distances are not easily measured, but embryos within a lobe are as much as several millimeters from the surface of the lobe (Chaffee and Strathmann, 1984), and a whole mass is several centimeters in diameter. The masses lie on the substratum unattached and are often stranded intertidally.

Apparatus

Different types of electrodes were available at different times during the study. A few observations were made with a microelectrode with external reference (Diamond General), but most were with micro-Clark electrodes (Diamond General Corp., Ann Arbor, Michigan, and Micro-Sense, Ramat Gan, Israel) with tip diameters of 100 or 150 μm. All electrodes were polarized by a Chemical Microsensor meter (Diamond General Corp.) connected to a chart recorder for permanent recording of data. All electrodes were calibrated in seawater at zero percent oxygen and at air saturation for a given temperature. Most zero calibrations were in nitrogen-bubbled seawater; some were in anoxic sediment with pablum (baby cereal) added to maintain anoxia. A few zero calibrations of the electrodes with external reference were with a 2% solution of sodium sulfite, but this method is incompatible with electrodes that have an internal reference. Zero and saturation calibrations were done at the beginning and end of each recording session to check for drift in electrode sensitivity. Drift was seldom a problem, and data were not used unless drift was negligible.

Stirring sensitivity was determined by the difference between readings in gel and in stirred water at air saturation. The differences between air saturation and zero oxygen were adjusted accordingly for measurements within gelatinous masses. With the Diamond General external-reference electrodes, the apparent mean drop in oxygen was 8.3% across the surface of gelatinous masses of *M. diomedea* at air saturation. With the Diamond General micro-Clark electrodes, the apparent mean drop in oxygen because of stirring was 4.2% at air saturation. No effect of stirring was detected for the Micro-Sense electrodes.

The chambers for measurements with microelectrodes were surrounded by a water bath to keep temperature constant. The chamber for most observations was a beaker submerged in a temperature-controlled water bath. Gas bubbled to maintain concentrations in solution was passed through a coil within the bath to maintain a constant temperature. Egg masses were held in a stream of gas bubbles to minimize the thickness of the boundary layer. The other chamber was a recirculating flow tank with a water jacket to maintain temperature. Most measurements were at 20°C, which is well within the range encountered in nature. A few measurements were made at the temperature of seawater in the aquarium system, which is lower and approximates temperatures at field sites at high tide.

Electrode tips were marked with evenly spaced black lines so that the position of the tip of the electrode could be determined for measurement of oxygen profiles through egg masses.

Measuring gradients

Gradients of oxygen concentration were measured in the globose masses of *Melanochlamys diomedea* with an electrode inserted into the mass along a radius from the outer surface toward the center along the short axis of the mass.

Oxygen concentration at the center of a mass was determined by recording continuously as the electrode was moved into the mass. Concentration was lowest at about the center of a mass, and this value was taken as the central oxygen concentration. Each paired comparison of central and peripheral oxygen concentration was made with a different mass.

Measuring the diffusion constant

The diffusion constant was estimated for the spheroidal masses of *M. diomedea.* The embryos and associated microorganisms were killed by microwaving the masses in a much larger volume of seawater for 8 min to temperatures approaching 60°C. The optical properties of the gel did not change discernibly from this heating. Subsequent microbial growth was controlled by 40 to 100 μg ml^{-1} of gentamycin in seawater (Leahy, 1986). Each mass was equilibrated with nitrogen-purged water and then reequilibrated to air-saturated water for diffusion of oxygen into the anoxic mass, and each mass was also equilibrated with air-saturated water and then reequilibrated to water purged of oxygen by nitrogen bubbling for diffusion of oxygen out of the mass. The diffusion constant was estimated by using the equation of Ingersoll *et al.* (1948, pp. 162–164 and appendix H). This was the method used by O'Dor and Balch (1985) to calculate heat transfer in a spherical egg mass. The equation is

$$(C_c - C_s)/(C_o - C_s) = 2[\exp(-\pi^2 Dt/R^2) - \exp(-4\pi^2 Dt/R^2) + \exp(-9\pi^2 Dt/R^2) - \cdots]$$

with C_c, C_s, and C_o the central, surface, and initial concentrations of oxygen, D the diffusion constant, t time, and R the radius of the sphere. We took the two short axes of the mass as the diameter of a sphere, an assumption that should underestimate the diffusion constant. Estimates of diffusion constants for a mass were consistent except near the asymptote of equilibration, where small errors in measurement of oxygen magnify errors in calculated diffusion constants. We therefore calculated diffusion constants with a reading of central oxygen concentration taken when the oxygen concentration was changing rapidly and was far from the initial and final equilibrium values. The times used were 0.75 or 1 h from the start of each run toward a new equilibration.

Effect of light

To examine the effect of light on oxygen concentrations within masses, we used a halogen lamp with fiber optics and a heat filter (2.5 cm of a solution of copper sulfate) to eliminate temperature changes. Although differences in direction and spectrum of light prevent exact comparisons between light in the laboratory and field, we tried to achieve an intensity of light similar to full summer sun at the water's surface. Readings from a photographic light meter for the arrangement with the fiber-optic light were close to readings from a cloudless summer sky. Black plastic sheets provided darkness. Dim light was the illumination from overhead fluorescent lights in the laboratory, the condition for most of our measurements of oxygen in egg masses. For measurements of effects of light on oxygen concentrations in the cephalaspidean egg masses, the light conditions were maintained until the oxygen concentration became constant, and then oxygen concentration was noted. The cephalaspidean masses were held in the temperature-controlled chamber in a stream of air bubbles. The masses of *Nereis vexillosa* were held in air-saturated water in a flow tank.

Consumption and production of oxygen were determined with a Clark-type oxygen electrode and a closed, well-stirred chamber of 4.1 ml, as described in Herbert and Waaland (1988). Consumption by the electrode was too small to be measured within intervals of several hours. The slope of a chart recording of oxygen concentration over time gave the rate of production or consumption. The concentration of oxygen was calculated from the solubility of oxygen in seawater at 1 atm, about 5.3 μl ml^{-1} at 20°C (Carpenter, 1966). A lamp in a slide projector provided visible light on egg masses at an intensity about half that of summer sun (about 1000 μmol photons m^{-2} s^{-1}) (Herbert and Waaland, 1988).

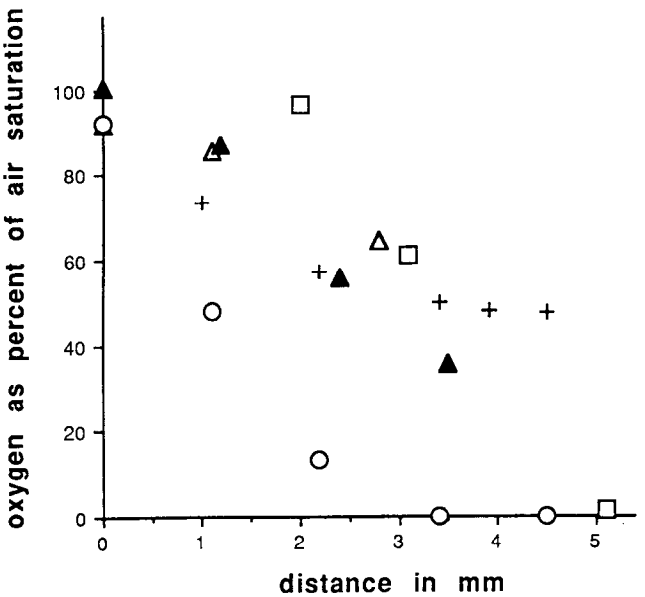

Figure 1. Oxygen concentrations from the surface toward the center along the diameter of five egg masses of *Melanochlamys diomedea* at 20°C. The stages of the masses were early cleavages (+), blastulae (△, open and filled), gastrula–trochophore (□), and veligers (○). The diameters, in the same order, were 9, 10, 8, 11, and 9 mm.

Following estimates of oxygen consumption for masses of *M. diomedea,* numbers of embryos were counted by freeing capsules from the gel with a 1% solution of sodium hypochlorite. The capsules were rinsed, and a suspension of capsules from a mass was subsampled for counts of embryos. The accuracy of subsampling was confirmed by a few counts of capsules from whole masses.

Results and Discussion

Gradient of oxygen concentration in masses

Oxygen concentrations in egg masses declined from the surface toward the center. The gradient in oxygen concentration was most easily and clearly demonstrated for masses of *Melanochlamys diomedea,* which were regular in shape and sufficiently thick that an electrode could be placed at measured distances from the surface toward the center (Fig. 1). The oxygen concentrations were similar near the surface in masses at all stages of development. Oxygen concentrations declined more rapidly from the surface toward the centers of masses at more advanced stages of development. Variations in oxygen concentration that were unrelated to stage of development could have resulted from variation in size of masses, concentrations of embryos, fouling by microorganisms, or errors in estimating the position of the electrode tip. For example, the near-saturation value at 2 mm in one mass at the gastrula–trochophore stage (Fig. 1) could have been due to an unusually thick embryo-free zone near the outer surface or an error in estimating the depth of the electrode tip.

To test for the significance of the effects of stage of development and distance from the surface, we used ANOVA for the outer and nearly linear portion of the oxygen gradient, eliminating the inner three points for the mass at early cleavage stages and the inner two points for the mass at the veliger stage. Distance from the surface, stage of development, and their interaction were all significant at $P < 0.01$ ($n = 15$, four stages, two masses at blastula stage lumped). This result was also obtained when the two masses at the blastula stage were treated as separate stages. The significant interaction confirmed the steeper gradient in the outer part of the egg mass at more advanced stages of development (Fig. 1).

Oxygen concentrations at centers of masses

Oxygen concentrations were extremely low at the centers of masses. Central oxygen concentrations were lower in masses at more advanced stages of development (Fig. 2). Central oxygen concentrations were low in both the thin and thick masses but lower in the thicker globose masses of *Melanochlamys diomedea* than in the thinner ribbons of *Haminaea vesicula* (Fig. 2). In an ANOVA with stage as a continuous variable, the effects of stage and species on central oxygen concentration were signif-

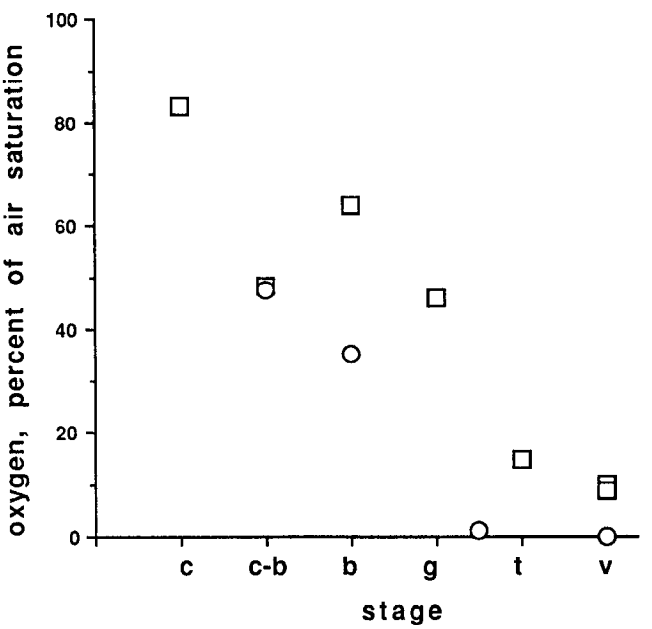

Figure 2. Oxygen concentrations at the centers of gelatinous egg masses versus stages of development. Stages are early cleavages (c), cleavages to blastula (c–b), blastula (b), gastrula (g), trochophore (t), and veliger (v). Circles are masses of *Melanochlamys diomedea;* squares are masses of *Haminaea vesicula.* Moving cilia develop between the gastrula and trochophore stages.

Table I

Estimated diffusion constants for oxygen in three egg masses of Melanochlamys diomedea *based on the equation for diffusion in a sphere of diameter equal to the two short axes of the masses*

Axes (mm)	Diffusion constant* (10^{-5} cm^2 s^{-1})	Estimated depths of electrode tip (mm)	Directions of equilibration
13 × 11.5 × 11.5	2.14, 2.01, 1.91	5.6, 5.1, 4.5	++−
19 × 9 × 9	1.92, 1.87	4.5, 4.5	+−
17.5 × 8 × 8	1.37, 1.51	4.5, 4	+−

* Each estimate is for a separate equilibration with directions of equilibration anoxic to air saturated (+) or air saturated to anoxic (−).

icant at $P < 0.001$ and $P = 0.021$ ($n = 11$, df 1, 8). The interaction term was omitted from the test because it was not significant in an ANOVA that included it. The diffusion distances were much less for the egg ribbons of *H. vesicula* (about 8 to 10 mm wide and 2 mm thick) than for the globose masses of *M. diomedea* (13 to 16 mm long and 8 to 11 mm in diameter). However, the concentration of embryos was much higher in ribbons of *H. vesicula* (see above). Central oxygen concentrations were low even though these masses were held in a stream of air bubbles so that water near the surface of the mass was air-saturated and well-stirred. All of these masses were at 20°C.

Diffusion constant

Diffusion constants for oxygen in egg masses were estimated from the rate of change in oxygen concentrations at the centers of egg masses first equilibrated at one oxygen concentration and then exposed to water at a different oxygen concentration. The reequilibration was from air saturation to anoxia or from anoxia to air saturation. Three egg masses from *M. diomedea*, in which embryos and microorganisms had been killed, were reequilibrated in both directions for at least two observations per mass, in all cases at 20°C. The estimates of the diffusion constant (Table I) were close to the 2×10^{-5} cm^2 s^{-1} reported for water at 20°C (Horne, 1969). There was little variation between estimates from separate equilibrations for an egg mass, but significant differences between estimates for different egg masses (ANOVA, $P < 0.01$).

This result indicates that the low supply of oxygen to central embryos is not the result of a low diffusion constant; however, several assumptions of the calculation could bias the estimate. We assumed that the masses were spheres with diameters equal to the short axes of the masses. The longer diffusion distance in the long axis should result in an underestimate in the diffusion constant, and the estimated diffusion constants were indeed lower for the two elongate masses than for the more nearly spherical mass (Table I). Misplacement of the electrode off-center should have the opposite effect, but repeat runs gave similar values even when the electrode was replaced at depths differing by up to 1 mm. The estimate of diffusion constant is sensitive to measurements of the diameter of the mass, and the inaccuracy of measuring the dimensions of gelatinous masses with calipers could bias estimates of the diffusion constant. Distortion and wobbling of the mass in the bubble stream could shorten diffusion distances, thus giving an overestimate of the diffusion constant. The estimates of the diffusion constant are approximate but support an assumed value close to the diffusion constant for oxygen in water.

Effect of light on oxygen concentration

Gelatinous masses become fouled with a variety of organisms. Diatoms were especially conspicuous among foulers of the opisthobranch and polychaete masses. Respiration and photosynthesis by these fouling organisms would be expected to affect net oxygen consumption by the whole mass, thereby affecting the supply of oxygen to internal embryos. We tested this hypothesis by measurements of oxygen concentrations in bright light in contrast to dim light or darkness. Oxygen concentrations within egg masses were affected by light (Table II). In the masses of the opisthobranchs, for which fouling was restricted to the surface, oxygen concentrations were affected both at the surface of the masses and at depth. Under some conditions the fouling organisms produced supersaturating amounts of oxygen near the outer surface, even in well-stirred and air-saturated water, as in the mass of *H. vesicula* with oxygen at 130% of air saturation. The egg masses of *Nereis vexillosa* were conspicuously fouled by diatoms and other organisms, which penetrated into the mass. Because the shapes of these egg masses were irregular with lobes and channels, the position of the electrode relative to the surface of the mass was not so easily char-

Table II

Effect of light on oxygen inside egg masses of Melanochlamys diomedea, Haminaea vesicula, *and* Nereis vexillosa

Species	Stage	Position of electrode	Percent of air saturation: Dark or dim light	Percent of air saturation: Bright light	Ratio of concentrations in light and dark
M. diomedea	veliger	2.2 mm inside	15.2	20.5	1.35
M. diomedea	veliger	near surface	38.1	47.5	1.25
H. vesicula	veliger	center	8.9	41.0	4.6
H. vesicula	veliger	near surface	—	130	—
N. vexillosa	with cilia	in a lobe	26	42	1.6
N. vexillosa	with cilia	in a lobe	21	49	2.3

Table III

Production and consumption of oxygen in a closed chamber by four masses of Melanochlamys diomedea *in light and dark*

Production in light (μmol O_2 min^{-1})	Consumption in dark (μmol O_2 min^{-1})	Volume of mass (mm^3)	Stage	Temperature (°C)
0.0048	0.0035	630	veliger	20
0.0065	0.0068	600	cleaving	20
0.0049	0.0030	271	hatching	20
0.0024	0.0040	352	veliger	12

Table IV

Consumption of oxygen per egg mass and per embryo for four masses of Melanochlamys diomedea *in a closed chamber in the dark; all were at early veliger stage*

Consumption (μmol O_2 min^{-1})	Volume of mass (mm^3)	Embryos per mass	Consumption per embryo (10^{-3} μl h^{-1})	Temperature (°C)
0.0037	527	14960	0.33	20
0.0052	471	18390	0.38	20
0.0059	670	9139	0.87	20
0.0047	452	23910	0.26	20
0.0029	452	23910	0.17	12

acterized, but light had a pronounced effect on oxygen concentrations within the mass.

Oxygen production and consumption in light and dark

Fouled masses of *M. diomedea* produced oxygen in bright light (Table III, Fig. 3). Microbial photosynthesis can be large relative to respiration by embryos. In the dark, the masses consumed oxygen (Table III, Fig. 3).

In the dark, the mean estimated respiration per embryo at 20°C was 0.46×10^{-3} μl h^{-1} (Table IV), which is close to that expected for hatched and swimming veligers of similar size (Bayne, 1983). This agreement may not reflect real similarity in the respiration rates of hatched and unhatched larvae. Instead, respiration by microorganisms may be inflating the oxygen consumption per unhatched veliger.

Oxygen consumption per embryo was calculated from consumption by whole masses, and the variation in estimates could therefore be a result of differences in fouling by microorganisms. The highest estimate (0.87×10^{-3} μl oxygen h^{-1}) exceeds that expected for hatched veligers of similar size (Bayne, 1983), and respiration by microorganisms may account for the excess. However, the oxygen consumption per embryo is inversely related to the estimated concentrations of embryos in the masses listed in Table IV. Possible explanations for this relationship are that (1) it occurred by chance, (2) higher concentrations of embryos depressed oxygen consumption, or (3) egg masses with fewer embryos per unit volume had more fouling organisms per embryo. We have no reason to suspect large errors in counts of embryos.

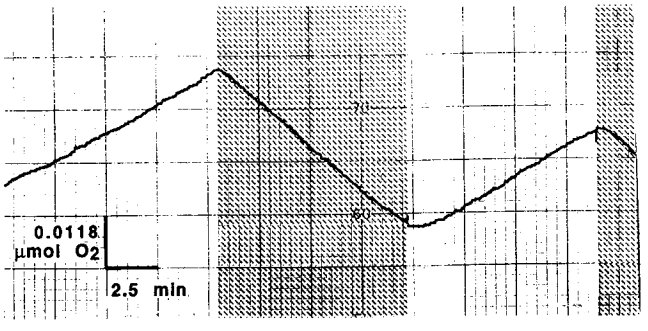

Figure 3. Trace of oxygen concentration in a closed chamber containing an egg mass of *Melanochlamys diomedea* in light and dark (interval shown by hatching).

Effect of boundary layer

An oxygen gradient around egg masses could be detected for egg masses in slow currents. For most of our observations, the boundary layer was minimized by holding masses in a stream of gas bubbles. To observe an external oxygen gradient around a mass, masses of *M. diomedea* were tethered in the sediments of a flow tank with a free-stream velocity of 8 cm s^{-1} at 18° to 19°C. Electrodes were positioned above the mass. Oxygen concentration dropped measurably as the electrode approached a mass (Table V), but the drop was much less than the decrease from the surface to the center of the mass (Figs. 1 and 2).

General Discussion

The measurements of oxygen concentrations within masses confirmed previous observations about the limits on the size and form of clutches of embryos. Oxygen concentrations were low near the center of embryo masses at

Table V

Gradients of oxygen concentration external to two egg masses of Melanochlamys diomedea

Axes of mass (mm)	Distance from mass (mm)	Oxygen (% of air saturation)
$13 \times 7 \times 7$	>10	85.0
	0 to 1	78.4
$16.5 \times 15 \times 15$	>10	100
	2	98.4
	1	96.5

the motile stages beyond gastrulation and in dim light. Even in air-saturated water, central embryos were near the oxygen concentration at which development is retarded (Strathmann and Strathmann, 1995). The measurements confirmed, approximately, assumptions on diffusion constants, oxygen gradients, and oxygen consumption that have been used to model the size and form of clutches (Strathmann and Chaffee, 1984). However, the observations also demonstrated that fouling microorganisms influence oxygen supply to embryos, a factor omitted from scaling models. We shall first discuss the properties of egg masses that influence oxygen supply and then discuss the effects of fouling microorganisms and other environmental factors.

The estimated diffusion constants for masses of *Melanochlamys diomedea* approach the diffusion constant for oxygen in water. Thus the low oxygen concentrations within the egg masses do not result from low diffusion constants for the material that separates and protects the embryos. The estimates for the diffusion constant are reassuring, because a diffusion constant close to that for oxygen in water has been a convenient assumption for models of oxygen supply to egg masses (Strathmann and Chaffee, 1984; Seymour and Bradford, 1995). The estimated diffusion constants were also close to Burggren's (1985) estimate of the diffusion constant for oxygen in frog egg jelly, which was 75% of that for oxygen in water. Our higher estimates for the diffusion constant surprised us because the diffusion constant in artificial gels decreases below that for water as the concentration of the polymer increases (Sato and Toda, 1983; Renneberg *et al.*, 1988). A possibility suggested to us by T. Hunter and P. Verdugo is that aligned macromolecules could allow more rapid diffusion in a gel. Another possibility is that biases in the measurement caused an overestimate, but the most obvious bias—the assumption that the masses were spheres of diameter equal to the two short axes—should underestimate the diffusion constant. A comparison of natural and artificial gel masses could determine whether the diffusion constants that we estimated for natural masses are indeed higher than for artificial gels of similar organic content or are in error.

Although features other than high diffusion constants may aid transport of oxygen to embryos, it is rate of diffusion that limits supply of oxygen for many clutches of embryos. Stirring by embryos in fluid-filled capsules may increase the rate of supply of oxygen (Burggren, 1985; Hunter and Vogel, 1986). However, embryos do not have motile cilia at early stages; stirring does not compensate for the increased metabolism at ciliated stages (Strathmann and Strathmann, 1995); and intracapsular stirring may be ineffective for transport through a mass if the oxygen concentration in each capsule is a local minimum (Seymour and Roberts, 1991). Pores that permit interstitial flow between embryos would impose a different kind of restriction on ventilation of masses (Strathmann and Chaffee, 1984). Although the gelatinous masses of amphibians have pores and the masses of *Nereis vexillosa* have irregular channels, most gelatinous masses lack pores.

The concentrations of oxygen at the centers of masses of *M. diomedea* were as low as those that retard development and cause smaller size at hatching (Strathmann and Strathmann, 1995). The observed concentrations were thus consistent with observations of retarded central embryos in masses from the field (Chaffee and Strathmann, 1984).

Even the thin ribbons of *Haminaea vesicula* were near the limit for a sufficient supply of oxygen to central embryos. The oxygen concentration was near 10% of air saturation at the center of the egg ribbon of *H. vesicula* when embryos were at the veliger stage in dim light. This oxygen concentration approaches the hypoxia at which development is retarded (Strathmann and Strathmann, 1995) despite the short diffusion distance to central embryos. Oxygen is depleted because the concentration of embryos in the ribbon is so high—much greater than in the thick globose egg mass of *M. diomedea*—about 70,000 g^{-1} compared with 23,000 g^{-1} (C. E. Lee and R. R. Strathmann, in prep.).

The discovery that the microorganisms fouling the masses played a large role in production and consumption of oxygen was in striking contradiction to the assumption made in previous models of diffusion in egg masses. Light increased oxygen supply to embryos by its effect on photosynthesis, and respiration by microorganisms must similarly decrease oxygen supply in darkness. Thus the limits on clutch structure that are set by oxygen supply cannot be predicted from the consumption of oxygen by embryos. Fouling microorganisms complicate predictions of scaling limits on the size, form, and concentrations of embryos in egg masses.

Light could switch masses from consumption of oxygen to production of oxygen. Deposition at sunnier sites could enhance oxygen supply by day, but respiration of microorganisms could decrease the supply of oxygen at night even at shallow sites. These effects of algal fouling have been observed for eggs of salamanders, with photosynthesis exceeding consumption in light and respiration severely depleting oxygen in darkness (Bachmann *et al.*, 1986; Pinder and Friet, 1994).

Visible algal fouling was limited to the surface of the masses of the opisthobranchs. For these masses, fouling changed the oxygen concentration at the boundary of the mass. In contrast, pennate diatoms and other fouling organisms penetrated the masses of *Nereis vexillosa* and changed the consumption and production of oxygen throughout the mass.

At high light intensities, damage from solar radiation could exceed the benefits from photosynthesis, but fouling algae could also protect embryos from solar radiation in the ultraviolet range. Solar radiation can be lethal for embryos in masses of some species (Biermann *et al.*, 1992; Blaustein *et al.*, 1994).

The concentrations of embryos and sizes of the masses examined here are near the limits at which the supply of oxygen supports full development rates and hatching sizes of central embryos (Strathmann and Strathmann, 1995). Other masses also approach these limits (C. E. Lee and R. R. Strathmann, in prep.). Photosynthesizing microorganisms, high oxygen concentrations in the surrounding water, and narrow boundary layers are factors that maintain an adequate supply of oxygen for development. Respiring microorganisms, low oxygen concentrations in the surrounding water, and weak velocity gradients around the masses tip the balance toward hypoxia and thereby retard or arrest development. These conditions influencing oxygen supply could affect the evolution of the size and shape of egg masses, the concentration of embryos, and the choice of deposition sites.

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