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OXYGEN AVAILABILITY AND EMBRYONIC DEVELOPMENT IN SAND SNAIL (*POLINICES SORDIDUS*) EGG MASSES

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Summary

The oxygen transport physiology of sand snail *Polinices sordidus* egg masses was investigated using oxygen microelectrodes and open-flow respirometry. *P. sordidus* eggs are laid in a jelly matrix that rapidly absorbs water and swells into a horseshoe-shaped sausage. The average diameter of these sausages is 37 mm. Eggs are enclosed in capsules that are distributed throughout the jelly matrix, but 65 % of the eggs are located within 3 mm of the outer surface. There is no circulatory or canal system within the matrix so all gas exchange between developing embryos and the environment must occur by diffusion through the jelly matrix.

Oxygen tension in the outer layer remains moderately high (P_{O_2}>10 kPa) throughout incubation but decreases rapidly in more centrally located regions, so that by day 4 embryos in this region are exposed to extremely hypoxic conditions (P_{O_2}<1 kPa). This hypoxia limits oxygen consumption of embryos to low levels and appears to slow embryonic development or even to arrest it. From day 4 onwards, the central region gradually become less hypoxic because the hatching of peripherally located embryos causes the outer layers of the jelly matrix to disintegrate and thus reduces the diffusion distance for oxygen between the centrally located embryos and the surrounding sea water. As the oxygen tension rises, development accelerates and the embryos eventually hatch as viable veligers, apparently unharmed by their prolonged exposure to hypoxia.

Key words: embryonic development, mollusc, snail, egg mass, hypoxia, diffusion, *Polinices sordidus*.

Introduction

Many gastropod molluscs (most notably the opisthobranchs) lay their eggs in capsules that are surrounded by a jelly-like material which swells to a variable extent on contact with sea water. The shape of these egg masses is often ribbon-like, the ribbons being attached to rocks or other suitable surfaces. The ribbon is frequently laid down in a coil because the parent crawls in a spiral whilst laying its eggs (Hurst, 1967). Embryos develop within these ribbons and eventually hatch as veliger larvae. The jelly in the ribbons is usually less than 3 mm thick and, thus, does not pose a serious problem for respiratory gas exchange to the developing embryos because the diffusion distance from the embryo to sea water is only 1–2 mm. A few gastropod molluscs lay their eggs in clumps of jelly attached to the substratum, and these clumps can reach dimensions that may seriously impair diffusion of respiratory gases to the innermost embryos which may, in turn, cause retardation of embryonic development (Chaffee and Strathmann, 1984; Strathmann and Chaffee, 1984). There is no circulatory system or system of water-filled channels within these egg masses that could be used to ventilate the innermost embryos with oxygen-rich water. Because the jelly matrix surrounding the eggs in these egg masses is primarily composed of water, it is likely that the diffusion coefficient for oxygen of the jelly is similar to that of water. If this is so, the maximum distance over which the oxygen demands of living tissues can be met solely by diffusion is of the order of 2–5 mm (Schmidt-Nielsen, 1990). Consequently, if oxygen is necessary for development, gastropod egg masses should be limited to radii of less than 5 mm. However, one species of gastropod mollusc, *Polinices sordidus* which inhabits intertidal sand and mud flats along the eastern coast of Australia, lays egg masses which have extraordinarily large dimensions. These egg masses are sausage-shaped, frequently exceed 100 g in mass (up to 210 g has been recorded) and have radii that frequently exceed 20 mm, while radii of up to 40 mm have been recorded (Shepherd and Thomas, 1989). Although most *P. sordidus* embryos are concentrated in a layer close to the outside surface of the egg mass, embryos do occur in the middle (Murray, 1962). This means that any oxygen reaching them must diffuse through 10 mm or more of jelly, a distance seemingly too great to support even an extremely low aerobic metabolism. The aims of the current study are to examine the oxygen transport physiology of these egg masses and to identify the strategy used by the innermost embryos to overcome the potentially severe limitation placed on oxygen transport by the large diffusion distance between themselves and the surrounding sea water.

Materials and methods

Collection and maintenance of egg masses

I collected egg masses at low tide from Nudgee Beach sand flat, Moreton Bay, Queensland (27˚21′ S, 153˚06′ E) between January and March 1994. Only egg masses that appeared to be freshly laid, as indicated by the outermost embryos still being at the early cleavage stage of development, were collected. Egg masses were transported in sea water to the laboratory, where individual masses were placed in plastic containers (17 cm×17 cm×10 cm) and incubated at 25±0.5 ˚C in a recirculating seawater system. The bottom and walls of the plastic containers were perforated by numerous holes which allowed circulation of sea water around the egg masses. An incubation temperature of 25 ˚C was chosen because this is close to the mean summer surface water temperature in Moreton Bay.

Morphometric measurements of egg masses

I weighed egg masses (±0.1 g) on an electronic balance and measured the circumference at the widest point with a piece of string. The length of string was then measured with a ruler (±1 mm). I then calculated egg mass radius by dividing the circumference by 2π. I determined egg mass volume by water displacement (±5 ml) in a 500 ml measuring cylinder and calculated egg mass density by dividing mass by volume. I determined the water content of egg masses by oven-drying at 70 ˚C for 48 h. To estimate the absolute density of eggs and the total number of eggs within a spawn mass, I cut 2–5 mm thick slices (1–2 g) at three places along the egg mass, weighed these slices (±0.0001 g) and counted the number of eggs in the slice under a dissecting microscope. Average egg density was calculated by dividing egg counts by slice mass and multiplying by the density of the egg mass. I noticed that eggs were distributed more numerously within 3 mm of the outside surface compared with the more central areas. To quantify this regional difference, I randomly cut cross-sectional slices and separated the outer 3 mm from the more central region. I then weighed each of these regions and counted the number of eggs in each. Egg density for each region was then calculated as described previously.

Oxygen partial pressure profile measurements

I tied egg masses to plastic Petri dishes perforated with numerous 5 mm diameter holes. These dishes were then placed on a grid suspended in a tank of vigorously aerated sea water maintained at 25±0.5 ˚C. I measured oxygen partial pressure (P_{O_2}) with a microelectrode (model 737, Diamond General Corporation, outside diameter 30 μm) connected to a polarizing amplifier (model OM200, Cameron Instrument Company), the output of which was connected to a computerized data acquisition system (Datacan V, Sable Systems). The microelectrode was attached to a micromanipulator and calibrated in nitrogen-saturated sea water (zero) and air-saturated sea water (span) at 25 ˚C immediately before and after measurements were made. Any drift between calibration times (typically less than 0.5 kPa) was assumed to be linear and an appropriate correction was applied. Immediately after the span calibration, the tip of the electrode, which was viewed through a horizontally mounted dissecting microscope, was lowered so that it just touched the outer surface of the egg mass and the P_{O_2} was recorded after the reading had stabilised (typically 1–3 min). I then advanced the electrode tip 1 mm into the egg mass, where another reading was taken after the reading had stabilised. This process continued until the electrode tip was 10 mm from the egg mass surface. After this, I advanced the electrode tip in 2 mm steps until a point 20 mm from the surface was reached. Egg masses that had been incubating for 14 days had lost a significant amount of their initial mass, so the electrode was only advanced to a point 10 mm from the egg mass's surface.

Oxygen consumption measurements

I measured oxygen consumption ($\dot{V}_{O_2}$) of egg masses at 25±0.5 ˚C with an open-flow system. The system consisted of three independent lines: a span line, which was used in calibration to set the span of the oxygen electrode; a blank line that contained a glass respirometry chamber (230 ml) full of sea water, so that microbial respiration could be corrected for; and a measurement line consisting of a glass respirometry chamber (230 ml) housing an egg mass and sea water. The outlet from each respirometry chamber was covered by an aquarium airstone, which prevented parts of the jelly mass from exiting the chamber. An air-equilibrated seawater reservoir and the respiratory chambers were placed in a constant-temperature water bath at 25±0.5 ˚C. Sea water was drawn through all three lines simultaneously by a peristaltic pump (model 7519, Cole Parmer) at a rate of 10 ml min^{-1}; the outflow of each line was directed in turn past an oxygen electrode (model 371, Diamond General Corporation) thermostatted at 25 ˚C. The electrode was connected to a polarizing amplifier (model OM200, Cameron Instrument Company), the output of which was recorded with a computerized data acquisition system (Datacan V, Sable Systems). I calibrated the oxygen electrode with water-saturated nitrogen gas (zero) and air-equilibrated sea water (span). Any drift between calibration times (typically less than 0.2 kPa) was assumed to be linear and an appropriate correction applied. The seawater lines were constructed of copper or stainless-steel tubing, except for 30 cm of Tygon tubing located in the pump head. I measured the salinity of the water in the reservoir with an optical refractometer (A.S.T. company) and used standard oxygen solubility tables (Cameron, 1986), taking into account salinity and temperature, to determine the oxygen capacitance of reservoir sea water. I calculated microbial $\dot{V}_{O_2}$ by multiplying flow rate by oxygen capacitance and the change in P_{O_2} of water entering and exiting the blank respirometry chamber. I calculated egg mass $\dot{V}_{O_2}$ by multiplying flow rate by oxygen capacitance and the change in P_{O_2} of water entering and exiting the measuring respirometry chamber, and corrected this value for microbial respiration of the volume of sea water in the chamber (seawater volume=230 ml minus the volume of

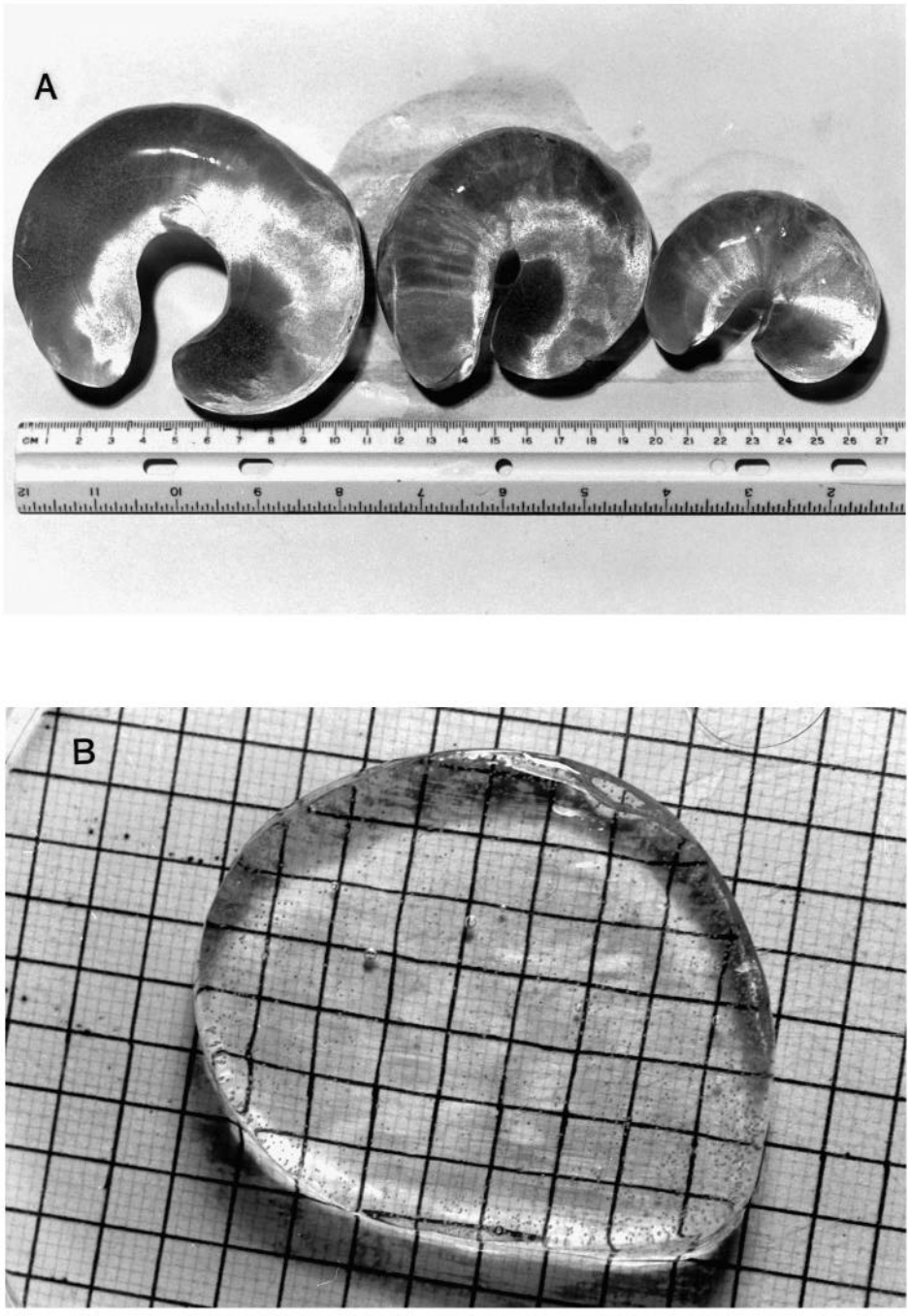

Fig. 1. (A) Freshly laid 'sausage blubber' egg masses of *Polinices sordidus*, indicating the typical size range found in Moreton Bay. (B) Cross section through a typical *P. sordidus* egg mass. Note that egg capsules are found throughout the jelly matrix, but are more numerous within 3 mm of the outside surface. Large dark squares are 5 mm×5 mm; small light squares 1 mm×1 mm.

the egg mass). In some experiments, I made measurements of $\dot{V}_{O_2}$ of the egg masses before and after the jelly matrix had been physically disrupted. In these experiments, I first measured the $\dot{V}_{O_2}$ of the intact egg mass, and then removed the egg mass from the chamber, placed it in 50 ml of air-equilibrated sea water at 25 °C and vigorously agitated it by hand with a scalpel for 60 s. The jelly mass rapidly broke up into small pieces (less than 2 mm in diameter) during this treatment. I poured the resultant liquefied jelly back into the chamber, topped the chamber up with air-equilibrated sea water and measured oxygen uptake again.

Results

Morphology

The size range and general morphology of the horseshoe-shaped 'sausage blubbers' characteristic of *P. sordidus* egg masses are illustrated in Fig. 1, along with a cross-sectional slice that shows the typical egg capsule distribution pattern within the gelatinous egg mass. The morphological variables of *P. sordidus* egg masses sampled are listed in Table 1. Egg masses are composed primarily of water (95.7 % on a wet mass basis), which is held in a gelatinous matrix. An average-sized egg mass (107 g) contains approximately 38 000 eggs, but these

Table 1. *Morphometric variables of* Polinices sordidus *egg masses*

Variable	Units	Mean	Standard error	Range	Sample size
Mass	g	107	4	24–188	57
Radius	mm	18.4	2.5	11.9–25.5	52
Density	g ml^{-1}	1.046	0.006	1.009–1.135	22
Mean egg density	eggs ml^{-1}	354	4	266–384	17
Water content	%	95.7	0.1	95.1–95.9	12

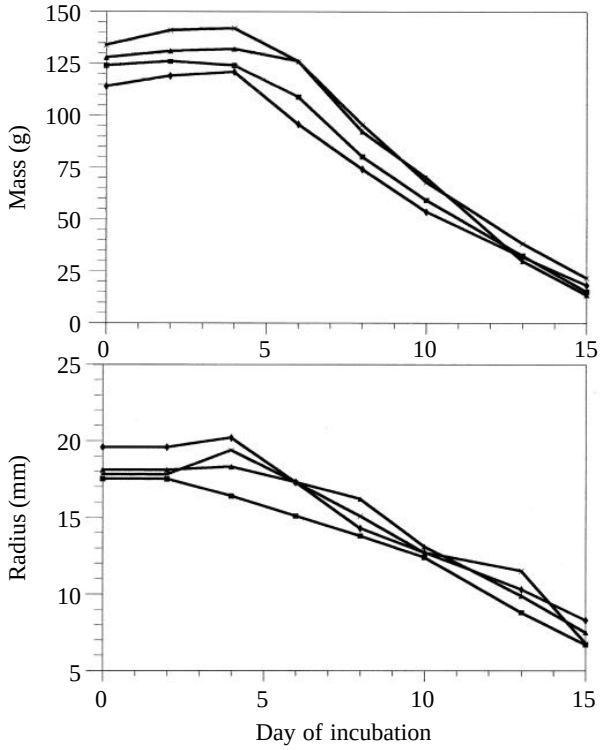

Fig. 2. Mass and radius variation throughout incubation in the four *Polinices sordidus* egg masses whose oxygen consumption was monitored throughout incubation.

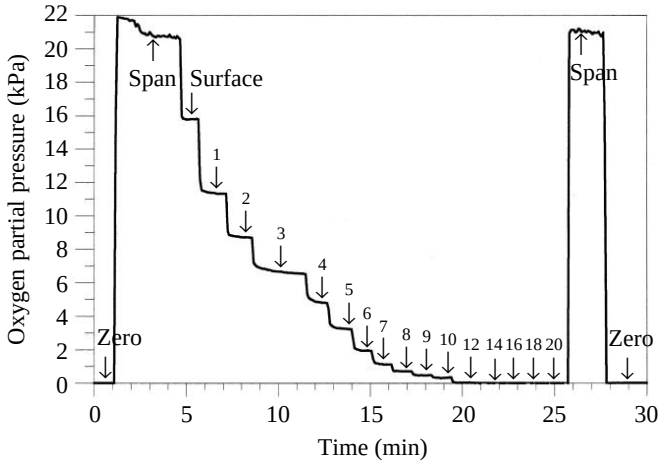

Fig. 3. A typical record of the oxygen partial pressure profile through a day 7 *Polinices sordidus* egg mass. After zeroing and setting the span, the profile was recorded and then the span and zero were checked again. Numbered arrows represent the distance in millimetres of the electrode tip from the surface of the egg mass.

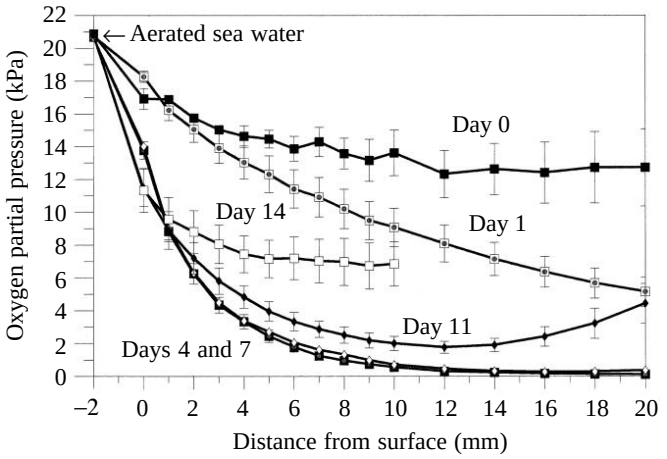

Fig. 4. Mean oxygen partial pressure profiles on various incubation days of 10 *Polinices sordidus* egg masses. By day 14, the egg mass radius had been reduced considerably so the oxygen electrode was only inserted 10 mm instead of the usual 20 mm. Error bars indicate standard error.

are not evenly distributed throughout the jelly matrix. Egg density within 3 mm of the outside surface (770 eggs ml^{-1}) is approximately four times greater than that of more centrally located eggs (190 eggs ml^{-1}). The mean radius of egg masses soon after being laid, when the jelly has swelled to its maximal size by absorbing sea water, is 18.4 mm. In egg masses that are incubated at 25 °C, the embryos on the outer surface of the egg mass begin to hatch on day 4, and this process dissolves the jelly matrix in that region so that, from this period onward, the mass and radius of the egg mass steadily decline (Fig. 2). Embryos continue to hatch from the outer surface until day 16–17 of incubation, by which time the entire jelly matrix has disintegrated.

Oxygen partial pressure profiles

A typical recording from an oxygen microelectrode as it was plunged into an egg mass is shown in Fig. 3. Oxygen partial pressure within egg masses decreases from a maximum on the outside surface to a minimum in the centre. Mean profiles of 10 egg masses throughout incubation are shown in Fig. 4. The precise time of laying is not known for these egg masses; however, when profiles were first measured (indicated by day 0 in Fig. 4), 12–24 h had lapsed since they were laid. There is a significant decrease in P_{O_2} from sea water to the surface of the egg masses even in freshly laid egg masses, and this difference increases as incubation proceeds. On day 0, there is a relatively small difference in P_{O_2} between the surface of the egg masses and the centre, but by the following day (day 1) P_{O_2} decreases in a steady and continuous manner towards the centre. The P_{O_2} profiles of egg masses on days 4 and 7 are

almost identical. P_{O_2} decreases very steeply near the surface, where the egg density is greatest, falling from 14 kPa at the surface to 4 kPa only 3 mm from the surface. P_{O_2} continues to decrease as the centre is approached and becomes highly hypoxic (<1 kPa) 10 mm from the surface. By day 11 of incubation, a large number of embryos have hatched from the peripheral area of the egg mass, causing the overall radius to decrease from its starting value of 18.5 mm to approximately 13 mm. Hence, by day 11 the diffusing distance for oxygen is significantly reduced and, as a result, the P_{O_2} in the central region increases slightly. The egg mass continues to disintegrate as incubation proceeds, so that by day 14 the egg mass radius has decreased to only 8 mm, further facilitating diffusive transport of oxygen to the central region and resulting in a rise in P_{O_2} to 8 kPa.

Oxygen consumption

Oxygen consumption (standardised per gram of egg mass on the day that $\dot{V}_{O_2}$ was measured) of whole egg masses increased over the first 6 days of incubation, but then remained almost constant for the remainder of incubation (Fig. 5). $\dot{V}_{O_2}$ of whole egg masses on day 6 of incubation was increased significantly (P<0.001, paired t-test) by an average of 300 % when the jelly matrix was disintegrated by physical agitation (Table 2).

Discussion

Eggs of *P. sordidus* are laid in fluid-filled capsules that are surrounded by a gelatinous matrix. The matrix is surrounded by a tough transparent membrane. Eggs are fertilized internally from sperm stored within the female reproductive tract (Murray, 1962). When first laid, the jelly matrix rapidly absorbs water and swells, stretching the outer membrane, and the egg mass takes on a characteristic horseshoe 'sausage blubber' shape (Fig. 1). The jelly matrix is 95.7 % water and embryos are distributed throughout, but they are more concentrated adjacent to the outside surface. In an averaged-sized egg mass that weighs 107 g and has a radius of 18 mm, 65 % of the eggs are located within 3 mm of the outer surface.

Embryonic development begins soon after laying, a process that requires oxygen. Oxygen must diffuse through the jelly matrix to reach the embryos because there is no convective transport system within the egg mass. Thus, the rate of oxygen delivery to embryos is determined by Fick's law of diffusion:

$$\dot{V}_{O_2} = K_{O_2} \times A/L \times (Pw_{O_2} - Pe_{O_2}),$$

where K_{O_2} is Krogh's diffusion coefficient, A is the effective gas-exchanging surface area, L is the diffusion path length, Pw_{O_2} is oxygen partial pressure at the surface of the egg mass, and Pe_{O_2} is oxygen partial pressure at the egg capsule surface.

Given that the dimensions of egg masses do not change over the first 4 days of incubation, and that Pw_{O_2} and K_{O_2} also do not change, then the only way in which an increase in oxygen transport can be achieved is for oxygen partial pressure at the embryos' surface to decrease. Cell division and differentiation during development require an increase in oxygen supply.

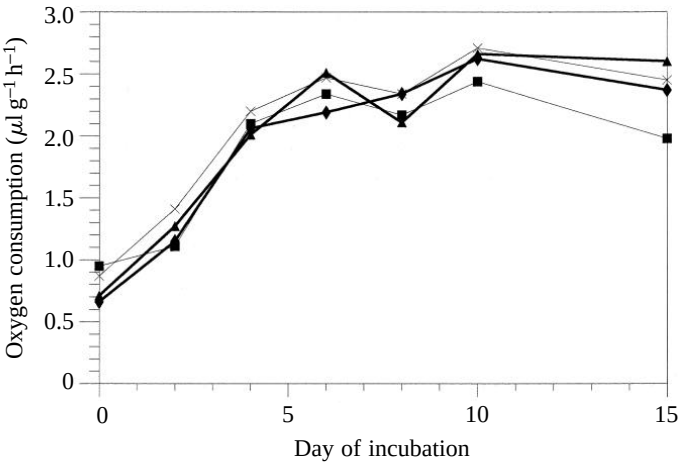

Fig. 5. Mass-specific oxygen consumption of four *Polinices sordidus* egg masses over the course of incubation. Mass-specific oxygen consumption was calculated by dividing absolute oxygen consumption by the mass of the egg mass on the day of measurement.

Table 2. *Oxygen consumption of* Polinices sordidus *egg masses at 25 °C on day 6 of incubation before and after jelly matrix disintegration*

	Mass of egg mass (g)	Oxygen consumption intact (μl g^{-1} h^{-1})	Oxygen consumption disintegrated (μl g^{-1} h^{-1})	Percentage increase in oxygen consumption
	73	1.68	5.61	333
	124	1.71	6.31	368
	115	1.97	4.03	204
	49	2.46	6.18	251
	32	2.24	7.22	322
	57	2.72	7.48	275
	100	2.31	5.63	244
	75	1.88	6.58	349
	89	1.77	6.08	343
	87	2.06	7.05	342
Mean	80	2.08	6.22	300

Hence, as a direct consequence of diffusion being the sole supply of oxygen, the farther away from the surface an embryo is located, the lower the Pe_{O_2} must become, a phenomenon clearly illustrated in Fig. 4. The majority of embryos do not experience severe hypoxia (P_{O_2}<5 kPa) because they are located within 3 mm of the surface, a distance over which the rate of oxygen diffusion is sufficiently high to support continuous embryonic development. However, the situation is quite different for the more centrally located embryos. Over the first 2 days of incubation, the oxygen demands of the embryos are moderate and P_{O_2} within the egg mass decreases moderately towards the central region, but by day 4, when the embryos closest to the surface have developed into mature veligers with vigorously beating cilia and relatively high oxygen demands, the P_{O_2} in the centre of the egg mass has dropped to an extremely low level (P_{O_2}<1 kPa; Fig. 4). Such low oxygen tensions clearly inhibit embryonic oxygen uptake

because, when egg masses are physically disintegrated, a process that greatly decreases the diffusion distance for oxygen between centrally located embryonic capsules and sea water, oxygen uptake increases threefold (Table 2). This increase in $\dot{V}_{O_2}$ is unlikely to be caused by an increase in microorganism growth, because such growth would take several hours to occur, whereas the increase occurred instantaneously. The instantaneous nature of the $\dot{V}_{O_2}$ increase clearly indicates that many embryos within the egg mass are oxygen-starved. The inability of centrally located embryos to increase their oxygen uptake owing to the hypoxic conditions within the egg mass early in incubation appears to inhibit embryonic development greatly because, although embryos at the surface of an egg mass have developed to mature veligers and begin to hatch on day 4 of incubation, centrally located embryos are yet to complete gastrulation at this time and do not hatch until days 16 and 17 of incubation. Similar developmental inhibition of centrally located embryos has been observed in the egg masses of another species of mollusc, *Melanochlamys diomedea* (Chaffee and Strathmann, 1984). In their study, Chaffee and Strathmann hypothesised that the diffusion limitation of oxygen and/or other small molecules may be responsible for developmental retardation of centrally located embryos, but they did not attempt to measure oxygen levels within egg masses in order to verify their hypothesis. The degree of developmental retardation in centrally located *M. diomedea* embryos is much less marked than in *P. sordidus*, probably because the physical dimensions of *M. diomedea* egg masses are much smaller than those of *P. sordidus*. In small egg masses such as those of *M. diomedea*, diffusion distances are smaller and the degree of hypoxia and consequent developmental retardation experienced by centrally located embryos are likely to be far less severe.

The oxygen tension experienced by *P. sordidus* embryos during the course of development very much depends on their position within the egg mass (Fig. 6). Embryos adjacent to the surface are exposed to relatively high P_{O_2} (>10 kPa) throughout their short incubation period, whereas embryos located 5 mm or more from the surface are exposed to severe hypoxia (P_{O_2}<5 kPa) for varying lengths of time and have a longer incubation period. Embryos in the centre of the egg mass experience the most severe hypoxia (P_{O_2}<1 kPa) for the longest period and, as a consequence, these embryos take the longest time to develop. Th oxygen tension in the central regions of the egg mass drops rapidly between days 1 and 4 of incubation (Figs 4 and 6), presumably because the relatively high density of embryos in the outermost 3 mm of the jelly mass creates a high oxygen demand and acts as an oxygen sink that inhibits oxygen from diffusing further into the egg mass. From day 4 onwards, the outermost embryos in *P. sordidus* egg masses begin to hatch, causing the jelly matrix surrounding these embryos to dissolve and disappear. From this time onwards, the radius of the egg mass steadily decreases (Fig. 2), reducing the diffusing distance for oxygen between sea water and the more centrally located embryos. Hence, the lowest oxygen tension experienced by centrally located embryos occurs on day 4 of incubation and thereafter oxygen tension increases steadily throughout the remainder of incubation.

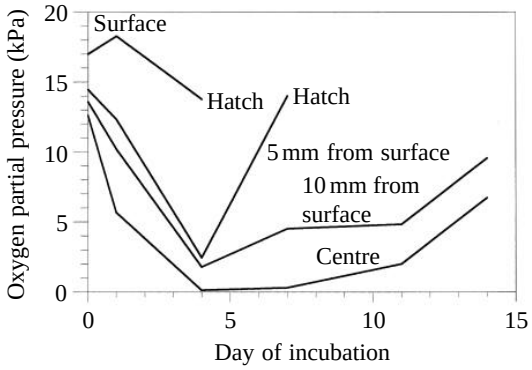

Fig. 6. Oxygen partial pressures experienced by *Polinices sordidus* embryos within an egg mass during the course of incubation. Embryos adjacent to the surface began to hatch on day 4; those 5 mm from the surface began to hatch on day 7.

The size, particularly the radius, of *P. sordidus* egg masses is important in determining the oxygen transport variables and incubation periods for embryos. My measurements of P_{O_2} profiles and $\dot{V}_{O_2}$ throughout incubation were limited to egg masses with radii in the range 16–20 mm. The natural range in size is much larger than this (10–40 mm; Shepherd and Thomas, 1989). Assuming that egg masses with small radii have similar egg densities to those examined in this study, the oxygen tension within the central region will be less severe than in this study because of the shorter diffusion distance and smaller absolute number of embryos. Thus, in small egg masses, centrally located embryos should be able to develop at a faster rate and hatch much sooner than 16–17 days. This appears to be the case, because an egg masses which had an initial mass of 60 g had completely disintegrated after just 7 days of incubation at 25 °C. Exactly the opposite should be true for egg masses with large radii. Oxygen tensions in the central region of egg masses with large radii will be rapidly reduced to very low levels and remain that way for a longer period. The result of this condition will be to increase the time to hatching for centrally located embryos to much longer than 17 days.

Strathmann and Chaffee (1984) present a model for predicting the maximum radius for spherical jelly egg masses and the maximum concentration of embryos within these masses if oxygen transport were to occur only by diffusion. Using this model under the extreme assumption that the oxygen concentration in the middle of the egg mass is zero, an average $\dot{V}_{O_2}$ of 1.7×10^{-6} μl s^{-1} embryo^{-1} (calculated from values in Tables 1 and 2) and an embryo capsule radius of 0.031 cm (Murray, 1962), the maximum predicted radius for a *P. sordidus* egg mass is 12 mm. Under the same conditions, but assuming an egg mass radius of 18 mm, the maximal concentration of embryos would be 145 eggs ml^{-1}. The maximum radius observed for *P. sordidus* in this study was 25.5 mm, and the average egg density was 354 eggs ml^{-1} (Table 1). The large differences between the predicted and observed values can be explained by one of the assumptions in

the model. The model assumes that the embryos are all consuming oxygen at the same rate at the same time, and this is clearly not the case in *P. sordidus* egg masses. The centrally located embryos are oxygen-deprived and, as a consequence, their $\dot{V}_{O_2}$ must be greatly reduced compared with that of peripherally located embryos. By having embryos that are tolerant to long periods of severe hypoxia, when development is arrested or greatly slowed, *P. sordidus* is able to lay a large egg mass with a relatively high density of embryos. If embryonic hypoxia were not tolerated, egg mass size and embryo density would have to be severely reduced to prevent embryonic mortality.

Given the generally small dimensions of the egg masses of other species of molluscs, a strategy that results in relatively synchronous and rapid development of embryos, the question arises of why gravid *P. sordidus* lay large egg masses when an alternative strategy would be to lay several smaller egg masses. Two potential answers to this question may be postulated. One possibility is that large-sized egg masses are less vulnerable to predation, the large amount of jelly matrix deterring predation of eggs and embryos. Unfortunately, information about predation on egg masses that would shed light on this hypothesis is not available. Another possibility is that increased dispersal of veliger larvae could be achieved by a slow release of larvae from the egg mass into the water column over a period of many days. The nature of tidal and wind-driven dispersing currents is likely to change over the course of several days and thus to ensure that larvae released from a single egg mass over several days are dispersed further than if the larvae were released over several hours. This is likely to be particularly true of *P. sordidus* egg masses, because they are not attached to the substratum and drift along the bottom with currents.

In summary, embryos within *P. sordidus* egg masses develop asynchronously: embryos adjacent to the surface begin to hatch after just 4 days of incubation, whereas the most centrally located embryos take 16–17 days to hatch. The development of centrally located embryos appears to be slowed by low oxygen tensions which develop within 1–2 days of oviposition and persist for most of the incubation period. As embryos in the outer regions hatch, the jelly matrix disintegrates, which decreases the oxygen diffusion distance between the central embryos and sea water, resulting in a steady rise in the oxygen tension surrounding the central embryos. This rise in P_{O_2} allows the pace of embryonic development to increase. The result of these two processes is a steady release of mature veliger larvae from the outer surface of the egg mass from day 4 onwards.

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References

CAMERON, J. N. (1986). *Principles of Physiological Measurement*. Orlando: Academic Press.

CHAFFEE, C. AND STRATHMANN, R. R. (1984). Constraints on egg masses. I. Retarded development within thick egg masses. *J. exp. mar. Biol. Ecol.* **84**, 73–83.

HURST, A. (1967). The egg masses and veligers of thirty northeast Pacific Opisthobranchs. *Veliger* **9**, 225–288.

MURRAY, F. V. (1962). Notes on the spawn and early life history of two species of *Conuber* Finlay and Marwick, 1937 (Naticidae). *J. malacol. Soc. Australia* **1**, 49–58.

SCHMIDT-NIELSEN, K. (1990). *Animal Physiology: Adaptation and Environment* (4th edition). Cambridge: Cambridge University Press.

SHEPHERD, S. A. AND THOMAS, I. M. (1989). *Marine Invertebrates of Southern Australian*. Adelaide: South Australian Government Printing Division.

STRATHMANN, R. R. AND CHAFFEE, C. (1984). Constraints on egg masses. II. Effect of spacing, size and number of eggs on ventilation of masses of embryos in jelly, adherent groups, or thin-walled capsules. *J. exp. mar. Biol. Ecol.* **84**, 85–93.