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Temperature–oxygen interactions in Antarctic nudibranch egg masses

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SUMMARY

The Southern Ocean is one of the coldest, most stable marine environments on Earth and represents a unique environment for investigating metabolic consequences of low temperature. Here we test predictions of a new diffusion–reaction model of O_2 distributions in egg masses, using egg masses of the Antarctic nudibranch mollusk, *Tritonia challengeriana*. When warmed from –1.5° to +1.5°C, embryos of *T. challengeriana* showed large increases in O_2 consumption (Q_{10} values of 9.6–30.0). Oxygen electrode measurements in intact masses showed, however, that O_2 levels were high throughout and virtually unaffected by temperature. The model suggested that both effects stemmed from very low metabolic densities in egg masses. Detailed morphological measurements of egg masses of *T. challengeriana* and a temperate congener, *T. diomedea*, revealed large differences in structure that may be related to O_2 availability. Egg masses of *T. challengeriana* were approximately twice as thick. However, the most dramatic effects were observed in embryos: embryos of *T. challengeriana* were >32 times larger (by volume) than embryos of *T. diomedea*. Antarctic embryos also were contained singly in large egg capsules (~500 μm diameter). Consequently, Antarctic embryos occurred at much lower densities, with very low metabolic densities.

Key words: Antarctica, Southern Ocean, McMurdo Sound, oxygen, diffusion, egg mass, nudibranch, marine, temperature, global warming, size, polar gigantism, *Tritonia*.

INTRODUCTION

Temperature has strong effects on the oxygen budget of ectothermic organisms through its effect on metabolic rate (=O_2 consumption). At higher temperatures, ectotherms can experience O_2 deficits if metabolic demand outstrips mechanisms of transport; thus high temperature, *via* effects on O_2 supply, places both short-term constraints on the environments that ectotherms can exploit and long-term evolutionary constraints on possible physiologies and morphologies. Cold temperatures, by contrast, affect O_2 supply–demand relationships by depressing O_2 demand from metabolism while potentially having little effect on O_2 supply by diffusion (Dejours, 1981; Clarke and Johnston, 1999; Woods, 1999; Peck and Conway, 2000; Peck, 2002; Pörtner, 2001; Pörtner, 2002). Thus, ecthotherms in cold environments may be released from some constraints on physiology and form experienced at warmer temperatures.

The Southern Ocean is one of the coldest, most stable marine environments on Earth (Sidell, 2000) and represents a unique environment for investigating metabolic consequences of low temperature. Sea temperatures near the Ross Ice Shelf at McMurdo Station are approx. –1.9°C year round (Littlepage, 1965), and have been <5°C for at least 10–14 MY (Sidell, 2000). Oxygen levels are high throughout the water column (Littlepage, 1965). This combination of conditions may have released Antarctic ectotherms from O_2-imposed constraints commonly experienced by warmer-temperature organisms. Several examples support this idea, including low erythrocyte counts of Antarctic notothenioid fish (Eastman, 1993), evolutionary loss of respiratory proteins in some Antarctic icefish (Ruud 1954; Cocca et al., 1995), and gigantism of many Southern Ocean invertebrates (Chapelle and Peck, 1999).

Here we examine O_2 supply–demand relationships in gelatinous egg masses of Antarctic nudibranchs (Mollusca). Gelatinous masses may serve a number of functions: retention of embryos in favorable microhabitats, chemical or structural protection from predation, reduction of the vulnerable planktonic larval phase, and protection from physical threats (Pechenik, 1979; Rumrill 1990; Rawlings, 1999; Woods and DeSilets, 1999; Przeslawski, 2004). In temperate waters, metabolism by embryos can establish steep O_2 gradients in egg masses. Hypoxia or anoxia has been observed directly, or inferred from developmental asynchrony of embryos, in egg masses of frogs (Seymour and Bradford, 1995; Seymour et al., 1995; Mitchell and Seymour, 2003), fish (Taylor, 1971), mollusks (Booth, 1995; Cohen and Strathmann, 1996; Moran and Woods, 2007), crustaceans (Fernández et al., 2003) and polychaetes (Strathmann, 2000), and may reduce the quality of juveniles, kill embryos directly (Strathmann and Strathmann, 1995), or increase mortality by prolonging development and exposure to benthic predators (Pechenik, 1999). These effects are important because early-stage survival and performance strongly influence population dynamics in marine and aquatic systems (Thorson, 1946; Strathmann, 1985; Roughgarden et al., 1987; Pechenik, 1999; Underwood and Keough, 2000; Moran and Emlet, 2001).

The preceding paper (Woods and Moran, 2008) described a numerical model capable of predicting full spatial and temporal profiles of oxygen in egg masses. The work reported here tests predictions of the model in two ways. The first focuses on intraspecific effects: as an egg mass is warmed, O_2 gradients should become steeper – i.e. central O_2 levels should be depressed. This effect should be especially strong in Antarctic egg masses, because recent studies suggest that metabolism in polar organisms can be much more temperature sensitive than in temperate or tropical organisms. Peck and Prothero-Thomas (Peck and Prothero-Thomas, 2002), for example, found that in larvae of the Antarctic sea star *Odontaster validus*, O_2 consumption rates increased by a factor of nearly 1.5 over

a temperature range between –0.5 and 2.0°C (Q_{10} of ~4.4). Likewise, Bosch et al. (Bosch et al., 1987) and Stanwell-Smith and Peck (Stanwell-Smith and Peck, 1998) found that the effect of temperature on developmental rate in polar echinoderms was much stronger than for temperate or tropical species: Q_{10} values of 10–15 between –2°C to +2°C, far outside the normal biological range (2–3). A counterexample is provided by the data reported in the previous paper (Woods and Moran, 2008) on another polar echinoderm, *Sterechinus neumayeri*, which showed that it had a low Q_{10} (~1.5).

How temperature effects on metabolism translate into temperature sensitivity of O_2 distributions in egg masses depends on an assumption of the model – that diffusive transport of O_2 is indeed insensitive to temperature. This assumption is derived from theoretical and empirical studies of molecules diffusing in substances like water. For molecules in biological structures, such as egg mass gel, the temperature sensitivity of diffusive transport also depends on temperature sensitivity of structural properties. For egg masses of both temperate and Antarctic *Tritonia*, we directly measured temperature effects on O_2 diffusion coefficients (Woods and Moran, 2008), finding negligible increases across the same temperature ranges that stimulated large increases in metabolic rates, thus confirming the assumption of temperature-insensitivity of O_2 transport. Here we measure all remaining model parameters – embryo metabolic rates, diffusion coefficients of O_2 and radial profiles of O_2 – at both ambient water temperature of McMurdo Sound (–1.8°C) and slightly warmer temperatures (+1.5–2°C) in egg masses of the Antarctic dendronotid nudibranch *Tritonia challengeriana* Bergh 1884. Using these measurements as parameters, we show that the model (1) accurately predicts radial O_2 profiles in egg masses of *T. challengeriana* and (2) explains the puzzling temperature insensitivity of these profiles.

The second model test is interspecific. We recently published work on O_2 distributions in egg masses of *Tritonia diomedea* Bergh 1894 (Moran and Woods, 2007), a temperate congener of *T. challengeriana* that inhabits subtidal areas along the West Coast of North America. Between 12 and 21°C, embryo metabolic rates rose approximately twofold (Q_{10} 2.1–2.5). For egg masses containing early-cleavage embryos, central O_2 levels were high (60–70% of air saturation) and only slightly affected by temperature. In egg masses containing veligers, central O_2 levels were lower (0–40% of air saturation) and more sensitive to temperature – approx. 30% of air saturation at 12°C and <10% at 21°C. The gross morphology of *T. diomedea* masses has been described by several authors (e.g. Hurst, 1967; Kempf and Willows, 1977; Lee and Strathmann, 1998), and we add more detailed descriptions in this study.

Our measurements allow detailed physiological comparisons of Antarctic and temperate egg masses. The model predicts that O_2 constraints observed *T. diomedea* (Moran and Woods, 2007) should disappear at low temperatures in the Southern Ocean. Specifically, it predicts that Antarctic egg masses will (1) have higher O_2 levels, (2) be thicker (i.e. larger radius), (3) contain embryos at higher densities, and (4) exhibit tougher, less O_2-permeable egg-mass gel, without incurring an increased O_2 deficit relative to masses of their warmer-water relatives. Our data supported only the first two predictions, although several additional observations, particularly on embryo size and density, suggest functional reasons for deviations from predictions 3 and 4.

MATERIALS AND METHODS

Natural history

Tritonia diomedea Bergh 1894 and *Tritonia challengeriana* Bergh 1884 are congeneric dendronotid nudibranchs (genus *Tritonia* Cuvier 1797; family Tritoniidae; suborder Dendronotina) that are similar to each other in adult size and appearance (Fig. 1). *T. diomedea* has planktonic larvae and a broad distribution in the temperate northern Pacific, from Alaska to Panama (McDonald, 1983). *T. diomedea* is subtidal and feeds on soft corals (specifically *Ptilosarcus gurneyi* and *Virgularia* sp.) (McDonald and Nybakken, 1980). The biology of *T. challengeriana* is poorly known. Recent studies have synonymized this species with *Tritonia antarctica* Bergh 1884 (Wägele, 1995; Schrödl, 2003), and this taxon has been reported from the Antarctic Peninsula, Patagonia, the Weddell Sea, South Georgia, the Falkland Islands, and Signy Island (Odhner, 1926; Wägele, 1995; Barnes and Bullough, 1996; Schrödl, 2003). Food sources of *T. challengeriana* are unknown, though it probably feeds on octocorals (McClintock et al., 1994; Barnes and Bullough, 1996). The developmental mode of *T. challengeriana* is also unknown, but the large egg size and larval morphology reported here suggest that it produces crawl-away juveniles or larvae with a short dispersive period.

The preceding paper (Woods and Moran, 2008) describes collection and holding methodology.

Egg mass morphology

Egg masses of the two species differed in size and overall morphology, and measurement techniques varied accordingly. For whole egg masses of *T. challengeriana*, which approximated long cylinders, we measured 'height' (the longest diameter) and 'thickness' (perpendicular to the height) in two ways: using calipers to directly measure intact egg masses (*T. challengeriana*), and by sectioning egg masses and measuring parameters from calibrated digital microphotographs.

For *T. challengeriana*, sections (Fig. 2) were cut with a razor blade along the 'height' axis, perpendicular to the long axis of the egg mass cylinder. Sections were photographed using a Wild M5A stereomicroscope and Nikon Coolpix 900 digital camera. We measured embryo length (longest diameter), embryo width (perpendicular to longest diameter), egg volume (estimated from length, width, and assuming the shape of a prolate spheroid), capsule length and width, capsule volume (estimated as for embryos), egg mass 'height' (longest diameter), mass 'width' (perpendicular to longest diameter) and mass wall thickness. We also measured the average thickness of the outer mucous covering of masses (see Fig. 2). All microscopic measures were made in BioSuite (Olympus, Inc.) on calibrated micrographs. On a subset of egg masses we also measured embryo density by counting embryos in mass sections of known length. Segment volume was estimated by multiplying cross-sectional area by section length. Embryo density (mm^{-3}) was calculated by dividing total number of embryos by section volume (mm^3). For each mass, average embryo density was calculated from one to three sections.

T. diomedea had smaller, more gelatinous egg strings than *T. challengeriana*, requiring slightly different methods. To measure gross egg mass morphology, we (1) measured masses directly using a calibrated ocular micrometer or (2) photographed whole masses under a calibrated stereomicroscope and measured parameters from digital images. Because cut *T. diomedea* masses did not hold their shape, cross-sectional diameters were impossible to measure directly. Instead, we assumed they were ellipsoid cylinders for volume calculations. All other measurements were made from microphotographs as described for *T. challengeriana*.

Embryo metabolic rates

Rates of O_2 consumption by embryos of *T. challengeriana* were measured using the end-point determination μBOD method (Marsh and Manahan, 1999) with some modification (see Moran and

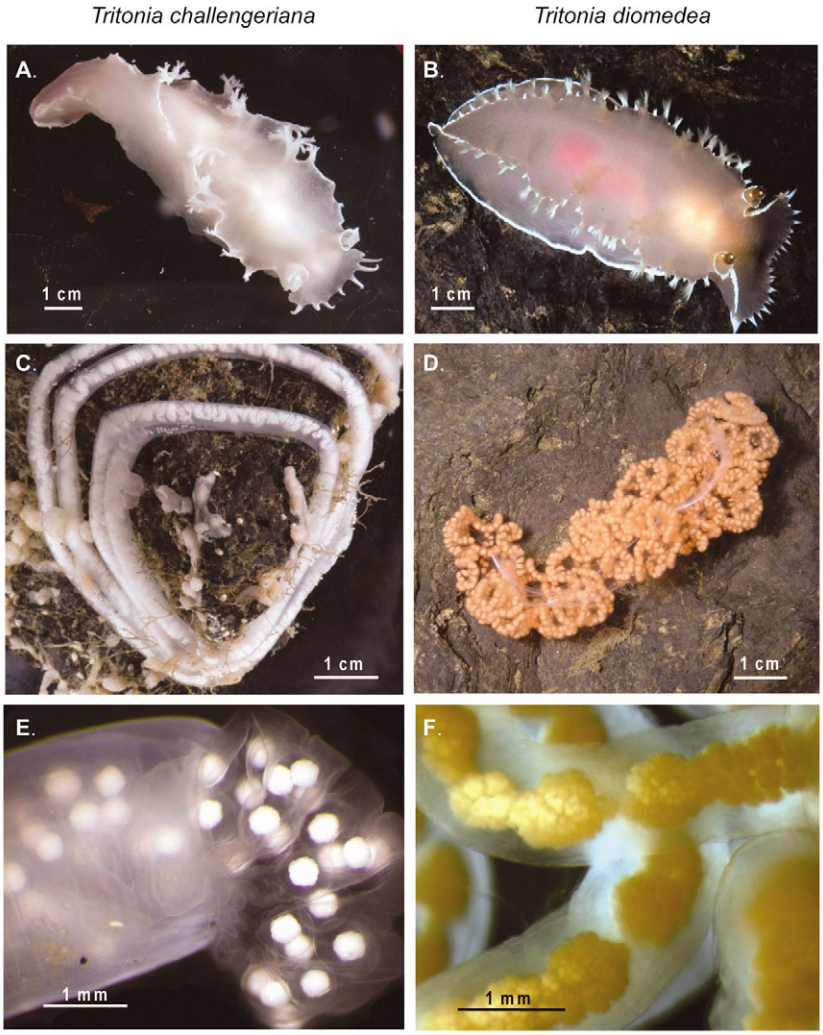

Fig. 1. Adults and egg masses of *Tritonia challengeriana* (Antarctic; A,C,E) and *T. diomedea* (temperate; B,D,F). (A,B) Adults. (C,D) Egg masses (macro view). The egg mass of *T. challengeriana* (C) is *in situ*; the egg mass of *T. diomedea* was removed from its substrate with a razor blade. (E) Cut section of a egg mass of *T. challengeriana* with embryos spilling out of the cut end, showing gelatinous egg string in which embryos are contained. White spheroids are individual embryos at an early cleavage stage. (F) Uncut sections of coiled egg mass of *T. diomedea,* showing embryos (small yellow dots) in egg capsules (clusters of yellow embryos) contained within the mucous sleeve.

Woods, 2007). Encapsulated embryos were removed from egg masses, placed in freshly filtered (0.2 μm pore diameter) seawater, and allowed to acclimate to the experimental temperature for 2 h. They were then pipetted into temperature-equilibrated glass microrespiration chambers (500–700 μl), and vials were capped and held at the experimental temperature for 5–14 h (total O_2 depletion <20% of fully saturated values). Subsequently, ~300 μl of water from each vial was removed with a temperature-equilibrated gas-tight syringe and injected into a respiration cell (MC-100, Strathkelvin, Glasgow, UK) containing a Clark-style oxygen microelectrode (Strathkelvin), kept at temperature with a recirculating water bath. Per-embryo respiration rate was calculated as the slope of the least-squares regression line of total respiration per vial plotted against number of embryos per vial (Marsh and Manahan, 1999).

Metabolism was measured at three developmental stages and two temperatures. Because development in *T. challengeriana* is very long (⩾1 year; H.A.W. and A.L.M., unpublished data), we used embryos from field-collected masses assigned to one of three stages: early (gastrula), mid (unshelled, ciliated veliger) and late (shelled, ciliated, and with a visible ciliated foot). For all three stages we compared metabolic rates at two temperatures, –1.5°C and +1.5°C. The low temperature is close to natural temperatures (–1.8°C); the warmer temperature (+1.5°C) represents summer temperatures animals might experience in more northerly parts of the Antarctic peninsula (Palmer Station LTER sea temperature dataset, http://pal.lternet.edu/).

Oxygen profiles in egg masses and capsules

P_{O_2} in egg masses of *T. challengeriana* was measured using Clark-style O_2 microelectrodes (10, 25 or 50 μm tips; Unisense, Aarhus, Denmark) connected to a picoammeter (model PA2000, Unisense). Electrodes were calibrated, at experimental temperature, before and after each set of measurements in seawater bubbled with air or pure N_2. Calibration water was held at constant temperature with a water-jacketed calibration cell connected to a recirculating water bath. Picoammeter output was logged once per second. Water temperature was also logged using a T-type thermocouple connected to a thermocouple meter (TC-1000, Sable Systems, Las Vegas, NV, USA). Thermcouples were calibrated in a seawater ice bath (–1.9°C). Pieces of egg masses, or individual egg capsules (in general, each capsule contained a single embryo), were submerged and P_{O_2} values were measured as described (Moran and Woods, 2007).

Paired pieces of egg mass (several cm long, cut ends tied with dental floss) were equilibrated overnight in air-bubbled seawater at either –1.5 or +2.0°C (N=7 pairs). Subsequently, pieces were transferred to the temperature-controlled stage described (Moran and Woods, 2007) and pinned onto a piece of Nitex mesh. A micromanipulator was used to position an oxygen electrode at the egg mass surface, and the tip was advanced in increments of 0.5 mm to the center of the mass. In separate experiments, individual egg capsules that had been removed from egg masses were pierced with a 10 μm tip electrode and lifted from the substrate (to avoid substrate-induced boundary layers).

Modeling oxygen profiles in egg masses of *T. challengeriana*

Measurements of egg-mass morphology, embryo metabolic rates, and O_2 diffusion coefficients were used to parameterize the model developed in the preceding paper (Woods and Moran, 2008). Our interests were primarily in determining how similar modeled radial profiles were to measured profiles across temperatures. All modeling was done in the R statistical package (v2.3.1), as described previously.

RESULTS

Egg mass morphology – *T. challengeriana*

Egg masses were elongate white- or off-white cylindrical tubes affixed to substrate along one axis. Embryos could be seen faintly inside masses, but the outer mucous covering was almost opaque

Table 1. Morphology of *Tritonia challengeriana* and *T. diomedea*

	T. challengeriana		*T. diomedea*	
	Mean ± s.e.m.	*N*	Mean	*N*
Short diameter (mm)	2.88±0.07	9	–	
Long diameter (mm)	3.12±0.07	9	1.62±0.09	4
Mass cross-sectional area (mm^2)	6.73±0.73	7	–	
Mass wall thickness (μm)	183±17.4	15	–	
Embryo diameter (μm)	339±6.8	15	102.3±1.0	4
Embryo volume (mm^3)	0.015±0.001	15	0.0037±0.0004	4
Embryo density (mm^{-3})	9.24±0.96	6	215.2±39.3	4
Embryo density, inner (mm^{-3})	14.45±1.55	7	–	
Capsule packing density (% filling)	83±8	5	–	

Values are means ± s.e.m.

– so the details of interior morphology could not be seen. Masses were laid in either loose spirals (Fig. 1C) or, less commonly, wrapped around small rocks, sponges, or hydroids. Embryos were white to off-white and each was contained individually in a large, transparent, ellipsoid capsule. Within the mass, embryo capsules were contained in a long, loosely coiled membranous sheath (Fig. 2A). Capsules were loose within the membranous string; once egg masses were cut, capsules fell freely out. Capsules were densely packed within the mass (Fig. 2A). Measurements of embryo length and volume, capsule diameter and volume, mass diameter and cross-sectional area, and embryo density are given in Table 1.

Egg mass morphology – *T. diomedea*

Morphological data (Table 1) were consistent with previous descriptions (Hurst, 1967; Strathmann, 1985; Lee and Strathmann, 1998). Overall, *T. diomedea* had narrower egg cords than *T. challengeriana*. Mass coverings of *T. challengeriana* were tough and translucent whereas those of *T. diomedea* were transparent and jelly-like. *T. diomedea* also had substantially smaller embryos, many more embryos per capsule, and much higher embryo densities than *T. challengeriana* (Table 1).

Embryo metabolic rates – *T. challengeriana*

Metabolic rates increased with both embryo age and temperature (Fig. 3). O_2 consumption ranged from 3.4–51.1 pmol $embryo^{-1}$ h^{-1} and respiration rates were highly temperature-sensitive; for the three developmental stages we examined, Q_{10} values of respiration rate ranged from 9.6 to 30.0, depending on developmental stage. Respiration rates of *T. diomedea* are given elsewhere (Moran and Woods, 2007).

Oxygen profiles in egg masses and capsules – *T. challengeriana*

O_2 levels in egg masses of *T. challengeriana* were high throughout and only slightly affected by temperature (Fig. 4). A linear mixed-effects model (S-Plus v6.2) with radial position and temperature as main effects and mass identity as a random effect, showed that temperature had a statistically significant effect on O_2 levels ($F_{1,46}$=26.7, P<0.001). Masses used in this experiment contained embryos of various developmental stages, though most were early to mid-veligers; embryo stage had no effect on level of O_2 depression. Capsules freed from egg masses had very small O_2 gradients from surrounding seawater to intracapsular fluid. For each of seven masses, gradients were measured on three separate capsules and averaged, giving a mean gradient (±s.e.m.) of 0.20±0.11 kPa. This value represents a 1% drop from air-saturated values (21 kPa). Oxygen profiles in egg masses and across capsule walls of *T. diomedea* are given in Moran and Woods (Moran and Woods, 2007).

Modeling oxygen profiles in egg masses of *T. challengeriana*

The cylindrical diffusion–reaction model developed in the previous paper requires four parameters – egg mass radius, O_2 diffusion coefficient, metabolic density (a combination of embryo density and embryo metabolic rates), and a term describing the half-saturation constant (K_m) of metabolism. We measured all parameters directly except for K_m. For the simulations, we assumed a very low K_m (=20 nmol O_2 cm^{-3}), based on older studies of embryo O_2 sensitivity (e.g. Tang and Gerard, 1932; Yanigasawa, 1975; Palumbi and Johnson, 1982). Under all combinations of embryo age and temperature, simulated profiles showed high levels of O_2 throughout the egg masses (Fig. 5), similar to observed radial profiles (Fig. 4). Early- and mid-stage embryos in colder conditions had almost no central O_2 depression, and the warmer conditions led to only slight additional O_2 depression. Late-stage embryos showed a larger but still modest effect of temperature.

One systematic difference between modeled and measured profiles was shape; modeled profiles showed smoothly declining O_2 levels whereas measured profiles showed sharper decreases near the egg-mass surface and flat internal O_2 profiles, suggesting that more resistance to O_2 flux is localized in the egg-mass wall or external boundary layers and less in the packed capsules. Stirring by embryos may also flatten internal O_2 profiles.

DISCUSSION

The cold, well-oxygenated waters of Antarctica provide an ideal natural experiment for assessing how changes in O_2 supply–demand relationships affect organismal physiology. We took advantage of this 'experiment' to examine the oxygen biology of Antarctic nudibranch egg masses. Analyses were carried out in the context of the reaction–diffusion model developed in the preceding paper. The model's predictions were driven by the twin assumptions, now confirmed, of (1) high temperature sensitivity of metabolic O_2 consumption and (2) low temperature sensitivity of diffusive O_2 transport. The present paper uses detailed measurements of egg-mass morphology and physiology of the Antarctic nudibranch *Tritonia challengeriana* to evaluate two model-derived hypotheses. The first is intraspecific, focusing on how warming affects O_2 profiles in egg masses. The second is interspecific, focusing on morphological and physiological comparisons of *T. challengeriana* (Antarctic) and a temperate congener, *T.*

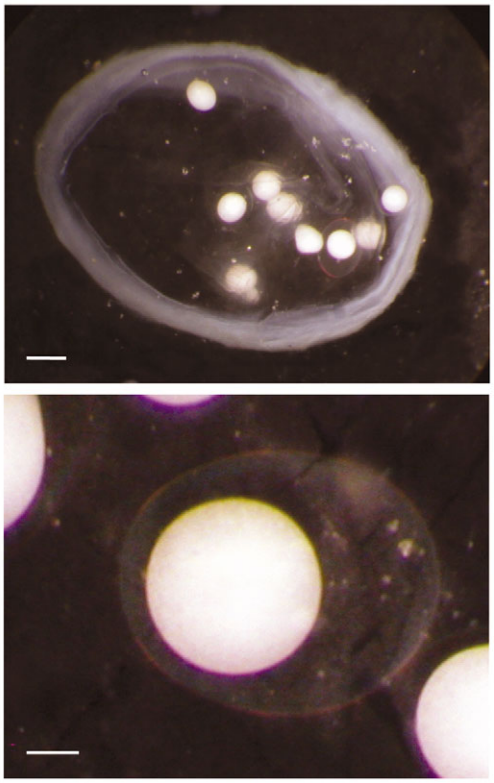

Fig. 2. (A) Cross section of egg mass of *T. challengeriana* showing central embryos (many of which have fallen out) and the robust mass wall. Scale bar, 500 μm. (B) Single egg of *T. challengeriana* surrounded by egg capsule. Scale bar, 100 μm.

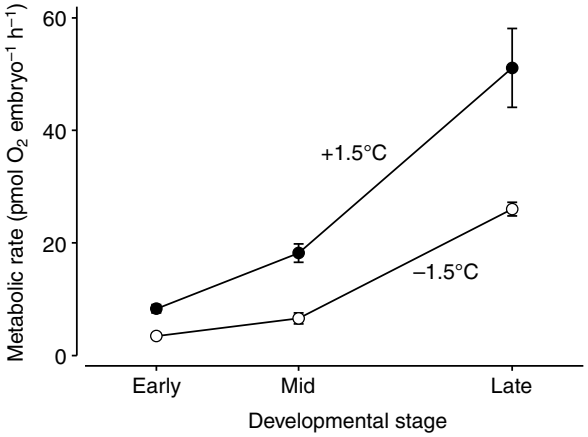

Fig. 3. Metabolic rates of early, mid- and late-stage embryos of *T. challengeriana* at two different temperatures. Each point and its associated error bars represent the slope (and error) of a linear regression of oxygen consumption *versus* number of embryos per vial (6–8 vials per point). See Marsh and Manahan (Marsh and Manahan, 1999) for details of the method. Q_{10} values for metabolism were 18.8, 30.0 and 9.6 for early-, mid- and late-stage embryos, respectively.

diomedea. Although the core idea of the model – differential temperature sensitivity of O_2 consumption *versus* transport – was supported, the data provided several surprises that were only partially resolved by the model.

Intraspecific effects of warming on oxygen in egg masses

Metabolic rates of Antarctic species can be quite sensitive to temperature (Bosch et al., 1987; Stanwell-Smith and Peck, 1998; Peck and Prothero-Thomas, 2002), though the degree of sensitivity varies among species. The strong effect of temperature on O_2 consumption by embryos of *T. challengeriana* – coupled to temperature insensitivity of O_2 diffusion coefficients (Woods and Moran 2008) – suggested that egg-mass O_2 profiles should be affected by even small changes in temperature. This prediction, however, was refuted by both direct measurement (Fig. 4) and model simulation (Fig. 5). A resolution can be found in the very low metabolic density (per embryo metabolic rate × embryo density) of *T. challengeriana* egg masses. In particular, low metabolic density gave trivially small O_2 drawdown under cold (natural) conditions, and even large factorial increases in O_2 consumption caused little additional drawdown (Fig. 5). An implication is that increasing sea temperatures could increase development rates without offsetting costs from hypoxia. For example, if metabolic rate and development rate are coupled, a 3°C rise in temperature could halve time to hatching. Whether this increase would be beneficial is unclear; generation times would shorten but shifts in hatch timing could result, e.g. in mismatches with seasonal food availability (Rivkin 1991; Both et al., 2006).

Interspecific differences between Antarctic and temperate *Tritonia* species

The temperature difference between the Antarctic site (shallow subtidal near McMurdo Station) and the temperate site (Friday Harbor Labs) is 10–14°C. The model predicts that, all else being equal, Antarctic egg masses will have higher O_2 levels, be thicker, contain embryos at higher densities, and exhibit tougher egg-mass gel that is less permeable to O_2, without incurring increased O_2 deficits. Only two of these four predictions were supported.

The supported predictions were O_2 levels in the egg mass and egg mass size. At ambient environmental temperatures (–1.5°C), egg masses of *T. challengeriana* contained high O_2 levels (Fig. 4) across all stages of development. Warming induced slightly steeper O_2 gradients, but levels never dropped below 17 kPa (~75% of air saturation). By contrast, warming egg masses of the temperate *T. diomedea* gave areas of extreme hypoxia and anoxia (Moran and Woods, 2007). High O_2 in egg masses of *T. challengeriana* were attributable to moderate per-embryo O_2 demand at Antarctic temperatures coupled to very low embryo density. Supporting the prediction that Antarctic egg masses should be large, we found that egg masses of *T. challengeriana* were on average twice the diameter of those of *T. diomedea* (radii of 1.5 and 0.8 mm, respectively). This pattern is consistent with the phenomenon of polar gigantism (Chapelle and Peck, 1999), and may be related to release from O_2 constraints.

Support for both predictions – higher O_2 levels and larger egg-mass size – suggests, however, that size of Antarctic masses has not increased to the extent that would be permitted by relief from O_2 constraints. The model offers a way to evaluate this idea quantitatively. For example, the model indicates that an egg mass of *T. challengeriana* containing mid-stage embryos could be sixfold thicker (18 mm) and still have some O_2 at its center. Under conditions of higher O_2 demand, i.e. late stage embryos at +1.5°C, egg masses could still be two- to threefold thicker before the onset of central anoxia. At their actual sizes, therefore, it appears that natural masses are ‘overconstructed’ with regard to O_2 supply to embryos (see also Seymour and Bradford, 1995). Several processes may explain this pattern. First, laboratory experiments were

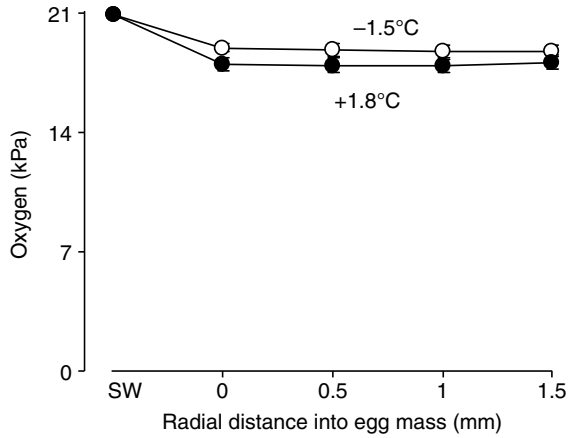

Fig. 4. Oxygen profiles in paired pieces of egg mass (*N*=7) from *T. challengeriana* at two temperatures. Values are means ± s.e.m., just visible behind the data symbols. SW, air-bubbled seawater around the egg mass.

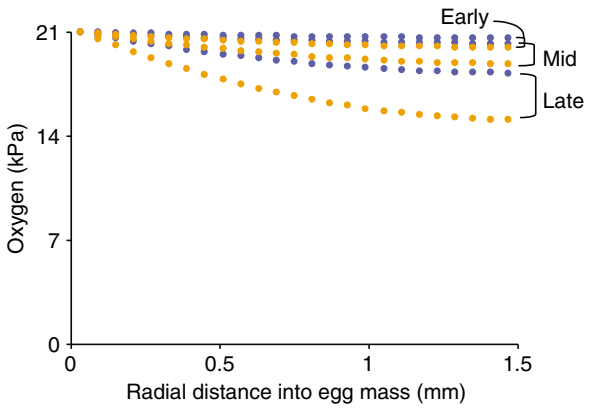

Fig. 5. Simulated radial O_2 profiles in egg masses of *T. challengeriana*. Early, mid and late refer to developmental stage. Blue indicates simulations based on measured values at –1.5°C, and orange indicates values at +1.5°C.

performed in full O_2 saturation with stirring; in the field, O_2 concentrations and flow may be lower. Respiration by other neighboring organisms may draw local oxygen levels down further. Direct field measurements of O_2 concentration in egg masses *in situ* would constitute a good test. Second, egg-mass cords often fold back on themselves or are laid in closely apposed spirals (see Fig. 1). Third, Antarctic embryos themselves may be more sensitive to low O_2 availability than are temperate embryos. Fourth, morphological constraints in the adult reproductive tract may preclude generating larger-diameter egg masses. None of these possibilities has been tested.

Now consider the unsupported predictions, embryo density and gel impermeability. Contrary to expectation, we found that embryos in Antarctic species were 23-fold *less* dense than their temperate congener (9.2 embyros mm^{-3} compared with 215.2 embryos mm^{-3} for *T. diomedea*). A likely proximate explanation stems from embryo sizes of the two species. Embryos of *T. challengeriana* were >32-fold larger (by volume) than embryos of *T. diomedea*, and single embryos were contained in very large (~500 μm diameter) egg capsules. Masses of *T. challengeriana* also had stiffer, thicker (~183 μm) egg-mass walls, and tightly packed capsules within. We calculated the packing density of embryos by measuring the volume of the internal cavity of each mass and the volume of embryos contained in it, and found that *T. challengeriana* capsules filled an estimated 83.0% (±8.0%) of available space. The theoretical maximum packing of jammed disordered ellipsoids is ~74% (Donev et al., 2004); thus, while capsules were somewhat flexible and could clearly be packed at high densities, embryos could not occur at much higher densities without reduction in capsule size. The functional role of large capsules is unknown, but their size places a limit on embryo density that is lower than the theoretical limit imposed by O_2 supply–demand dynamics in our model.

The second prediction, that Antarctic egg masses would use O_2 surplus to construct especially tough egg-mass gel that was less oxygen-permeable, was also rejected. The logic was that (1) predation is important in Antarctic ecosystems; (2) slow-developing embryos would need substantial protection, i.e. tough egg-mass walls, if they were to survive to hatching; and (3) construction of tough walls would result in reduced permeability to O_2. Egg mass walls of *T. challengeriana* indeed appeared tougher than those of *T. diomedea*. However, direct measurement of O_2 diffusion coefficients (D) in intact egg masses of *T. challengeriana* (Woods and Moran, 2008) showed that D was almost as high as in pure seawater. Egg-mass wall toughness may be irrelevant to predation risk if masses are chemically defended, as some Antarctic adult nudibranchs appear to be (Bryan et al., 1998).

Physiology of Antarctic egg masses: conclusions and caveats

Antarctic egg masses were thicker (2×) than those of a temperate congener and had high O_2 levels throughout, at least under laboratory conditions. In addition, embryos in the Antarctic masses were much larger (32×) than the temperate congener. It is possible that large embryo size is facilitated by release from O_2 constraints. In this aspect, our focus on egg-mass size may be too narrow; perhaps the appropriate organizational level is on embryos and the O_2 gradients surrounding them (Seymour and White, 2006). We are presently developing new analyses to evaluate this idea.

An obvious caveat is that our conclusions are drawn in part from a two-species comparison, so that firm evolutionary conclusions are impossible (Garland and Adolph, 1994). We focused instead on developing detailed physiological and morphological datasets grounded in a modeling context. Our evolutionary conclusions are thus subject to additional, ongoing studies of larger species sets.

A second caveat concerns implicit connections between O_2 and egg-mass structure and function. Other factors unrelated to O_2 may also be at work. For example, predation plays an important role in structuring Antarctic benthic ecosystems (Dearborn, 1977; Dayton et al., 1994; McClintock, 1994; McClintock et al., 2005). High predation rates on early life-history stages are thought to select against strategies in which reproductive effort is packaged into few, large clutches rather than more numerous, smaller ones (Smith and Fretwell, 1974). Thus, production of thick masses in the Antarctic, which our model suggests is physiologically possible, might be disadvantageous because of the high probability that a parent's entire reproductive output could be consumed by a single predator (although parents could avoid this problem by producing short, thick masses). Likewise, if predation rates on Antarctic masses are high, any reduction of internal O_2 concentrations may be detrimental if it prolongs development and increases vulnerability to predation.

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