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Victoria University of Wellington
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University of Hawai'i at Mānoa
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PRIMARY RESEARCH PAPER

Oxygen production from macrophytes decreases development time in benthic egg masses of a marine gastropod

Nicole E. Phillips · Amy L. Moran

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Abstract Oxygen stress can result in slowed development or death of embryos in benthic egg masses; one potential mechanism to alleviate hypoxic stress is by photosynthesis of associated macrophytes. We tested whether the presence of macroalgae increased central oxygen saturation and decreased development time of embryos in benthic egg masses of the intertidal gastropod *Siphonaria australis*. Central oxygen saturation was highest when masses were maintained in the presence of algae and under lit conditions. When masses were kept in the dark or without algae, mass centers were anoxic. Central oxygen saturation was also related to the age of masses; younger masses (2 days old) had 3× higher central oxygen than older ones (6 days old). Similar results were seen in masses in natural tide pools. Almost all (98%) embryos from masses that were cultured with algae had reached late developmental stages at the onset of hatching, whereas in masses without algae, development of central embryos was comparatively delayed. These data support the hypothesis that by increasing oxygen throughout the egg mass, macroalgae (1) mitigate the effects of oxygen gradients that cause developmental asynchrony and (2) increase the proportion of offspring that are developmentally mature at the onset of hatching.

Keywords Hypoxia · Hyperoxia · Embryonic development · Egg mass · Tide pool · Algae · Gastropod · Marine invertebrates

Introduction

Oxygen supply is an important limiting factor for aquatic organisms that can be highly variable in both space and time, over large and small scales. Low oxygen conditions can regulate the distribution, trophic interactions, and community structure of animals in marine and freshwater (Tunnicliffe, 1981; Kolar & Rahel, 1993; Diaz & Rosenberg, 1995; Breitburg et al., 1997); many aquatic habitats routinely experience anoxia or hypoxia, e.g., temporary ponds, swamps and marshes, water bodies under ice, deep ocean basins, nearshore open ocean environments, and mid-water oxygen minimum zones in the ocean (Rosenberger & Chapman, 1999; Levin, 2003; Smith & Able, 2003; Apodaca & Chapman, 2004). Excess oxygen, or hyperoxia, can arise from algal blooms in shallow lakes, ponds and coastal lagoons. In some habitats hyperoxia occurs on a regular seasonal basis,

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N. E. Phillips (✉)
School of Biological Sciences and Coastal Ecology Laboratory (VUCEL), Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand
e-mail: nicole.phillips@vuw.ac.nz

A. L. Moran
Department of Biology, University of Hawaii, Mānoa, Honolulu, HI 96822, USA

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often lasting for days (Huntington & Miller, 1989; Davies & Gates, 1991). On smaller scales, oxygen production by photosynthesising macrophytes can also lead to supersaturation in seasonally ephemeral or tidal pools (Truchot & Duhamel-Jouve, 1980; McMahon, 1988; Keeley & Sandquist, 1991).

Mobile animals such as fish, amphibians, and aquatic invertebrates have a variety of physiological and behavioral mechanisms that serve as a buffer for variable oxygen conditions (Burggren & Mwalukoma, 1983; Pihl et al., 1991; Fischer et al., 1992; Eriksen et al., 1996). However, many mobile aquatic species deposit their developing young in benthic egg masses that are affixed to the substrate. Embryos in benthic egg masses are unable to change location in response to variable oxygen conditions and may be particularly vulnerable due to the rapid and major morphological and physiological changes associated with development. Because embryos in masses are reliant on the comparatively slow process of diffusion to supply oxygen required for metabolism and development (Moran & Woods, 2007; Woods & Moran, 2008a, b), they frequently experience hypoxia, often with negative effects. Exposure to low oxygen causes embryonic mortality and developmental delays for egg masses of amphibians and invertebrates (Chaffee & Strathmann, 1984; Pinder & Friet, 1994; Cohen & Strathmann, 1996; Seymour et al., 2000). Hyperoxic conditions can also have negative effects on some fish and invertebrates [e.g., gas bubble disease: reviewed by Weitkamp & Katz (1980)], particularly in aquaculture where oxygen supplementation is common (Salas-Leiton et al., 2009). Much less has been reported about effects of hyperoxic conditions on benthic embryos, although in some amphibians, oxygen production by symbiotic algae is thought to be the only source for the innermost embryos in an egg mass, which otherwise would not be able to develop (Pinder & Friet, 1994).

In marine ecosystems, tide pools are habitats that are known to vary widely in oxygen conditions and abundance of macroalgae, and they also house a variety of benthic invertebrate egg masses. Thus, tide pools provide a model system in which to examine the influence of macroalgal oxygen dynamics on benthic embryos. Because tide pools are isolated from the sea during low tide, photosynthesis by algae may increase oxygen saturation during the day and benefit embryos. However, this may be balanced by low tides at night when respiration by algae may reduce oxygen saturation. Egg masses from a variety of taxa can occur on or near macrophyte, and under these conditions, photosynthesis by macrophytes can drive rapid increases in oxygen in egg masses (Woods & Podolsky, 2007). Nevertheless, the long-term consequences of this association for the development and survival of offspring have been little studied. In one of the only examples, Fernandes & Podolsky (2011) showed benefits of association with eelgrass for embryos of the mollusc *Haminoea vesicula* that were dependent on light conditions; embryos exhibited more rapid development under moderate to high light conditions but decreased development rate and hatching size in low light. This suggests that there is a balance between potential benefits and costs for benthic embryos associated with macrophytes that is mediated by the environment where egg masses are deposited.

Siphonaria australis (Quoy & Gaimard) is an intertidal, pulmonate limpet on rocky shores in New Zealand that deposits embryos in gelatinous egg masses where they develop for about a week before hatching as planktonic larvae. Egg masses are deposited intertidally and are commonly found in tide pools. Previous work has shown that *S. australis* embryos developing in tide pools can experience high mortality in summer, due to elevated temperature, salinity, and UV radiation that can occur during low tide (Russell & Phillips, 2009; Fischer & Phillips, 2014). In this risky habitat, development time is critical, and factors that increase developmental rate will likely be beneficial for larvae. Here, we used a series of laboratory and field experiments to examine the role of macroalgae in mediating the rate of embryonic development in *S. australis*. The aims of this study were to (1) determine whether the presence of macroalgae decreases the length of development of embryos in benthic egg masses; (2) examine how egg mass oxygen saturation varies with light conditions and the presence of algae; and (3) determine whether oxygen saturation in the water of tide pools is correlated with oxygen saturation in egg masses from the field.

Methods

Effect of algae on development time and hatching

To determine whether the presence of algae can influence hatching success of benthic egg masses, we

conducted laboratory experiments in December 2010 (austral summer). We collected adult *S. australis* from the field (Te Raekaihau Point: 41°20′46″S, 174°47′28″E), brought them to the Victoria University Coastal Ecology Laboratory (VUCEL), and maintained them in running seawater in a shallow sea table. The sea table was checked every day for egg mass deposition, and when multiple adults had deposited masses, a scalpel was used to remove the egg masses intact from the surface. Egg masses were used in two experiments. The first experiment was performed under controlled conditions in a constant temperature room (16.5°C) with a fixed light schedule of 16 h light: 8 h dark ($n = 10$ egg masses). The second experiment was conducted outside in a shallow sea table with flowing seawater where temperature and light fluctuated naturally ($n = 8$ egg masses). Daytime temperature in dishes outside ranged from 16.8°C (min, early morning) to 20.5°C (max, afternoon). For both experiments, egg masses were divided in half with a scalpel, and each half was placed in individual glass dishes with approximately 150 ml filtered seawater (FSW, filtered to 0.4 μm). One half of each egg mass was placed in a treatment with algae, and the other half was placed in a treatment with no algae. For the algae treatment, we collected live *Ulva* sp., (common green seaweed in tide pools) from the field and maintained it in a separate sea table in running seawater. *Ulva* was rinsed in FSW to remove epifauna before use in experiments, and similar amounts of algae were placed in each dish (a $\sim 10 \times 10$ cm piece, ~ 6 g). Water was changed, and fresh algae collected and replaced in dishes every 2 days.

Egg masses were checked twice daily for hatching (in the morning and evening), and when swimming veligers first appeared in the dishes, hatching was considered to have begun, and egg masses were immediately preserved with 2% buffered formalin. Preserved egg masses were later sliced through the thickest region, several mm from the edge, and the section laid flat on a slide. On a microscope at 100× magnification, we ran a straight-line transect across each section from edge to edge through the center and staged every embryo encountered. Stages were identified as trochophore (no distinguishing features under light microscopy, ball-shaped), pre-veliger (distinct forming velar lobes but no distinguishable foot or other structures), early pre-shelled veliger (foot, velum, and visceral mass distinguishable but no shell), early shelled veliger (lightly pigmented shell, velum, and foot not retracted after exposure to formalin), and late veliger (fully formed darkly pigmented shell, animal withdrawn entirely into shell). 17–36 embryos were staged in each transect. We then calculated the percentage of the embryos in each transect that were shelled (late stage and likely to survive if hatched) and unshelled (early stage and unlikely to survive if hatched). A paired *t* test was used to examine the effect of algae on the proportion of shelled veligers separately for each experiment.

Effect of algae on oxygen saturation in egg masses

To examine how egg mass oxygen saturation varied with the presence of algae and different light conditions, we measured the oxygen in the centers of egg masses from the laboratory experiment above at 2 days old and again at 6 days old. On both days, oxygen was measured after 8 h of equilibration in the dark and again after 4 h in the light. To determine whether laboratory measurements were similar to field conditions inside egg masses, we also measured central oxygen concentration from egg masses in the field at low tide from a single tide pool both during the day ($n = 10$ egg masses) and at night ($n = 9$ egg masses).

Oxygen concentrations were measured in egg masses in the field and laboratory using an underwater-capable field picoammeter connected to a Clark-style microelectrode (50 μm tip, UWMeter, Unisense, Inc., Denmark). The meter and probe were calibrated with air- and nitrogen-bubbled water across the range of temperatures found in the tide pools in this study. Salinity was also measured in each tide pool, and oxygen saturations were adjusted accordingly. To estimate oxygenation of seawater in pools, we took three measurements at different, arbitrarily chosen points within each pool and averaged the values. To measure internal oxygen concentrations in masses, the tip of the probe was pushed by hand slowly through the thickest part of the egg mass until it was at the center, at which point the measured oxygen level was recorded. The probe was then removed from the mass, and this step was repeated twice, for a total of three replicate internal measurements for each mass. The means of the three tide pool and the three internal readings were used for analyses.

We used linear models on days 2 and 6 to examine whether the presence of algae affected central oxygen

saturation in egg masses, and whether there was an effect of light conditions. Egg mass identity was a random factor in the model. Data were logit transformed before analysis (Warton & Hui, 2011) and fulfilled assumptions of normality and equality of variance. To determine whether egg mass age affected oxygen availability to central embryos, we compared central oxygen concentrations measured for each egg mass on day 2 to the same mass on day 6 (with algae and in light only) using a paired *t* test.

Relationship between oxygen saturation in tide pools and in egg masses

To determine whether oxygen saturation in tide pools drives oxygen saturation in egg masses in the field, we measured oxygen in both the water in tide pools ($n = 6$) and in *Siphonaria* egg masses in those pools ($n = 2$–10 per pool) using the Unisense picoammeter and probe as above. Oxygen concentrations were calculated as the average of three measurements taken at different points in each pool. After measurement, egg masses were collected and brought to the laboratory where they were staged under a microscope as pre-veliger, early veliger, or late veliger based on the latest stage observed. ANCOVA was used to examine the relationship between water oxygen saturation (continuous variable) and egg mass central oxygen saturation (dependent variable) with developmental stage as a categorical factor. Raw data were normal and homoscedastic so did not need to be transformed.

Results

Effect of algae on development time and hatching

At the beginning of laboratory experiments, embryos ranged in developmental stage from newly laid (4–8 cell stage) to early post-gastrulation. There was little variation in developmental stage within individual young masses. Hatching began after 6–8 days, both in the lab and in the sea table outside. In all cases, the first appearance of hatchlings occurred at the same sampling interval for both sister egg mass halves (i.e., the half with and the half without algae).

Examination of the central section of the preserved masses (all of which were preserved as soon as hatching was observed) revealed that across masses and treatments, embryos from more central locations were at earlier stages of development. Gradients of embryonic development were found in all egg masses, such that the outermost embryos were at the hatching stage, and the earliest stage embryos were most central (Fig. 1). The earliest developmental stage found in egg masses with algae was early pre-shelled veliger; this stage was found in 30% of masses from the lab and 14% from the outdoor sea tables. For the treatment without algae, the earliest stage was trochophore, and trochophores were present in 60% of lab egg masses and 30% of those in the outdoor sea tables. For both experiments, egg mass halves that were in water with algae had a higher proportion of embryos with shells at the onset of hatching, compared to their counterparts that were in water with no algae (lab: $t\ (9) = 9.28$, $P < 0.0001$; outside: $t\ (6) = 8.83$, $P < 0.0001$). On average, for transects across egg masses with algae, 98% was shelled, compared with 59–66% of embryos from treatments without algae (Fig. 2).

Effect of algae on oxygen saturation in egg masses

On both day 2 and day 6, there was a significant interaction between presence of algae and light treatment (day 2: $F_{1,23} = 37.70$, $P < 0.0001$; day 6: $F_{1,25} = 49.3$, $P = 0.01$). In the light, egg mass halves with algae had higher central oxygen concentrations

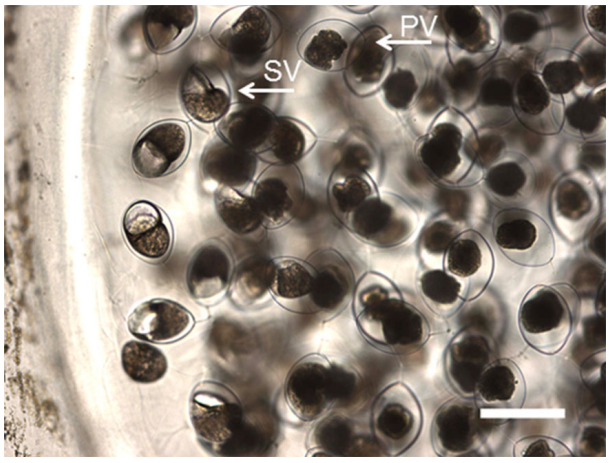

Fig. 1 Cross section of a hatching *Siphonaria* egg mass, showing a gradient in development from embryos ready to hatch near the mass edge (*left*) to early pre-shelled veligers toward the center of the mass (*right*). The *arrows* indicate examples of a shelled veliger (SV) and pre-shelled veliger (PV). Control mass from the lab experiment, reared without algae. *Scale bar* 200 μm

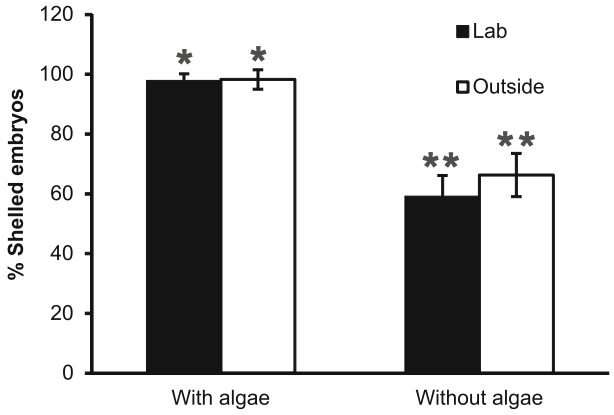

Fig. 2 Mean percentage of embryos (±95% CI) that were shelled veligers when hatching began, from two treatments and for two different experimental conditions (controlled conditions in the lab, or variable conditions outside). *Asterisks* indicate significant differences between treatments for each condition (*t* tests, $P < 0.0001$ in both cases). Lab: $n = 10$, outside: $n = 7$

than the paired halves kept without algae, but in the dark central oxygen was low in all egg masses with or without algae (Fig. 3). Regardless of light, mean oxygen saturation of water in dishes without algae was similar and close to normoxic (dark: 98.0%, SD = 1.1%; light: 97.5%, SD = 1.4%). Water in dishes with algae in the dark was hypoxic (mean: 46.0% saturation, SD = 12.3%), whereas in the light, it was hyperoxic (mean: 264.1%, SD = 52.3%). On average, for egg masses in dishes with algae and in the light, younger egg masses (day 2) had 3× higher central oxygen saturation than older (day 6) egg masses (t (8) = 3.86, $P = 0.005$).

Ambient dissolved oxygen in the tide pool was 179% of saturation in the day and 46% of saturation at night. Early egg masses from the pool (pre-veliger and early veliger stages) had a mean central oxygen saturation of 141.7% (SD = 29.7%, $n = 10$) in the day and 2.2% at night (SD = 2.3%, $n = 9$).

Relationship between oxygen saturation in tide pools and in egg masses

From pools in the field measured during the day (temperature: 13.9–17.5°C, $n = 6$), central oxygen saturation was significantly higher in pre-veliger stage compared to early and late veliger stage egg masses ($F_{2,28} = 8.93$, $P = 0.001$, 166.6 versus 115.0 and 116.1%, respectively; Tukey test: $P < 0.05$) and increased with pool oxygen saturation ($F_{1,28} = 100.36$, $P < 0.0001$). Pool oxygen saturation explained 68% of the variation in central oxygen saturation of pre-veliger egg masses, 90 and 87% of early and late veliger egg masses, and varied from 112.4% (SD = 3.4%) to 245.5% (SD = 3.0%; Fig. 4).

We measured oxygen saturation at low tide on a sunny summer day in an additional 11 small tide pools that were isolated from ocean exchange and which we qualitatively observed to vary in macroalgal cover. Oxygen saturation varied from 118% (SD = 2.1%) in a pool with no visible macroalgae to 243% (SD = 29.6%) for a pool with abundant algae (primarily *Ulva* sp.).

Discussion

It has long been recognized that hypoxia in aquatic environments can have devastating effects on a range of taxa that undergo benthic development. What has

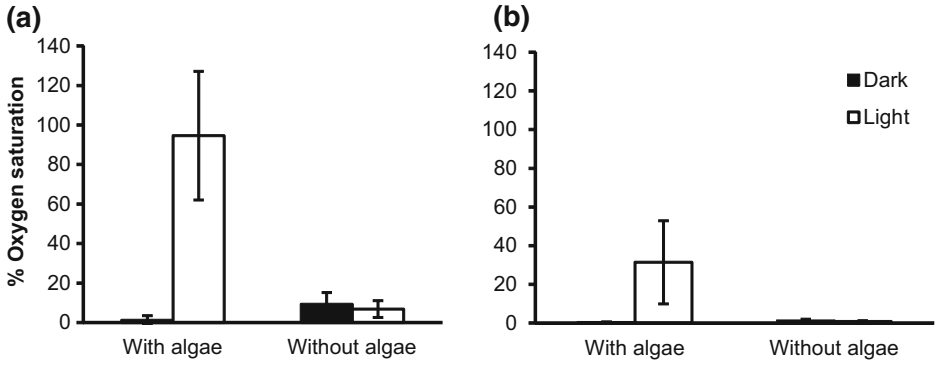

Fig. 3 Mean (±95% CI) central oxygen saturation in egg masses in two different algal treatments and after either 8 h in dark or 4 h in light, **a** at 2 days old and **b** at 6 days old. These egg masses were from controlled conditions in the laboratory experiment (day 2: $n = 10$, day 6: $n = 9$). For both days, there was a significant interaction between light and algal treatments (2-way ANOVA, $P < 0.01$ in both cases)

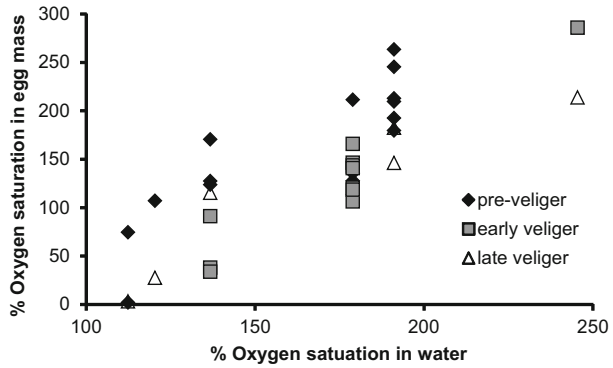

Fig. 4 Oxygen saturation in the water of tide pools drives oxygen saturation in the centers of egg masses in the pools (ANCOVA, $P < 0.0001$), for egg masses containing a majority of embryos at each of three different developmental stages (ANCOVA, $P < 0.001$): pre-veliger *black diamonds* ($n = 15$), early veliger *gray squares* ($n = 11$), and late veliger *open triangles* ($n = 6$)

received far less attention is the potentially crucial role of oxygen supplied by photosynthesizing macrophytes in mitigating that risk. In this study, association with macrophytes increased oxygen saturation in benthic egg masses during development under lighted conditions. While the presence of algae did not affect the length of development for the outermost embryos in masses, egg masses reared with algae had higher proportions of late stage larvae at the commencement of hatching. In all cases, hatchlings appeared simultaneously from paired egg mass halves regardless of whether algae were present, likely because the short diffusion distance from the water surrounding masses to the outermost embryos allowed these embryos to obtain sufficient oxygen regardless of the presence of algae. If depletion of oxygen by dark respiration of algae slowed development of embryos, these effects were subtle, suggesting that embryos of this species can regulate their oxygen consumption down to fairly low levels. In contrast, Fernandes & Podolsky (2011) reported accelerated hatching of the gastropod *Haminoea vesicula* when associated with eelgrass. Because oxygen was not directly measured in that study, it is not possible to evaluate whether this different response indicates a species-specific difference in developmental effects of hypoxia or contrasting dynamics of photosynthesis and respiration in their eelgrass treatments. Further, in that study, hatching time was evaluated as the number of days at which 50% of embryos had hatched, whereas in the present study, we stopped the experiment when hatching began.

Siphonaria australis egg masses begin to disintegrate soon after hatching begins (Phillips, personal obs); after loss of structural integrity, water movement would break up the mass and free the remaining embryos. Once freed from the mass, embryos that were not yet ready to hatch or commence a planktotrophic lifestyle—particularly those that had not formed a shell or swimming structures—might be at higher risk for mortality. We saw developmental gradients in all egg masses, but this pattern was significantly stronger when masses were reared without algae; remarkably, 50% of the egg masses in the control treatments (no algae) contained central embryos that were at the very earliest embryonic stages (i.e., trochophores) when their outermost siblings were hatching. By contrast, almost all central embryos in their counterparts reared with algae had reached a much more advanced, shelled stage. These gradients are not a lab artifact because developmental gradients were also observed in all *S. australis* egg masses collected from the field (Phillips & Moran, unpublished data) and are known to occur in other species (e.g., Chaffee & Strathmann, 1984). The oxygen provided by algal photosynthesis measurably increased the amount available to central embryos and was likely the main factor that reduced the steepness of the developmental gradient in masses in the presence of algae. In nature, this would permit a higher proportion of embryos to reach the shelled veliger stage prior to the breakup of the egg mass. Because larger egg masses tend to be more oxygen-limited (all else being equal; Moran & Woods, 2007; Woods & Moran, 2008a, b), the benefit of increased oxygen provided by algae will be mediated by egg mass size, and algae will be more beneficial for large egg masses.

In the laboratory, older egg masses (day 6) had lower central oxygen levels than younger ones (day 2), regardless of treatment. Similarly, in the field, although oxygen saturation in the water directly drove central oxygen concentrations in egg masses, late stage egg masses had lower saturation overall than early ones, probably because metabolic activity increases as embryos age (e.g., Moran & Woods, 2007). Not only are older masses more hypoxic in general, but embryos of many types of molluscs also tend to become more sensitive to hypoxia as development progresses, probably due to a decrease in surface area/volume ratios and/or to metabolic demands that increase as embryos develop (e.g., Wang &

Widdows, 1991; Cancino et al., 2003). Thus, the presence of macroalgae is likely to be more beneficial to older masses than to younger ones. Similarly, macroalgae may have a more substantial effect on masses of species with direct development that complete metamorphosis in masses or capsules than on species which hatch as swimming veligers.

Regardless of age or size, the centers of egg masses kept without algae (controls) were always hypoxic and were completely anoxic after embryos developed past the trochophore stage. When masses were kept with algae, central oxygen varied depending on lighting conditions. When the surrounding water was hyperoxic from several hours of algal photosynthesis, oxygen in the centers of masses ranged from 95% saturation for newly laid masses to 30% of saturation for older egg masses. After a simulated night (several hours in the dark), central O_2 concentrations of masses kept with algae resembled that of controls. These results closely mirrored field data, where oxygen saturation from a tide pool and egg masses within it also ranged from hyperoxic in the day to hypoxic at night. Any effects on developmental rate of algal-induced hypoxia in the dark, however, appear to be entirely offset by the benefits of access to oxygen from photosynthesis during the day.

Oxygen saturation in tide pools in the field is highly variable, both temporally (due to day/night light cycles, changes in temperature, and metabolic activity of organisms, as well as degree of daytime illumination), spatially from pool to pool (reviewed in Metaxas et al., 1994), and even within pools (e.g., Irwin & Davenport, 2010). All pools in our study were comparatively small and shallow, and pool-to-pool variation was probably largely driven by biotic factors, particularly the relative abundance of macrophytes and metabolic oxygen consumption by those and other organisms (microorganisms, invertebrates, fish). As an example of pool-to-pool variation, on a warm sunny day in summer, we found some pools that were near normoxic and others with oxygen levels at $\sim$2.5 times saturation. Egg masses were found in both high and low oxygen pools, suggesting that for *S. australis* the intertidal environment is a complex landscape in which offspring could potentially either benefit from algae or not depending on where parents choose to deposit their egg masses. While some species of gastropods are selective as to egg mass deposition site (von Dassow & Strathmann, 2005), to our knowledge, no studies in benthic marine invertebrates have focused on environmental oxygen availability as a criterion for parental choice in egg deposition site. *Siphonaria* spp. in Australia and New Zealand do not avoid depositing masses under conditions of high temperature, desiccation, and UV radiation (Przeslawski & Davis, 2007; Russell & Phillips, 2009).

Our oxygen measurements demonstrate that in nature, and through development, *Siphonaria* embryos routinely experience wide fluctuations in environmental oxygen availability from anoxia to 2–3$\times$ saturation. For an individual embryo, oxygen supply will depend both on mass deposition site and position within the mass. For an embryo near the periphery of an egg mass, oxygen levels may be near normoxic throughout development or may alternate between moderate hypoxia and hyperoxia. Central embryos, in contrast, are likely to be oxygen-limited regardless of deposition site and may experience long periods of complete anoxia when macroalgae are absent. Although we did not test for sublethal effects of hyper- and hypoxia on larval performance, most mature embryos of *S. australis* appeared to be fully functional on hatching, suggesting that they have a high tolerance for both. Intertidal marine molluscs in general show a high capacity for metabolic down-regulation or arrest in the face of hypoxia or anoxia (reviewed in Storey, 1993), and embryos of freshwater pulmonates can withstand long periods of near-anoxic conditions (Goldberg et al., 2008). However, the presence of adult mechanisms for metabolic down-regulation (e.g., reversible enzyme phosphorylation) has not, to our knowledge, been investigated in molluscan embryos. Similarly, while molluscs have numerous physiological defences against hyperoxia (Abele & Puntarulo, 2004) and hyperoxia has been shown to negatively affect larval growth in at least one mollusc (Huntington & Miller, 1989), overall, these mechanisms have received little attention in embryos. *S. australis* embryos experience dramatic temporal and spatial variance in oxygen supply in their natural habitat, and therefore these and other organisms that develop in tide pools would be interesting focal species for understanding metabolic adaptations to tolerance of hypo- and hyperoxia in aquatic ecosystems.

There is a wealth of literature on physiological and behavioral responses of aquatic animals to hypoxia. However, to date there has been much less focus on the

consequences or drivers of variable oxygen conditions. Macrophytes are fundamental components of aquatic habitats that play a key role in determining oxygen variability, particularly in smaller, more isolated or ephemeral habitats (Truchot & Duhamel-Jouve, 1980; Keeley & Sandquist, 1991). We have shown that oxygen supplied by aquatic macroalgae can have positive effects on benthic embryos. A large number of taxa deposit egg masses; oxygen is critical to successful development and often limiting for the bulk of interior embryos which have little capability to respond to abiotic stress. Further, hypoxic conditions are common in many aquatic habitats and increasing in some with global change (Altieri, 2008). Thus, the oxygen-mediated relationship between macrophytes and aquatic animals is likely to be an important, yet not well-recognized beneficial interaction for a range of taxa.

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References

Abele, D. & S. Puntarulo, 2004. Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. Comparative Biochemistry and Physiology Part A 138: 405–415.

Altieri, A. H., 2008. Dead zones enhance key fisheries species by providing predation refuge. Ecology 89: 2808–2818.

Apodaca, C. K. & L. J. Chapman, 2004. Larval damselflies in extreme environments: behavioural and physiological response to hypoxic stress. Journal of Insect Physiology 50: 767–775.

Breitburg, D. L., T. Loher, C. A. Pacey & A. Gerstein, 1997. Varying effects of low dissolved oxygen on trophic interactions in an estuarine food web. Ecological Monographs 67: 489–507.

Burggren, W. & A. Mwalukoma, 1983. Respiration during chronic hypoxia and hyperoxia in larval and adult bullfrogs (*Rana catesbeiana*), 1. Morphological responses of lungs, skin and gills. Journal of Experimental Biology 105: 191–203.

Cancino, J. M., J. A. Gallardo & E. A. Torres, 2003. Combined effects of dissolved oxygen concentration and water temperature on embryonic development and larval shell secretion in the marine snail *Chorus giganteus* (Gastropoda: Muricidae). Marine Biology 142: 133–139.

Chaffee, C. & R. R. Strathmann, 1984. Constraints on egg masses, I. Retarded development within thick egg masses. Journal of Experimental Marine Biology Ecology 84: 73–83.

Cohen, C. S. & R. R. Strathmann, 1996. Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. Biological Bulletin 190: 8–15.

Davies, R. W. & T. E. Gates, 1991. Intra- and interspecific differences in the response of two lentic species of leeches to seasonal hyperoxia. Canadian Journal of Fisheries and Aquatic Science 48: 1124–1127.

Diaz, R. J. & R. Rosenberg, 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Marine Biology: An Annual Review 33: 245–303.

Eriksen, C. H., V. H. Resh & G. A. Lamberti, 1996. Aquatic insect respiration. In Merritt, R. W. & K. L. Cummins (eds), An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque: 29–40.

Fernandes, D. A. O. & R. D. Podolsky, 2011. Developmental consequences of association with a photosynthetic substrate for encapsulated embryos of an intertidal gastropod. Journal of Experimental Marine Biology and Ecology 407: 370–376.

Fischer, J. & N. E. Phillips, 2014. Carry-over effects of multiple stressors on benthic embryos are mediated by larval exposure to elevated UVB and temperature. Global Change Biology 20: 2108–2116.

Fischer, P., K. Rademacher & U. Kils, 1992. In situ investigations on the respiration and behaviour of eelpout *Zoarces viviparous* under short-term hypoxia. Marine Ecology Progress Series 88: 181–184.

Goldberg, J. I., S. A. Doran, R. B. Shartau, J. R. Pon, D. W. Ali, R. Tam & S. Kuang, 2008. Integrative biology of an embryonic respiratory behaviour in pond snails: the 'embryo stir-bar hypothesis'. Journal of Experimental Biology 211: 1729–1736.

Huntington, K. M. & D. C. Miller, 1989. Effects of suspended sediment, hypoxia, and hyperoxia on larval *Mercenaria mercenaria* (Linnaeus, 1758). Journal of Shellfish Research 8: 37–42.

Irwin, S. & J. Davenport, 2010. Oxygen microenvironment of coralline algal tufts and their associated epiphytic animals. Journal of Environmental Biology 110B: 185–193.

Keeley, J. E. & D. R. Sandquist, 1991. Diurnal photosynthesis cycle in CAM and non-CAM seasonal-pool aquatic macrophytes. Ecology 72: 716–727.

Kolar, C. S. & F. J. Rahel, 1993. Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia 95: 210–219.

Levin, L. A., 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanography and Marine Biology 41: 1–45.

McMahon, B. R., 1988. Physiological responses to oxygen depletion in intertidal animals. American Zoologist 28: 39–53.

Metaxas, A., H. L. Hunt & R. E. Sheibling, 1994. Spatial and temporal variability of macrobenthic communities in tidepools on a rocky shore in Nova Scotia, Canada. Marine Ecology Progress Series 105: 89–103.

Moran, A. L. & H. A. Woods, 2007. Oxygen in egg masses: interactive effects of temperature, age, and egg-mass morphology on oxygen supply to embryos. Journal of Experimental Biology 210: 722–731.

Pihl, L., S. P. Baden & R. J. Diaz, 1991. Effects of periodic hypoxia on distribution of demersal fish and crustaceans. Marine Biology 108: 349–360.

Pinder, A. W. & S. C. Friet, 1994. Oxygen transport in egg masses of the amphibians *Rana sylvatica* and *Ambystoma maculatum*: convection, diffusion and oxygen production by algae. Journal of Experimental Biology 197: 17–30.

Przeslawski, R. & A. R. Davis, 2007. Does spawning behavior minimize exposure to environmental stressors for encapsulated gastropod embryos on rocky shores? Marine Biology 152: 991–1002.

Rosenberger, A. E. & L. J. Chapman, 1999. Hypoxic wetland tributaries as faunal refugia from an introduced predator. Ecology of Freshwater Fish 8: 22–34.

Russell, J. & N. E. Phillips, 2009. Synergistic effects of ultraviolet radiation and conditions at low tide on egg masses of limpets (*Benhamina obliquata* and *Siphonaria australis*) in New Zealand. Marine Biology 156: 579–587.

Salas-Leiton, E., B. Canovas-Conesa, R. Zerolo, J. Lopez-Barea, J. P. Canavate & J. Alhama, 2009. Proteomics of juvenile Senegal sole (*Solea senegalensis*) affected by gas bubble disease in hyperoxygenated ponds. Marine Biotechnology 11: 473–487.

Seymour, R. S., J. T. Roberts, N. Mitchell & A. J. Blaylock, 2000. Influence of environmental oxygen on development and hatching of aquatic eggs of the Australian frog, *Crinia georgiana*. Physiological and Biochemical Zoology 73: 501–507.

Smith, K. J. & K. W. Able, 2003. Dissolved oxygen dynamics in salt marsh pools and its potential impacts on fish assemblages. Marine Ecology Progress Series 258: 223–232.

Storey, K. B., 1993. Molecular mechanisms of metabolic arrest in mollusks. In Hochachka, P. W., P. L. Lutz, T. Sick, M. Rosenthal & G. van den Thillart (eds), Surviving hypoxia: mechanisms of control and adaptation. CRC Press, Boca Raton: 253–269.

Truchot, J.-P. & A. Duhamel-Jouve, 1980. Oxygen and carbon-dioxide in the marine intertidal environment: diurnal and tidal changes in rockpools. Respiration Physiology 39: 241–254.

Tunnicliffe, V., 1981. High species diversity and abundance of the epibenthic community in an oxygen-deficient basin. Nature 294: 354–356.

von Dassow, Y. J. & R. R. Strathmann, 2005. Full of eggs and no place to lay them: hidden cost of benthic development. Marine Ecology Progress Series 294: 23–34.

Wang, W. X. & J. Widdows, 1991. Physiological responses of mussel larvae *Mytilus edulis* to environmental hypoxia and anoxia. Marine Ecology Progress Series 70: 223–236.

Warton, D. I. & F. K. C. Hui, 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92: 3–10.

Weitkamp, D. E. & M. Katz, 1980. A review of dissolved gas supersaturation literature. Transactions of the American Fisheries Society 109: 659–702.

Woods, H. A. & R. D. Podolsky, 2007. Photosynthesis drives oxygen levels in macrophyte-associated gastropod egg masses. Biological Bulletin 213: 88–94.

Woods, H. A. & A. L. Moran, 2008a. Oxygen profiles in egg masses predicted from a diffusion-reaction model. Journal of Experimental Biology 211: 790–797.

Woods, H. A. & A. L. Moran, 2008b. Temperature-oxygen interactions in Antarctic nudibranch egg masses. Journal of Experimental Biology 211: 798–804.