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Extreme low oxygen and decreased pH conditions naturally occur within developing squid egg capsules

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ABSTRACT: Young animals are the foundation of future cohorts and populations, but are often particularly susceptible to environmental changes. This raises concerns that future conditions, influenced by anthropogenic changes such as ocean acidification and increasing oxygen minimum zones, will greatly affect ecosystems by impacting developing larvae. Understanding these potential impacts requires addressing present tolerances and current conditions in which animals develop. Here, we examined changes in oxygen and pH adjacent to and within normally-developing squid egg capsules, providing the first observations that the egg capsules, housing hundreds of embryos, have extremely low internal pH (7.34) and oxygen concentrations (1.9 µmol l^{-1}). While early-stage egg capsules had pH and oxygen levels significantly lower than the surrounding seawater, late-stage capsules dropped dramatically to levels considered metabolically stressful even for adults. The structure of squid egg capsules results in a closely packed unit of respiring embryos, which likely contributes to the oxygen-poor and CO_2-rich local environment. These conditions rival the extremes found in the squids' natural environment, suggesting they may already be near their metabolic limit, and that these conditions may induce a hatching cue. While squid may be adapted to these conditions currently, further climate change could place young, keystone squid outside of their physiological limits.

KEY WORDS: Cephalopod · Climate change · Hypoxia · Boundary layer · Eggs · Larva

INTRODUCTION

Shifts in oceanic chemistry, such as changes in available oxygen (O_2) and decreasing pH, are of growing concern given their potential impacts on marine organisms and the ecosystems they support (Pörtner et al. 2004, Seibel & Childress 2013, Rosa et al. 2014). Dissolved O_2 is necessary for cellular respiration, but in many oceanic regions O_2 levels are declining and oxygen minimum zones (OMZs) are expanding (Stramma et al. 2012). These changes are being attributed to several factors, including lower sea-surface O_2 concentrations, local eutrophication events, and reduced ventilation caused by ocean warming. The effects of decreasing O_2 levels are compounded by increasing carbon dioxide (CO_2) concentrations, largely from fossil fuel burning, which drives ocean acidification (Caldeira & Wickett 2003). Despite concerns regarding future ocean conditions, the baseline environmental conditions that organisms currently face still require substantial attention, especially in highly dynamic coastal ecosystems where parameters such as O_2 and pH fluctuate dramatically (Gobler et al. 2014, Wallace et al.

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2014). Consequently, there is a great deal of uncertainty when predicting the influence of current and future environmental changes on key marine taxa. Collecting baseline data affords a better understanding of these animals' current tolerances and contributes to the reduction of these uncertainties.

In nearly all marine environments, young, developing animals appear to be particularly susceptible to changing ocean conditions, with effects such as impaired development and reduced size having been shown for a variety of species (Kurihara 2008, Ries et al. 2009, Rosa et al. 2013). These impacts have been suggested to reduce recruitment success, and consequently, could reduce population abundances (Munday et al. 2010). Marine invertebrates that deposit calcareous skeletons have received much attention recently, with their young showing vital changes (i.e. growth, structure) when raised under ocean acidification or hypoxic conditions (Hoegh-Guldberg et al. 2007, Ries et al. 2009, Gobler et al. 2014). Impacts to soft-bodied invertebrates are seemingly less understood, yet they too have calcified structures, and are often physiologically limited by pH, aragonite concentrations, and O_2 levels (Radtke 1983, O'Dor et al. 1995, Pörtner et al. 2004, Rosa et al. 2013). Any mechanism that may even slightly reduce the early-life survival rates of marine organisms can have major repercussions on adult population sizes, and in the case of keystone taxa, overall ecosystem health (Houde 1987, 2008).

Cephalopods, particularly squid, are an ecologically and economically key taxon, providing a central trophic link in many marine food webs and 15 to 20% of global fisheries landings and values (Boyle & Rodhouse 2005, Hunsicker et al. 2010). The loliginid squid are a primary commercial cephalopod in the western North Atlantic and support a fishery of approximately 16600 mt yr^{-1} (NOAA 2010). Occasionally cited as keystone taxa, squid play a central role in food webs as predator and prey to a wide array of taxa that occupy different trophic levels (Clarke 1996). Cephalopods are no exception to the potential impacts of changing ocean conditions. Increased pCO_2 can cause significant increases in development time, decreases in hatchling size, and changes to statolith structure in squid (e.g. *Doryteuthis pealeii*; Kaplan et al. 2013). In adults, decreased pH can impair the O_2 binding capacity of haemocyanin, a respiratory protein that transports oxygen in squid (Pörtner 1990). Even in today's oceans, adult squid are considered to live near the edge of O_2 limitation, particularly during muscular exercise (Pörtner et al. 1991, Seibel 2007, Seibel & Childress 2013). Hence, lower metabolic rates have been found across different squid taxa as respective environmental O_2 concentrations decrease (Seibel et al. 1997). Small decreases in ambient pH or O_2 are thought to restrict the ability of haemocyanin to bind sufficient O_2 or otherwise limit O_2 uptake, which would likely impair the squid's high energetic demand (Pörtner et al. 2004, Seibel & Childress 2013).

Squid recruitment is largely driven by environmental factors (Dawe et al. 1990); environmental conditions also play a large role in migration, distribution, growth, and spawning (Boyle & Rodhouse 2005, Zeidberg et al. 2011). Because cephalopod abundances are directly tied to the success of early life history, growth, and survival (Boyle & Rodhouse 2005, Foote et al. 2006), environmental changes such as ocean acidification or decreased O_2 availability could directly impact populations. With growing evidence that ocean acidification will be amplified by hypoxia and eutrophication in coastal waters, it is becoming increasingly important to consider the interaction of these environmental parameters (Cai et al. 2011, Melzner et al. 2013, Wallace et al. 2014), and particularly effects on the susceptible early life stages. Adjacent, near-shore estuarine habitats where squid such as *D. pealeii* are occasionally found may vary substantially in pH and O_2, conditions that are exacerbated by eutrophication (Wallace et al. 2014, Baumann et al. 2015). Yet, for *D. pealeii* and many other squid species, there are few data addressing the epi-benthic, coastal environment (pH, O_2, flow) where the majority of reproductive adults are harvested, and thus, the conditions that most egg capsules naturally experience.

There is a growing body of literature that addresses O_2 availability or O_2 and pH conditions associated with developing mollusks (Booth 1995, Cohen & Strathmann 1996, Moran & Woods 2007). Work on cephalopods has largely focused on cuttlefish (Cronin & Seymour 2000, Gutowska & Melzner 2009, Dorey et al. 2013), a taxon in which a single embryo develops in individual capsules. Adult loliginids, however, like many coastal squid, lay their eggs in gelatinous capsules on the benthos, with each egg capsule densely housing 150 to 200 embryos (Hanlon & Messenger 1996). These animals undergo rapid growth, becoming fully developed in 12 to 14 d at 20°C (McMahon & Summers 1971), with the capsule expanding in size to accommodate this growth (Hanlon et al. 1983). Although most hatching occurs during the night, and certain physical disturbances (such as handling) may induce hatching (Hanlon & Mes-

senger 1998, Zeidberg et al. 2011), the natural cues or catalysts for squid egg hatching (besides full development) are not well established. Recent work has considered the respiration of squid embryos, although individuals were removed from their capsules for respirometry measurements, creating an environment unlike that they experience in nature, and potentially promoting premature stress and hatching (Rosa et al. 2012, 2014). Thus, to date, squid embryos consume an unknown amount of O_2 and produce an unquantified amount of CO_2, all within a semi-permeable capsule — the structure of which likely alters the exchange of O_2 and CO_2. Furthermore, it remains poorly understood how a population of fast growing, highly active cephalopod embryos inside a capsule influences the pH and O_2 within the capsule or adjacent water, and how this influence may vary with development, embryo size, and increases in O_2 demand and CO_2 respiration. Studies of this would require a detailed profile of the egg capsule and the surrounding physical boundary layer of intact capsules, as has been done with metabolically slower gastropods and polychaetes (Chaffee & Strathmann 1984, Booth 1995, Cohen & Strathmann 1996, Moran & Woods 2007). For example, in many marine gastropods that lay benthic egg clutches, intracapsular O_2 availability substantially affects embryo development rates (Booth 1995, Strathmann & Strathmann 1995). Local environmental conditions can also affect O_2 uptake and consequent embryo condition (Cohen & Strathmann 1996, Cancino et al. 2011). Moreover, the physical boundary layer surrounding egg capsules is a function of the physical characteristics of water flow rates and the roughness of the capsule surface, and may significantly alter exchange across organismal boundary layers. The variation of this boundary layer due to fluctuating flow rates in coastal ecosystems has not been considered in experiments of changing ocean conditions or metabolism on cephalopod egg capsules.

To address these unknowns and provide a better understanding of the natural pH and O_2 conditions associated with a densely populated cephalopod egg capsule, we sought to quantify (1) the O_2 and pH levels within egg capsules where embryos develop, (2) egg capsule O_2 consumption and pH change across embryonic development, and (3) the pH and O_2 levels in the boundary layer adjacent to the capsule. Results were then placed in the context of current data on thresholds and on pH and O_2 limits for squid, to highlight what data are still required for a better understanding of this critical developmental stage.

MATERIALS AND METHODS

Experiments were conducted at the Woods Hole Oceanographic Institution (WHOI), MA, USA in August and September 2014. Adult *Doryteuthis pealeii* squid were collected under Massachusetts Division of Marine Fisheries research permit #152087. Husbandry and animal care were performed in accordance with guidelines approved by WHOI's Institutional Animal Care and Utilization Committee. Squid were trawl-caught in Vineyard Sound, MA, on 2 occasions in 10 to 20 m of water. Adults in healthy condition (free of cuts and scrapes) were hand-selected from the group, gently placed in individual buckets, and immediately transported to a 500 l holding tank at WHOI, where they were maintained in 14°C cooled, filtered, flowing local water. Within ~48 to 72 h, the squid bred, laying egg capsules in a mass on the tank bottom. Egg masses (~30 capsules $mass^{-1}$) were transferred to either a 38 l aquarium in which water was replaced daily, or a 100 l flow-through aquarium, both of which were filled with local filtered seawater maintained at 20°C, the average temperature for Vineyard Sound during the study period (mean ± SD: 19.4 ± 0.68°C; data from Martha's Vineyard Coastal Observatory, www.whoi.edu/page.do?pid=70177). Individual egg capsules (Fig. 1a) were separated from the egg mass immediately prior to profiling, attached on top of 5 mm rigid plastic mesh using zip ties at the leading edge of the capsule, and transferred to a 0.5 l glass container or a custom 9.5 l recirculating micro-flume, both filled with the same filtered 20°C seawater. All measurements and incubations were done under ambient laboratory light conditions. A micromanipulator (Unisense) was used to vertically profile up to and within the egg capsules (Fig. 1b).

A FireStingO_2 optical oxygen sensor (50 µm sensing tip) and meter (Pyroscience) and liquid ion exchange (LIX) pH sensors (5 to 20 µm sensing tip) were used to measure profiles. LIX pH sensors were constructed and used following Gieseke & de Beer (2004). Briefly, glass capillaries were pulled to a tip diameter of 5 to 20 µm and the glass was silinized using N,N-dimethyltrimethylsilylamine (Sigma) in a sealed glass container at 200°C to make the glass hydrophobic. PVC-stabilized H^+ sensitive membranes (H^+ ionophore II; Sigma) were pulled into the capillary tips, and the electrode was back-filled with a 300 mmol l^{-1} potassium chloride, 7.0 pH, 50 mmol l^{-1} phosphate buffer. The micro electrodes were finished by sealing a silver chloride plated 0.25 mm diameter silver wire into the back of the capillary.

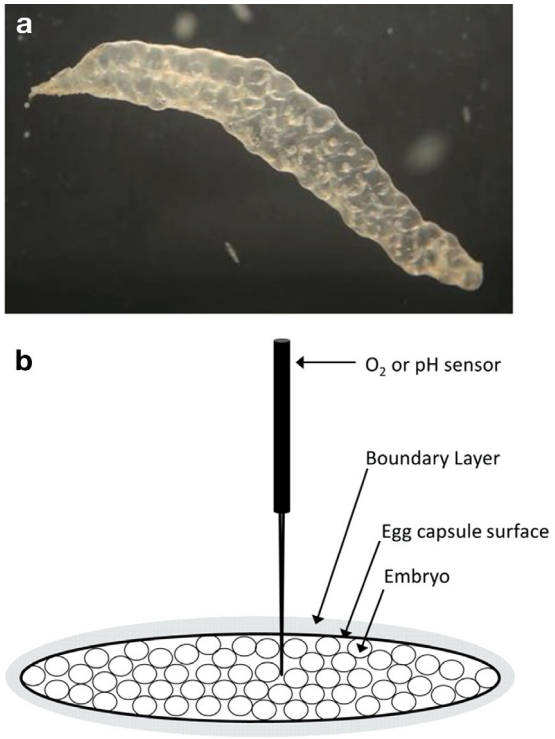

Fig. 1. (a) Squid *Doryteuthis pealeii* egg capsule photograph, and (b) schematic of profiling, boundary layer (grey shading), and egg capsule. Photo credit: D. Cojanu

The electrochemical circuit was completed with a reference electrode consisting of a glass capillary filled with a saturated potassium chloride solution, a microporous glass frit (Princeton Applied Research) tip, and sealed with a silver chloride plated 0.25 mm diameter silver wire. The millivolt response of the electrodes (>50 mV per pH unit) was measured using a high-impedance millivolt meter.

The pH sensors were calibrated using National Institute of Standards and Technology (NIST)-traceable buffers at 20°C and cross-checked before and after each profile with a commercial pH sensor (Hach). The Pyroscience O_2 optodes were calibrated using a saturated sodium ascorbate solution (0% O_2) and water-saturated air according to manufacturer's instructions. To ensure sensors were not damaged or otherwise affected by profiling through the egg capsules, sensors were returned to the ambient water after reaching the center of the egg capsules to confirm that the sensors showed consistent readings with the beginning of the profile.

The recirculating mini-flume consisted of a divided 9.5 l aquaria connected by a passage 0.07 m high, 0.07 m wide, and 0.3 m long. Water was pumped between the aquaria halves using a small pump, and the flow was adjusted using a ball-valve. Flow rates were evaluated with simple discharge–area–time relationships. Sensors were located using the micromanipulator and a forward-looking adjustable (0–25×) dissection scope (Zeiss), all mounted to a sturdy microprofiling base station (Unisense). The mini-flume water was changed daily using filtered, local seawater at 20°C. Static (no-flow) profiles were determined in 0.5 l glass containers within the same microprofiling base station, and filled with water from the aquaria in which the capsules were incubated. Egg capsules were incubated in the flume at a specific flow rate for at least 1 h prior to profiling.

A coarse vertical profile (1000 µm increments) was measured in the water column down to the capsule 'boundary layer', or the fluid layer around the egg capsule where diffusive transport is of primary importance — defined here by the location where large O_2 concentration changes were observed (e.g. Gieseke & de Beer 2004) (see Fig. 2). At the boundary layer, measurement increments were decreased to 100 µm to better resolve the concentration gradient. The sensors were then pushed into the egg capsule and the profile continued until reaching the egg capsule center, which was determined using a micrometer in the dissection scope, the egg capsule diameter, and the visible location of the sensor tip. Care was taken to prevent puncturing individual embryos; the small sensor movements allowed the embryos to shift laterally, allowing the sensors to remain within the intracapsular fluid. Each profile was done on a new egg capsule in the range of 1–3 or 10–13 d old.

Differences between the ambient conditions and conditions in the center of the different aged capsules were determined by 1-way ANOVAs, with differences between these groups determined by Tukey's post hoc tests. Due to the seasonal cessation of squid breeding, testing of flow effects on egg capsule profiles could only be conducted with unfertilized egg capsules. However, these provided initial baseline profiles of egg capsule respiration due to microbial biomass (i.e. no metabolism of developing embryos) and the effects of water flow past capsules at low (0.01 m s^{-1}) and high (0.1 m s^{-1}) current velocities utilizing the recirculating micro-flume.

RESULTS

Newly-laid egg capsules demonstrated significantly lower O_2 concentrations and pH relative to the ambient water, with O_2 dropping from ~200 to 160 µmol l^{-1} O_2 and pH decreasing from 8.0 to 7.8 at the capsule

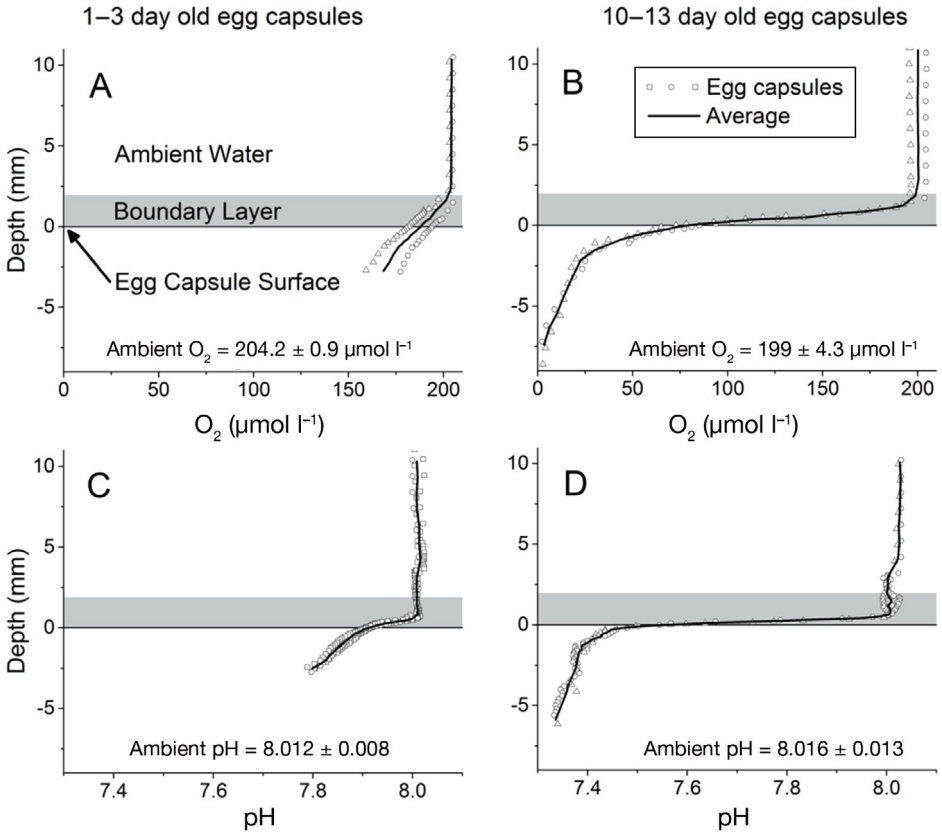

Fig. 2. Profiles of (A,B) oxygen and (C,D) pH in 1–3 d old (left) and 10–13 d old (right) egg capsules. Shapes indicate individual profiles in different egg capsules; solid lines: average profile

center (Fig. 2A,C). This difference increased substantially with egg development (Fig. 2B,C); after 10 to 13 d of development, egg capsule centers contained only trace amounts of O_2 (mean ± SD: 1.9 ± 1.1 µmol l^{-1}) and had a pH of 7.34 ± 0.01.

The steep gradients between the ambient water and the egg capsule allowed for the calculation of diffusive O_2 flux across the boundary layer around the egg capsule (Fig. 2). The dominant transport process in this boundary layer is diffusion; therefore Fick's Law of Diffusion can be used to determine exchange across the egg capsule surface. The flux = $\delta O_2/\delta x \times D$, where D is the diffusion coefficient of O_2, and x is the depth (Gieseke & de Beer 2004). The depth of the boundary layer (x) was determined from the concentration profiles, and the O_2 gradient was determined from the O_2 gradient between the ambient water and the egg capsule surface. The fluxes revealed a 10-fold increase in egg capsule O_2 consumption over a 10 d period (0.060 to 0.595 µmol cm^{-2} min^{-1} for the 1–3 and 10–13 d old capsules, respectively). Applying Fick's Law of Diffusion to the capsule boundary layer allows for the determination of the time point at which the maximum physical transport into the capsule is exceeded by the capsule's metabolic requirement (indicating significant hypoxic stress). For example, using the measured boundary layer thickness, the maximum possible O_2 gradient (~200 µmol l^{-1} mm^{-1}), and assuming a linear increase in O_2 consumption with capsule age, the time when the maximum physical transport of O_2 is exceeded by egg O_2 consumption (0.84 µmol cm^{-2} min^{-1}) was 15.8 d

The unfertilized egg capsules had similar O_2 concentration changes and profiles to the 1 to 3 d old egg capsules (Fig. 3, Table 1). The former was primarily due to the metabolism of capsule-associated microbial communities (Barbieri et al. 2001), and suggests a relatively small measureable metabolic contribution by the 1–3 d old embryos. The calculated O_2 fluxes were 0.073, 0.088, and 0.098 µmol cm^{-2} min^{-1} for unfertilized egg capsules under no-flow, low-flow, and high-flow conditions, respectively. The thickness of the boundary layer decreased with flow (2.0, 0.7, and 0.4 mm for the no-flow, low-flow, and high-flow conditions, respectively).

All egg capsules used in this study hatched viable squid paralarvae, with the exception of the unfertilized capsules (characterized by no change in size or visible growth). Hatching success was not evaluated as a part of this work. The developing egg capsules visibly increased in volume as the embryos grew (Table 1), leading to the deeper profiles in the 10–13 d old egg capsules.

DISCUSSION

Both O_2 and pH conditions in the full-term embryo capsules were unexpectedly low, reaching levels that are often considered adverse to many pelagic taxa (Stramma et al. 2012). O_2 levels were below that of the water adjacent to the capsules and that of the local environment in which these capsules are often found, and were even lower than that of Atlantic

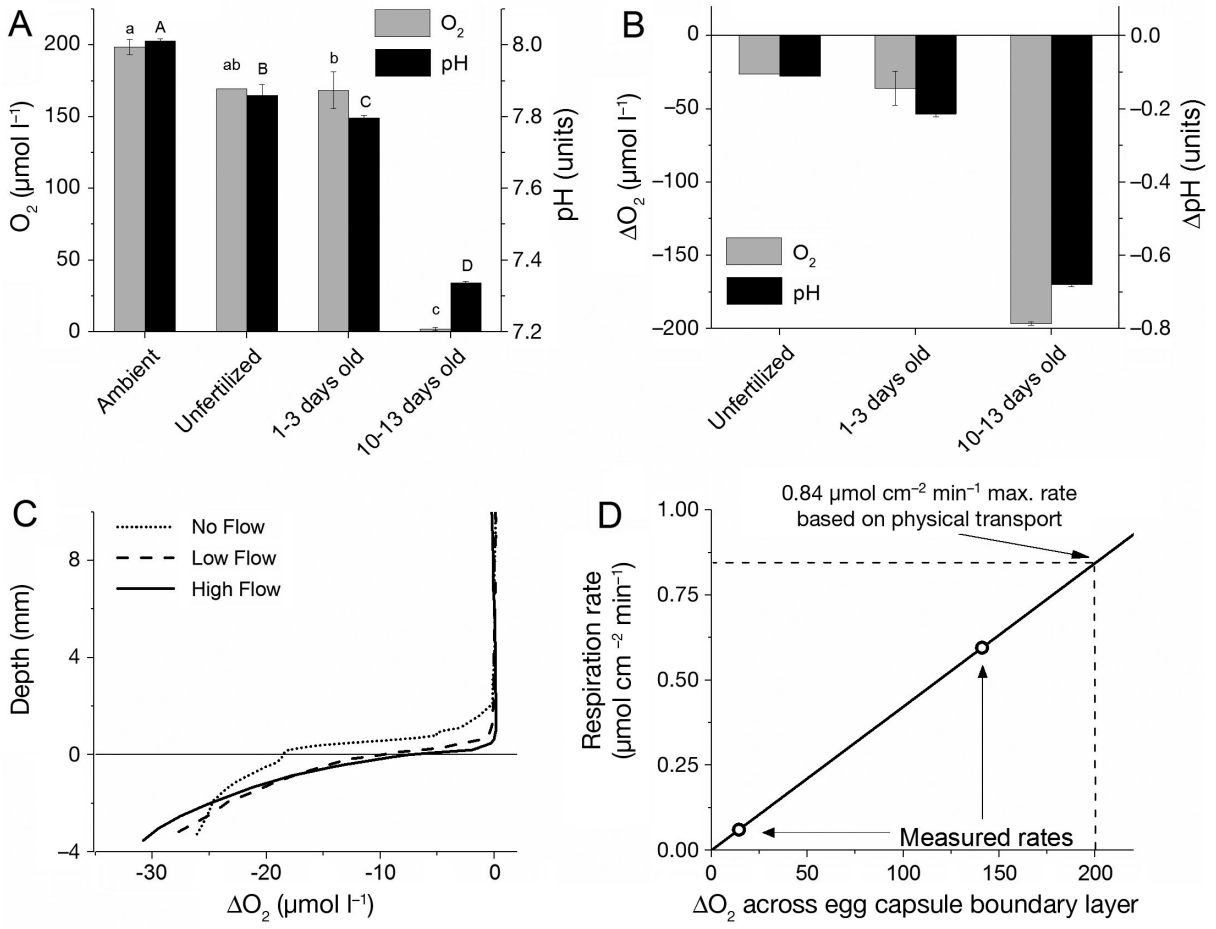

Fig. 3. (A) Oxygen concentrations and pH in ambient water and the center of different aged squid *Doryteuthis pealeii* egg capsules showing significant variation in both oxygen and pH (see Table 1); different letters above bars indicate significant differences between groups (Tukey's test). (B) Change in oxygen and pH in the center of egg capsules relative to ambient water conditions. (C) Effect of different flow rates on oxygen profiles in unfertilized egg capsules and compression of the boundary layer; each line represents the average of 3 profiles. (D) Maximum respiration rate under no-flow conditions (see 'Results') indicating egg capsules ~16 d old will be significantly stressed during hydrodynamically calm periods

OMZs (Karstensen et al. 2008). Although some oceanic deep-sea squid species have shown tolerances and even affinities to low O_2 levels (Gilly et al. 2012, Seibel 2013), active, coastal, adult loliginid squids are typically considered to be near the edge of their metabolic O_2 capabilities and somewhat intolerant of the conditions measured here (Pörtner 2002). The decrease of O_2 levels by 99% to near-anoxic conditions during growth is larger than the hypoxic conditions observed for similar cephalopod species that have a single egg per capsule (75% decrease, Dorey et al. 2013; 86% decrease, Cronin & Seymour 2000; 62% decrease, Gutowska & Melzner 2009; 85% decrease, Rosa et al. 2013), suggesting the densely packed egg capsule structure leads to an extremely high O_2 demand, as has been found in a number of gastropod and polychaete species housing multiple eggs per capsule (Chaffee & Strathmann 1984, Booth 1995, Cohen & Strathmann 1996, Moran & Woods 2007). The observation of hatching and healthy paralarvae was surprising based on previous results from other studies on cephalopods, and suggests these conditions may not have induced extreme stress, similar to results found in other multiple-egg-per-capsule species.

The boundary effects of the capsule suggest that encapsulation of the many embryos likely contributes to the lower O_2 and pH environment (Chaffee & Strathmann 1984, Booth 1995, Moran & Woods 2007), resulting in conditions that are substantially lower than that observed for single-egg-per-capsule species (e.g. cuttlefish; Rosa et al. 2013). The encapsulation of embryos has been proposed as a mechanism to protect embryos against ocean acidification

Table 1. Oxygen and pH in the ambient water and center of squid *Doryteuthis pealeii* egg capsules. *F* and p-values indicate significant differences determined by ANOVAs; different superscript letters indicate significant differences between groups determined by Tukey's post hoc tests; number in parentheses: no. of egg capsules

	Egg capsule diameter (mm)	Oxygen (μmol l^{-1})	pH (units)
Ambient seawater	–	198.5 ± 5.5^{a} (3)	8.01 ± 0.01^{a} (6)
Unfertilized egg capsules	6.53 ± 0.30^{a} (3)	169.3 ± 0.0^{ab} (1)	7.86 ± 0.03^{b} (2)
1–3 d old egg capsules	5.22 ± 0.28^{a} (5)	168.3 ± 12.8^{b} (2)	7.80 ± 0.01^{c} (3)
10–13 d old egg capsules	13.7 ± 1.86^{c} (5)	1.9 ± 1.1^{c} (2)	7.34 ± 0.01^{d} (3)
F_3	69.6013	299.6569	1640.154
p-value	<0.0001	<0.0001	<0.0001

through the buffering capacity of intracapsular fluids (Ellis et al. 2009, Fernandes & Podolsky 2012), but our data suggest that encapsulation actually causes reduced pH conditions around embryos, rather than buffering against it. We would expect to find even lower pH and O_2 values inside the egg capsules of squid raised in elevated ocean acidification or low oxygen conditions (as seen in some taxa, e.g. Rosa et al. 2013, Noisette et al. 2014). However, it is not clear whether these lower pH or O_2 conditions would lead to greater impacts or perhaps support adaptation to future, changing conditions.

Squid, particularly muscular, shallower species such as the taxa studied here, are considered relatively intolerant to small changes in pH (Pörtner et al. 2004). The blood pH for these adult squid is typically near 7.6 (Pörtner 1990), with some exceptions for specialized species living in OMZs or in the deep ocean (Seibel 2013, Seibel & Childress 2013). The intracapsular levels of pH 7.34 noted here were unexpected for such an energetic coastal squid, and were also well below environmental levels that have induced developmental changes in young squid (Kaplan et al. 2013, Rosa et al. 2014). This implies that during these prior studies (Kaplan et al. 2013, Rosa et al. 2014), pH values inside the experimental capsules were even lower — perhaps suggesting that despite these extreme conditions, young squid may be more tolerant than previously considered. Furthermore, the levels measured in this study were near the limit of predicted pH-dependent blood pigment O_2 affinity (Pörtner 1990); at a lower pH, blood might not effectively take up O_2. These embryos may already be at relatively inefficient metabolic levels due to the combined effects of low pH and O_2, or their haemocyanin pigment may have an improved affinity compared to adults, for example, due to the presence of different isoforms of haemocyanin in cephalopod embryos that may be more efficient at O_2 binding (Thonig et al. 2014). However, the higher surface area-to-volume ratio and importance of cutaneous O_2 uptake in squid embryos indicates that pigment-mediated O_2 exchange would likely be less important. While pH is a likely stressor for many squid, the low O_2 concentrations may dominate in limiting metabolism and energetics (Seibel et al. 1997, Seibel & Childress 2013). Thus, the low O_2 levels of egg capsules seen here reinforce concerns of expanding OMZs and deoxygenation of ocean waters, particularly if the limits of organismal adaptation are reached.

Certainly these squid may be adaptable to changes, as seen with tolerance to low O_2 conditions in the young of some of the more specialized squid species (Seibel et al. 1997, Seibel 2013, Trübenbach et al. 2013). While the conditions shown here are relatively extreme for the open ocean, they may be less stressful for coastal and estuarine organisms, which may have a greater range of tolerances (Murray et al. 2014). Based on data from adult squid and multiple other taxa (Pörtner et al. 2004, Seibel & Childress 2013), these squid may already be near their physiological limit, and thus less adaptable in the face of future ocean biogeochemical changes. Neither the optimal nor threshold O_2 and pH levels for embryonic development have been defined, so we do not know whether these conditions place substantial stress on the developing squid. The effect of the low intracapsular pH and O_2 observed here requires further study to determine how squid may be affected by future O_2 and CO_2 conditions. Understanding the mechanisms and the potential for developing squid embryos to withstand these conditions will inform our expectations for how these and other organisms may cope with projected global ocean changes.

Both pH and O_2 levels decreased over time, reflecting the increased size and energetic demands of the developing squid. As shown elsewhere (Pörtner et al. 2004, Seibel & Childress 2013), there are limits to squid pH and oxygen tolerances; we suggest that these levels may even act as an embryonic hatching cue. The maximum time to hatching was estimated from the maximum physical O_2 gradient across the egg capsule boundary layer (Fig. 3D). This maximum exchange rate under no-flow conditions suggests a maximum hatching time of 15.8 d under the observed conditions, which is consistent with previously observed hatching times of 12 to 14 d (Kaplan et al.

2013). The decrease to such low levels within the capsule may act as an embryonic hatching cue, and compounding O_2 and pH changes in the surrounding environment may induce premature hatching. Water flow over the capsule likely plays a role, since the capsule boundary layer thickness decreased with increasing flow (Fig. 3C) and O_2 exchange increased in the unfertilized egg capsules, suggesting enhanced exchange across the capsule surface due to current flow. Previously, hatching has been observed at night, which was explained as a mechanism to avoid consumption by visual predators (Zeidberg et al. 2011). Conversely, hatching may be induced during hydrodynamically calm periods during the night when O_2 is reduced by ecosystem respiration and photosynthesis is absent; for example, during a nighttime slack tide. The enhanced exchange with flow also suggests that egg laying locations that have active hydrodynamics may lead to faster embryonic development and reduced stress by low pH and O_2 conditions (Chaffee & Strathmann 1984, Cohen & Strathmann 1996, Zeidberg et al. 2011). Therefore, ocean acidification and hypoxia experiments should consider the effects of flow-enhanced exchange between organisms and the ambient seawater.

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