© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

RESEARCH ARTICLE

Both maternal and embryonic exposure to mild hypoxia influence embryonic development of the intertidal gastropod *Littorina littorea*

James C. S. McCoy*, John I. Spicer, Oliver Tills and Simon D. Rundle

ABSTRACT

There is growing evidence that maternal exposure to environmental stressors can alter offspring phenotype and increase fitness. Here, we investigate the relative and combined effects of maternal and developmental exposure to mild hypoxia (65 and 74% air saturation, respectively) on the growth and development of embryos of the marine gastropod *Littorina littorea*. Differences in embryo morphological traits were driven by the developmental environment, whereas the maternal environment and interactive effects of maternal and developmental environment were the main driver of differences in the timing of developmental events. While developmental exposure to mild hypoxia significantly increased the area of an important respiratory organ, the velum, it significantly delayed hatching of veliger larvae and reduced their size at hatching and overall survival. Maternal exposure had a significant effect on these traits, and interacted with developmental exposure to influence the time of appearance of morphological characters, suggesting that both are important in affecting developmental trajectories. A comparison between embryos that successfully hatched and those that died in mild hypoxia revealed that survivors exhibited hypertrophy in the velum and associated pre-oral cilia, suggesting that these traits are linked with survival in low-oxygen environments. We conclude that both maternal and developmental environments shape offspring phenotype in a species with a complex developmental life history, and that plasticity in embryo morphology arising from exposure to even small reductions in oxygen tensions affects the hatching success of these embryos.

KEY WORDS: Developmental plasticity, Gastropod, Maternal effects, Planktotroph

INTRODUCTION

Phenotypically plastic responses in morphology, physiology and behaviour may enable organisms to persist under unfavourable environmental conditions (Ghalambor et al., 2007; Chevin et al., 2010; Merila and Hendry, 2014; Seebacher et al., 2015). Such responses may provide a mechanism by which species can adapt over relatively short time scales, and facilitate the evolution of novel life history traits when exposed to a rapidly changing environment (Price et al., 2003; Wund, 2013). Also, the environment perceived by previous generations is known to be significant in shaping offspring phenotype through transgenerational epigenetic inheritance (transgenerational plasticity, TGP) (Munday et al., 2013). As a subset of TGP (Rossiter, 1996), maternal effects are well documented (Bernardo, 1996; Mousseau and Fox, 1998; Marshall and Uller, 2007) and are potentially important drivers of plastic responses to new environments (Mousseau and Fox, 1998; Price et al., 2003). Mechanistically, maternal effects can manifest as: the transmission of somatic factors including beneficial proteins and hormones; the transmission of epigenetic marks that influence gene expression in offspring (Munday, 2014); and alterations in the levels of maternal investment, in the form of differential levels of nutrient provisioning (Moran and McAlister, 2009). Increases in levels of nutrient provisioning will influence early developmental stages of offspring and may buffer them against the potentially damaging effects of the environment perceived by the mother (Guisande and Harris, 1995; Räsänen et al., 2005; Allen et al., 2006; Marshall and Bolton, 2007; Burgess and Marshall, 2014).

Whilst many studies have focused on phenotypic plasticity in adult life stages, there is growing interest in earlier stages of development (Pechenik, 2006; Hassel et al., 2008; Hettinger et al., 2012; Truebano et al., 2018). In marine systems, a large number of taxa exhibit complex biphasic lifestyles. Marine invertebrates typically have a larval stage, during which physiological systems and morphological structures vary significantly from that of the adult (Strathmann, 1993; Strathmann, 2007). The transition from larval to adult stages often co-occurs with a transition in environment; for example, benthic adult stages are more liable to encounter variable environmental conditions, such as levels of dissolved oxygen, relative to their larvae suspended in the water column (Bertram and Strathmann, 1998). The ability for mothers to provision their young for environmental conditions they have not themselves experienced remains an important question in characterising the responses of species to perturbations in environmental conditions. Consequently, investigating species with biphasic lifestyles provides an excellent opportunity to study the effects of maternal and developmental exposure to environmental stress.

Phenotypically plastic responses to hypoxia are relatively well documented (Wu et al., 2003; Hassel et al., 2008; Leung et al., 2013; Segura et al., 2014). Instances of 'mild' hypoxia occur naturally in aquatic environments via seasonal fluctuations, or restricted water movements, although these occurrences may be exacerbated by future climate change scenarios (Mahaffey et al., 2020). Exposure to sublethal hypoxia during sensitive periods of early development can result in differences to overall development time, and the size and timing of appearance of aspects of embryonic and larval morphology of aquatic animals (Travis, 1994; Rundle and Spicer, 2016), and marine animals in particular (Chan et al., 2008; Hassel et al., 2008; Funch et al., 2016). Furthermore, exposure to relatively mild reductions in oxygen tensions are also known to influence the embryonic and larval development of marine species. Many aquatic species exhibit plasticity in the architecture and sizes of structures implicated in respiratory gas exchange following exposure to reduced oxygen tensions, resulting in more efficient oxygen uptake and

Marine Biology and Ecology Research Centre, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK.

*Author for correspondence (james.mccoy@postgrad.plymouth.ac.uk)

J.C.S.M., 0000-0002-3238-9781; J.I.S., 0000-0002-6861-4039; O.T., 0000-0001-8527-8383; S.D.R., 0000-0002-9570-7070

Received 17 January 2020; Accepted 17 August 2020

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

removal of carbon dioxide (Bond, 1960; McDonald and McMahon, 1977; Burggren and Mwalukoma, 1983; Sollid et al., 2003; Chapman, 2007; Chan et al., 2008). For example, larvae of a tropical freshwater teleost *Trichopodus trichopterus* cultured in hypoxia exhibited significantly larger lamellae, and accelerated growth of these respiratory structures during embryonic development (Blank and Burggren, 2014). Such developmental differences generally may be causally associated with variations in fitness in new environments (Antonovics et al., 1988). Plasticity in the timing of appearance and size of these structures could therefore provide useful quantitative measures of performance under reduced oxygen tensions.

Understanding how maternal and developmental environments influence the phenotype will be crucial in assessing the vulnerability of populations to future climate change scenarios (Bernardo, 1996; Pechenik, 2006; Doney et al., 2012; Munday et al., 2013). Early life stages of marine species possessing planktotrophic stages of development are vulnerable to reductions in oxygen arising from seasonal fluctuations in oxygen levels, and the expansion of areas of coastal hypoxia (Díaz, 2001; Díaz and Rosenberg, 2008; Mahaffey et al., 2020). Characterising the effects of environmental hypoxia on the early development of planktotrophic species will be of importance in predicting how these populations respond to environmental change, as these early life stages are likely to experience markedly different conditions from their mostly benthic adult life stages (Bertram and Strathmann, 1998; Díaz, 2001; Díaz and Rosenberg, 2008). Recent studies have shown that developmental exposure to reduced oxygen tensions can result in significant alterations to the timings of appearance of embryonic traits, and the size of morphological characters in developing embryos of intertidal marine gastropods (Chan et al., 2008; Rudin-Bitterli et al., 2016), and that these effects persist into later life history stages (Pechenik, 2006; Li and Chiu, 2013; Segura et al., 2014). However, the relative influences of both maternal and developmental exposure to sublethal, mild environmental hypoxia on early development of intertidal marine gastropods is not currently known.

Consequently, the main aim of this study was to investigate the effect of chronic maternal and developmental exposure to reduced oxygen on the embryonic development of an invertebrate with a biphasic lifestyle. Possessing both an encapsulated, lecithotrophic embryonic development and a planktotrophic veliger stage, the intertidal marine gastropod *Littorina littorea* (Linnaeus 1758) provides a tractable model species (Lebour, 1937). Pre-hatch embryos were exposed to mild hypoxia (74% air saturation), and a number of key morphological and life history traits were quantified. Specifically the growth of the shell and the size of the multi-function (respiratory gas exchange, feeding and locomotion) transitory structure, the velum, and its associated pre-oral cilia were measured, as were the time of onset of key developmental events. Relationships between levels of nutrient provisioning and embryo morphology were also investigated: *Littorina* eggs contain a distinct albuminous layer surrounding the developing embryo (Lebour, 1937; Moran, 1999), and consequently the amount of yolk is easily quantifiable, and provides a proxy for the level of nutrient provisioning in each egg. Finally, a comparison of morphological traits in embryos that died under mild hypoxia with those that survived was made in order to allow inferences to be made over the selective advantages of particular morphological traits.

MATERIALS AND METHODS

Animal collection and maintenance

Adult *Littorina littorea* snails (N=100) were collected by hand from the mid-intertidal zone at Mount Batten, Plymouth (50°21′25.3″N, 4°7′33.1″W) in early March 2018 and transferred to the laboratory. Upon arrival, snails were kept in a number of plastic aquaria (volume=8.5 litres) filled with natural seawater (S=35, pH 8.1) continually aerated, via a diffuser coupled to an air pump (Mistral 4000, Aqua Medic, Bissendorf, Germany; flow rate 6 l min^{-1}) and maintained at a temperature (T) of 15°C. Water changes took place every 48 h. Snails were exposed to a 12 h:12 h light:dark cycle and fed seaweed (*Fucus serratus*) from the collection site *ad libitum* for 7 days before use in any experiments.

Experimental design

Maternal and embryonic exposures to reduced oxygen were applied using a reciprocal transplant design, which allowed their single and combined effects to be elucidated. Treatment codes were as follows where the first letter denotes maternal exposure and the second embryonic exposure: N–H, H–H, H–N, N–N [where N (normoxia) denotes 100% air saturation and H (hypoxia) denotes 65% air saturation (maternal) or 74% air saturation (embryonic)]. We term the level of oxygen used in this study 'mild hypoxia' as it is double the value of the operational definition of aquatic hypoxia (2.8 mg O_2 l^{-1} or approximately 35% air saturation) (UNEP, 2011). Furthermore, intertidal gastropod molluscs and their embryonic and/or larval stages may experience considerably greater levels of hypoxic stress in the wild. Adult females were also exposed to a lower oxygen level than developing embryos, as adult life stages occupying intertidal areas are likely to experience more pronounced reductions in oxygen levels than egg capsules that are dispersed into coastal waters (Morris and Taylor, 1983; Mahaffey et al., 2020).

Following laboratory acclimation, six female snails were transferred to each of three airtight containers (18.0×11.5×7.5 cm) per treatment filled with filtered (20 µm) seawater (T=14°C, S=35, pH 8.1) under a 12 h:12 h light:dark cycle. Female snails were then exposed to either normoxia or mild hypoxia (see below) for 14 days. During the exposure period water was changed every 48 h from 4 litre stock containers and snails were fed *F. serratus ad libitum.*

Production of eggs was continuous through this maternal exposure period; however, embryos for use in experiments were collected after 14 days to ensure maximum exposure time of the mothers. Following this, water containing eggs was filtered (20 µm) from each of the three airtight containers per treatment (normoxia or mild hypoxia) and combined into a single volume. From this volume eggs containing embryos at the four-cell division stage (N=80) were selected haphazardly under low power magnification (×10–40) using a micropipette (Sigma-Aldrich). By combining water containing embryos from each of the replicate aquaria into a single volume, we aimed to maximise representativeness from the female population. Embryos were then distributed evenly across treatments (N=20 per treatment) in individual wells of two microtitre plates per treatment (Nunc, Microwell, 96 wells, 350 µl per well), containing filtered (20 µm) seawater (T=14°C, S=35, pH 8.1) and sealed with gas-permeable film (Aeraseal, Excel Scientific, Sigma-Aldrich) to prevent evaporation. Plates were then placed into an airtight container (18.0×11.5×7.5 cm).

Manipulation of seawater P_{O_2}

Embryos were exposed to either normoxia or mild hypoxia for the duration of their embryonic development, as follows.

Normoxic seawater in aquaria containing female snails was produced by aspirating air, supplied by an air pump (Mistral 4000; flow rate ∼2 l min^{-1}), through the seawater within each of the airtight containers. Mildly hypoxic seawater within female rearing aquaria was produced by combining bottled N_2 gas with air supplied

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

from an air pump (Mistral 4000; air flow rate ∼1.4 l min^{-1}, N_2 flow rate ∼0.8 l min^{-1}). Rates of flow of bottled N_2 and air were adjusted using variable area flow meters (FR2000, Key Instruments). Normoxia or mild hypoxia was achieved within aquaria by supplying air or N_2-enriched air directly into them through an opening within their lids. All air supplied was scrubbed using a 3 mol l^{-1} solution of KOH to remove CO_2 and standardise between gas mixtures with respect to CO_2.

Within each airtight container a small 25 ml container filled with filtered (20 µm) seawater and sealed with gas-permeable film (Aeraseal) was used for measurements of percent air saturation, salinity, temperature and pH every 24 h, using a handheld dissolved oxygen meter (YSI Pro2030). This container was also used to replenish water within each microtitre well (90%) every 24 h of the embryonic exposure period, using a micropipette (Sigma-Aldrich).

Embryo staging and measurement

Microtitre plates containing developing embryos were removed from their respective airtight containers every 24 h for observations and measurements. An image sequence of individuals was acquired daily (30 s at 2.5 frames s^{-1}) using a QImaging Retiga R6 digital camera attached to a light microscope (×160 magnification; 1392×1040 pixels, Leica M205 C, Leica Microsystems) controlled using Ocular Scientific Image Acquisition Software (QImaging, 2018; https://www.photometrics.com/products/ocular). Water in each well was replenished (90%) with water from the relevant treatment container, before microtitre plates were returned to their respective airtight containers.

Individual images from each image sequence were used to measure pre-oral cilia length (µm), velum area (µm^2) and shell length (µm) every 24 h. Measurements were taken at three time points: (i) T_1 – within 24 h of appearance – the first point at which measurements were possible; (ii) T_2 – between appearance and hatching – 50% of that individual's total development time; (iii) and T_3 – within 24 h of hatching – the last measurement made before hatching. Velum area was measured using the freehand selection tool in ImageJ (Schindelin et al., 2012) for a single frame in which both velar lobes were fully presented (i.e. parallel to the field of view); a single lobe in this frame was measured and this value doubled (Fig. 1D). A standardised measure of velum size was also calculated as a function of shell length (velum area/shell length), to determine whether differences in velum size were explained by increases in overall body size. Pre-oral cilia length and shell length were quantified using the segmented line tool (Fig. 1B,C) and presented as the mean of 10 haphazardly selected pre-oral cilia. Albumen diameter was also measured within 24 h of fertilisation using ImageJ (Fig. 1A).

Embryo survival and hatching

Embryo mortality and hatching were recorded every 24 h from the image sequences acquired. Mortality was defined as absence of activity (cilia beating, rotational behaviour and gut peristalsis) between one video and the next (24 h later). Shell length at T_3 was used as a proxy for size at hatching.

Statistical analysis

Analyses were carried out using R (version 1.0.136; https://www.r-project.org/). A Shapiro–Wilk test for normality and Levene's test for homogeneity of variance were used to assess normality and equality of variances. Differences in temperature, salinity, pH and percent air saturation between treatments during maternal, embryonic and veliger exposures were analysed using a repeated-measures ANOVA. The effects of maternal and developmental environments and their interaction on embryonic traits were analysed using a two-

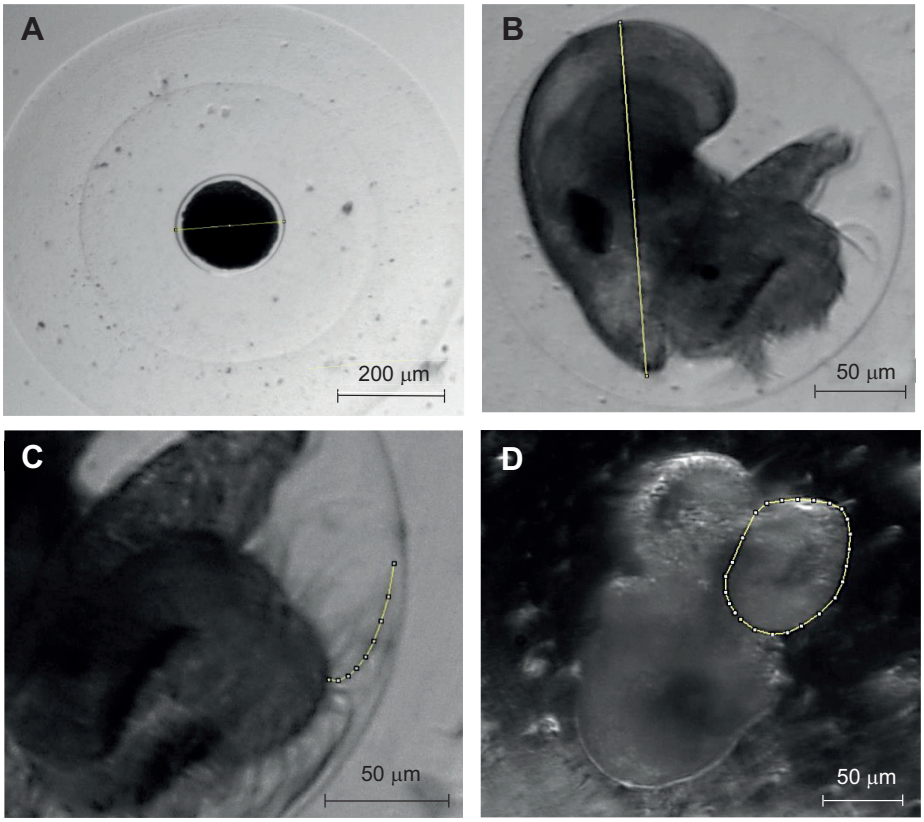

Fig. 1. Morphological measurements of *Littorina littorea* embryos. Measurements were made using the image analysis software ImageJ (Schindelin et al., 2012). (A) Albumen diameter. (B) Shell length. (C) Pre-oral cilia length. (D) Velum area.

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

Table 1. Summary of physical measurements between treatments of maternal and embryonic exposures

	Measurement	Normoxia (100%)	Mild hypoxia (70%)	d.f.	*F*	*P*
Maternal exposure	Temperature (°C)	13.64±0.04	13.67±0.03	1	0.193	0.108
	Salinity	34.97±0.23	34.42±0.23	1	2.785	0.664
	pH	8.09±0.02	8.10±0.03	1	0.012	0.92
	Air saturation (%)	94.67±1.04	65.26±2.24	27	0.89	0.607
Embryonic exposure	Temperature (°C)	14.06±0.037	14.09±0.039	1	0.161	0.69
	Salinity	34.68±0.11	34.34±0.119	1	2.712	0.107
	pH	8.09±0.03	8.09±0.04	1	3.502	0.0679
	Air saturation (%)	102.27±0.367	73.94±1.55	27	1.09	0.401

Temperature, salinity and pH: one-way ANOVA. Air saturation (%): repeated measures ANOVA, $P<0.05$.

way ANOVA. Trait values in hatched veligers were analysed using a three-way ANOVA to test for effects of maternal and developmental environments as fixed factors and status (hatched or died) nested within treatments. For significant interactions, Tukey's honest significant difference (HSD) tests were used to test for significant pairwise differences between treatments. Linear regressions were used to test for relationships between albumen diameter (µm) and morphological traits of developing embryos.

RESULTS

Physico-chemical measurements

Mean values of temperature, salinity and pH in culture conditions did not vary significantly between treatments for either the maternal or embryonic exposure periods (maternal: temperature, $F_{1,44}=0.193$, $P=0.108$; salinity, $F_{1,44}=2.785$, $P=0.664$; pH, $F_{1,44}=0.012$, $P=0.92$; embryonic: temperature, $F_{1,44}=0.161$, $P=0.69$; salinity, $F_{1,44}=2.712$; $P=0.107$; pH, $F_{1,44}=3.502$, $P=0.0679$). Mean percent air saturation for control and mild hypoxia treatments did not vary significantly for the duration of either maternal or embryonic exposure (repeated measures ANOVA: maternal normoxia, $F_{27,26}=0.89$; $P=0.607$; mild hypoxia, $F_{27,26}=0.98$, $P=0.523$; embryonic normoxia, $F_{26,25}=1.09$, $P=0.401$; mild hypoxia, $F_{26,25}=0.92$, $P=0.622$). Within mildly hypoxic maternal exposure tanks, percent air saturation was 65.26±2.24%, and the percent air saturation within microplate wells containing mildly hypoxic embryos was 73.94±1.55% (Table 1).

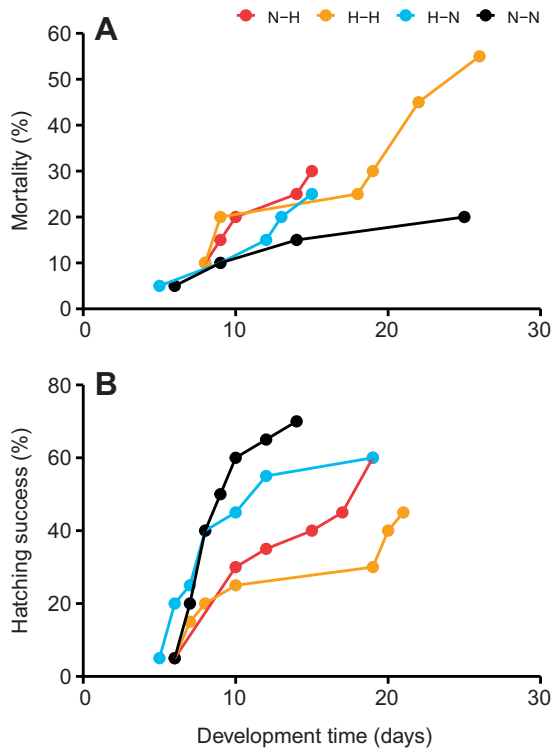

Fig. 2. Survival of embryos across treatments. (A) Percentage mortality of *L. littorea* embryos across treatments N–H ($N=19$), H–H ($N=17$), H–N ($N=20$) and N–N ($N=17$). (B) Percentage of successfully hatched *L. littorea* embryos across treatments N–H ($N=19$), H–H ($N=17$), H–N ($N=20$) and N–N ($N=17$).

Embryo survival and hatching

Mortality was higher among embryos reared under mild hypoxia (N–H: 30%) compared with those under normoxia (N–N: 20%) (Fig. 2A), and hatching success was reduced under mild hypoxia (N–H: 60%) compared with those reared under normoxia (N–N: 70%) (Fig. 2B). Maternal exposure to mild hypoxia increased mortality of embryos under normoxic (H–N: 25%; N–N: 20%) and mildly hypoxic (H–H: 55%; N–H: 30%) conditions, and reduced hatching success in those reared under normoxic (H–N: 60%; N–N: 70%) and mildly hypoxic (N–H: 60%; H–H: 45%) conditions.

Hatching time and size at hatching were only significantly influenced by embryonic environment. Embryos hatched significantly later under mild hypoxia, taking approximately 4 days longer to hatch than those reared under normoxia (N–H: 13.1± 1.3 days; H–H: 13.±2.3 days compared with H–N: 8.9±1.1 days; N–N: 8.8±0.6 days) ($F_{1,43}=11.26$, $P=0.0016$) (Fig. 3B). Hatchlings reared under normoxia were approximately 47% larger than those reared under mild hypoxia ($F_{1,33}=26.383$, $P<0.0001$).

Timings of appearance of embryonic traits

There was a significant interaction between maternal and embryonic environment on pre-oral cilia appearance ($F_{1,64}=6.212$, $P=0.015$), which was significantly delayed in embryos reared under mild hypoxia whose mothers had been maintained under normoxia (N–H: 2.6±0.1 days) compared with all other treatments (H–H: 2.2± 0.1 days; H–N: 2.3±0.1 days; N–N: 2.2±0.1 days) ($F_{3,64}=3.403$, $P=0.0221$) (Fig. 3A).

The timing of velum appearance was only influenced by maternal environment, with mildly hypoxic maternal exposure corresponding to a significantly delayed appearance of the velum (H–H: 4.5± 0.2 days; H–N: 4.8±0.3 days) relative to embryos from mothers maintained under normoxia (N–H: 4.1±0.1 days; N–N: 4.4± 0.2 days) ($F_{1,59}=5.496$, $P=0.022$) (Fig. 3A).

In contrast, only embryonic environment significantly influenced the timing of appearance of the shell. The shell started to form later in embryos reared under mild hypoxia (N–H: 5.5±0.3 days; H–H: 5.6±0.3 days) relative to those reared under normoxia (H–N: 5.1±0.1 days; N–N: 4.9±0.1 days) ($F_{3,53}=2.809$, $P=0.0466$) (Fig. 3A). There was no significant interaction between maternal and embryonic environment ($F_{1,53}=8.243$, $P=0.0056$), or main effect of maternal environment ($F_{1,53}=0.178$, $P=0.675$) on the timing of shell formation (Table 2).

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

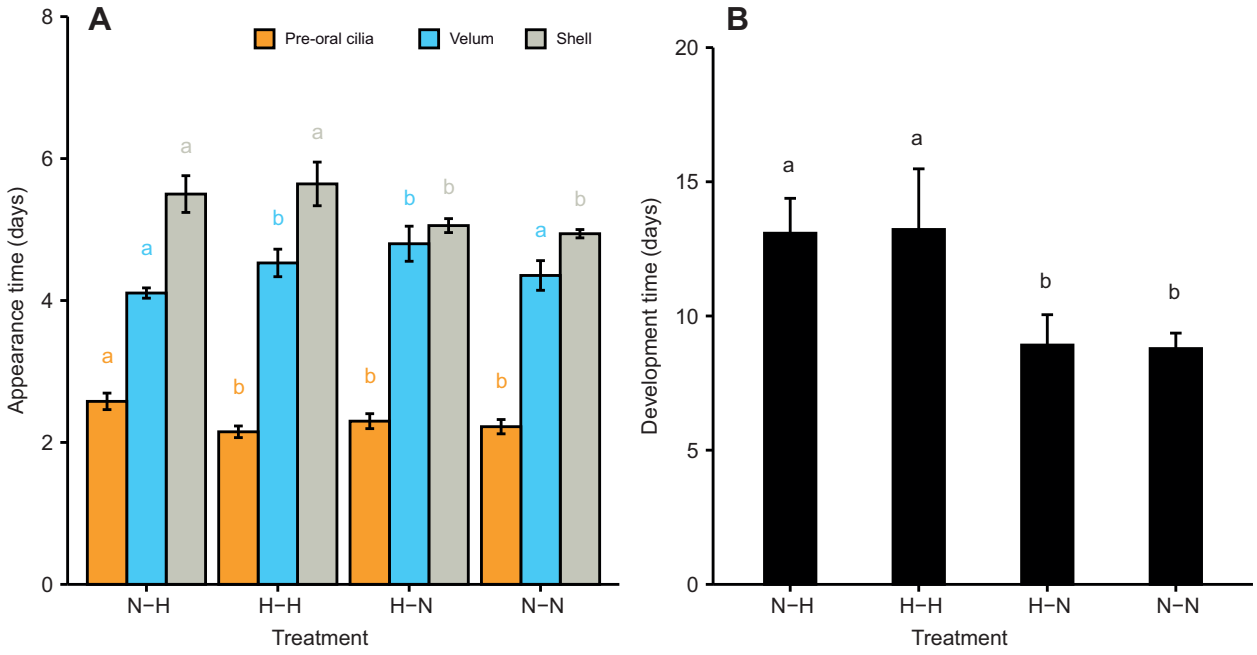

Fig. 3. Development time and appearance timings of aspects of embryo morphology between treatments. Treatments were N–H (N=19), H–H (N=17), H–N (N=20) and N–N (N=17) (means±s.e.m.). (A) Appearance time (days) of pre-oral cilia, velum and shell; pairwise significant differences (two-way ANOVA, P<0.05) between the appearance times of individual traits are denoted by lower case letters above treatments. (B) Overall development time (days); pairwise significant differences (two-way ANOVA, P<0.05) are denoted by lower case letters above treatments.

Embryo morphological traits

Pre-oral cilia length

Treatment effects on pre-oral cilia length were only detected early in T_1 individuals. There was a significant interaction between maternal and embryonic environment on T_1 pre-oral cilia length (µm) ($F_{1,60}$=4.881, P=0.032). Embryos reared under mild hypoxia whose mothers were maintained under mild hypoxia (H–H) had significantly longer pre-oral cilia compared with all other treatments ($F_{3,60}$=4.665, P=0.0069). Neither maternal nor embryonic environment was found to significantly influence the length of T_2 (maternal: $F_{1,54}$=1.002, P=0.323; embryonic: $F_{1,54}$=2.747, P=1.05) or T_3 pre-oral cilia (maternal: $F_{1,56}$=0.212, P=0.648; embryonic: $F_{1,56}$=3.882, P=0.069) (Fig. 4A, Tables 2 and 3).

Velum size

Only embryonic environment was found to significantly influence T_1 velum size ($F_{1,36}$=22.813, P<0.0001) with embryos reared under mild hypoxia having a significantly larger velum compared with those reared under normoxia ($F_{3,36}$=20.33, P=0.0016). This significant effect of developmental environment on velum size was also apparent at T_2 and T_3 time points (T_2: $F_{3,32}$=13.95, P<0.0001; T_3: $F_{3,32}$=41.358, P<0.0001) (Fig. 4B, Tables 2 and 3).

Shell length

Across all time points, only embryonic environment was found to be significant in influencing shell length (µm) (T_1: $F_{1,60}$=47.977, P<0.0001; T_2: $F_{1,40}$=47.294, P<0.0001). Embryos reared under mild hypoxia showed significantly reduced shell lengths relative to those reared under normoxia throughout their development ($F_{3,60}$=25.2962, P<0.0001; $F_{3,60}$=17.66, P<0.0001, respectively) (Fig. 4C, Tables 2 and 3).

Albumen diameter correlations

Albumen diameter did not change significantly during the course of development in any treatment (R^2=0.003, P=0.719). There were no significant relationships detected between albumen diameter and pre-oral length in any treatment, across all time points. However, embryos reared under mild hypoxia after maternal exposure to normoxia (N–H) showed a significant negative correlation between T_1 albumen diameter (µm) and T_3 velum area (µm^2) (R^2=0.5319, P=0.04006) (Fig. 5B). In those embryos reared under mild hypoxia (N–H), significant positive correlations between T_1 albumen diameter and shell length were also detected at time points T_2 and T_3 (R^2=0.4874, P=0.01689; R^2=0.3556, P=0.04909, respectively) (Fig. 5A, Table 4).

Comparisons of embryos that hatched and those that died

There were pre-hatch mortalities in all treatments (N–H: 6; H–H: 10; H–N: 5; N–N: 4), and so the opportunity was taken to compare embryos that successfully hatched with those that died to identify traits linked with successful hatching (see also Rudin-Bitterli et al., 2016). By presenting data for comparisons of those that successfully hatched and those that died, inferences can also be made over the selective advantages of morphological traits under mild hypoxia. Measurements for those that died were taken using image sequences <24 h preceding death.

Successfully hatched individuals reared under mild hypoxia had larger T_1 albumen diameters (N–H: $F_{9,19}$=12.08, P=0.0027) (Fig. 6A), and longer T_3 pre-oral cilia (N–H: $F_{1,13}$=4.007, P=0.0455; H–H: $F_{1,9}$=2.216, P=0.013) (Fig. 6B) and T_3 velum areas (N–H: $F_{1,10}$=5.511, P=0.0387) (Fig. 6C) relative to those that died. Shell length was comparable between embryos that survived and those that died under mild hypoxia (Fig. 6D) ($F_{1,23}$=3.630, P=0.0630).

DISCUSSION

Maternal or embryonic exposure?

This study aimed to investigate the extent to which chronic maternal and developmental exposure to mild hypoxia (65 and 74% air saturation, respectively) influenced the embryonic development of the intertidal gastropod *L. littorea*. Our findings suggest that plasticity in the size of morphological characters and overall development time

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

Table 2. Relative influences of maternal and embryonic exposure to mild hypoxia on timing and sizes of T_3 embryo morphological traits

Trait	d.f.	Sum of squares	Mean square	*F*	*P*
Development time					
Maternal	1	0.000	0.000	0.000	0.995
Embryonic	1	214.70	214.70	11.260	0.002
Maternal×Embryonic	1	0.000	0.000	0.000	0.998
Residuals	43	819.70	19.060		
Pre-oral cilia appearance					
Maternal	1	0.626	0.626	3.151	0.080
Embryonic	1	0.168	0.168	0.846	0.000
Maternal×Embryonic	1	1.233	1.233	6.212	0.061
Residuals	73	14.493	0.199		
Velum appearance					
Maternal	1	3.750	3.750	5.496	0.022
Embryonic	1	1.220	1.220	1.788	0.186
Maternal×Embryonic	1	0.000	0.000	0.003	0.953
Residuals	69	47.110	0.683		
Shell appearance					
Maternal	1	0.120	0.120	0.178	0.675
Embryonic	1	5.440	5.440	8.243	0.006
Maternal×Embryonic	1	0.000	0.000	0.005	0.944
Residuals	63	41.600	0.660		
Pre-oral cilia length					
Maternal	1	427	427	0.212	0.648
Embryonic	1	12,548	12,548	3.882	0.069
Maternal×Embryonic	1	1361	1361	0.673	0.417
Residuals	36	72,735	2020		
Velum size					
Maternal	1	231,557	231,557	1.684	0.206
Embryonic	1	5,688,420	5,688,420	41.358	0.000
Maternal×Embryonic	1	528,755	528,755	3.844	0.061
Residuals	26	3,576,099	137,542		
Shell length					
Maternal	1	7518	7518	0.080	0.779
Embryonic	1	2,465,581	2,465,581	36.740	0.000
Maternal×Embryonic	1	362,767	362,767	3.882	0.0573
Residuals	26	3,083,951	93,453		

Two-way ANOVA, $P<0.05$.

is driven primarily by exposure during embryonic development, whereas the timings of developmental events are shaped mainly by the maternal environment and its interaction with the embryonic environment. Plasticity arising from embryonic exposure to mild hypoxia included significant increases in velum size and development time, delays to the appearance of the shell, and significant reductions in shell length, whereas timings of appearance of morphological characters were found to be driven by interactive effects between maternal and developmental environment (pre-oral cilia), and only the maternal environment (velum).

Overall development and hatching success

Embryonic exposure to mild hypoxia significantly increased development time and saw higher mortality relative to embryos that developed under normoxia. Increases in development time and reductions in growth rate of developing embryos in response to

Table 3. Sizes of aspects of embryo morphology and associated sample sizes across all treatments and time points

	T_1		T_2		T_3	
	N	Mean±s.e.m.	*N*	Mean±s.e.m.	*N*	Mean±s.e.m.
Pre-oral cilia length (µm)						
N–H	11	34.61±0.99	12	59.75±1.39	10	61.81±1.38
H–H	10	40.74±0.94	9	57.95±1.59	7	62.44±1.47
H–N	11	36.43±1.55	12	58.85±1.73	9	57.42±1.36
N–N	13	35.54±1.04	15	54.24±1.51	14	59.19±1.35
Velum size (µm)						
N–H	10	179.37±19.69	9	207.93±20.48	8	203.91±14.94
H–H	7	120.31±21.03	7	168.83±23.96	7	156.61±26.55
H–N	10	95.84±6.27	10	91.41±2.94	10	97.45±7.14
N–N	14	90.33±2.81	12	83.06±4.23	11	91.03±5.74
Shell length (µm)						
N–H	12	128.75±8.55	11	129.56±10.25	9	137.29±8.54
H–H	9	135.89±8.29	9	143.76±12.59	7	155.38±13.69
H–N	11	169.58±6.69	11	186.92±8.69	10	185.59±10.32
N–N	13	186.34±3.43	14	215.70±7.88	11	207.67±8.60

N is the number of individual embryos.

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

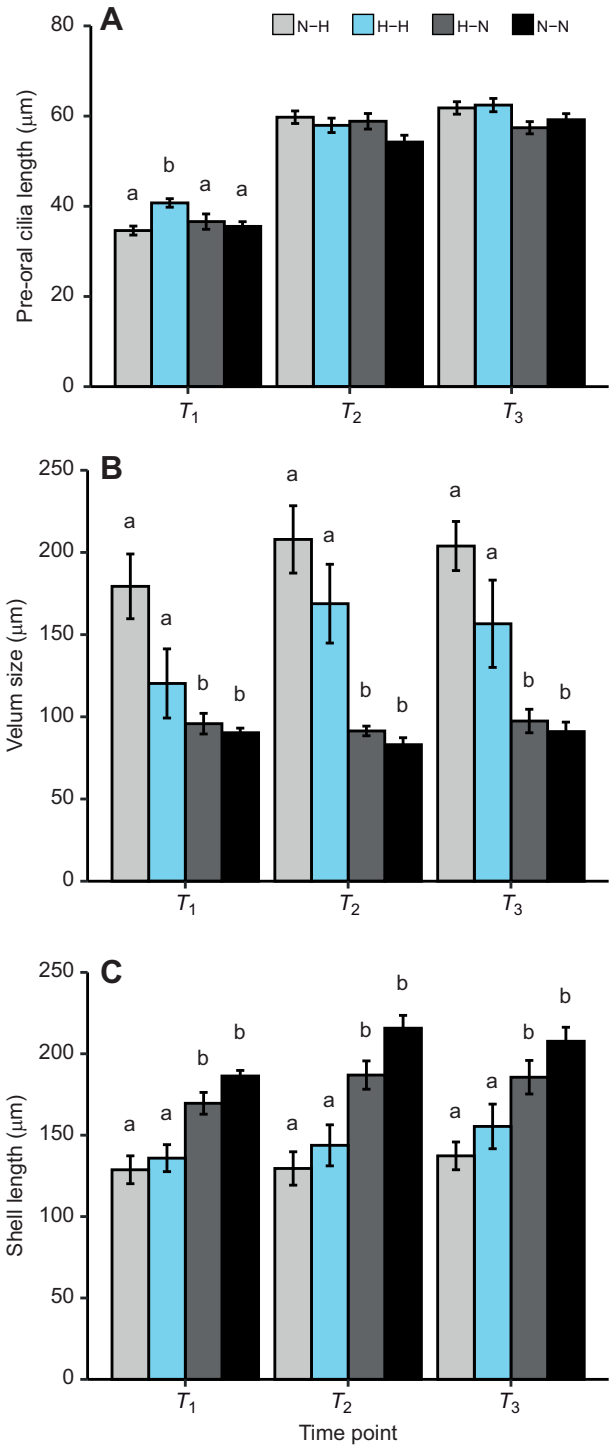

Fig. 4. Morphological traits of developing F1 embryos between treatments at three time points. Values are means±s.e.m. for time points T_1, T_2 and T_3. (A) Pre-oral cilia length (µm). (B) Velum size (µm) [calculated by dividing velum area (µm^2) by shell length (µm)]. (C) Shell length (µm). Pairwise significant differences (two-way ANOVA, P<0.05) within time points are denoted by lower case letters above treatments.

hypoxia have been observed in multiple taxa (Morrison, 1971; Widdows et al., 1989; Wang and Widdows, 1991; Shang and Wu, 2004). For example, when reared at a dissolved oxygen concentration of 3 mg l^{-1} (~37.5% air saturation), embryonic development of the gastropod *Reticunassa* (as *Nassarius*) *festivus* was significantly delayed relative to those reared under normoxia (Chan et al., 2008). Closer to the percentage saturation of oxygen used in the current study, Cancino et al. (2003) showed that when developmentally exposed to moderate hypoxia (57.5% air saturation), the muricid gastropod *Chorus giganteus* had a significantly delayed embryonic development and reduced hatching success. It is important to note that the oxygen saturation used in these experiments was considerably lower than that of the current study. This may indicate that intertidal gastropods possessing planktotrophic stages of development are more sensitive to reductions in oxygen than previous data would suggest. Extended development time under reduced oxygen may arise from reductions in aerobic performance, thereby limiting the amount of energy available for undisturbed growth (Calosi et al., 2013). In addition to the direct mortality resulting from development of *L. littorea* in mild hypoxia, retarded development may have consequences at later life history stages on the survival of these individuals. Predation pressure on pelagic veliger larvae is high (Thorson, 1950; Cowden et al., 1984) and an extended time spent within the plankton will probably increase mortality arising from predation. Those that metamorphose faster are exposed to predators for less time; therefore (according to the stage duration hypothesis: Leggett and Deblois, 1994), their chances of survival are greatly increased.

The observed increased mortality and reduced hatching success under mild hypoxia is in general agreement with previous studies (Huntington and Miller, 1989; Brante et al., 2008; Vasquez, 2013). In gastropod molluscs, the encapsulated larvae are released by three main mechanisms including: increases in osmotic pressure within the capsule by uptake of water (Kennedy and Keegan, 1992); chemical dissolution of the capsule by the production of hatching enzymes (Vaughn, 1953); and mechanical action on the capsule by the larvae (Pechenik, 1975). Mild hypoxia may act to impair these mechanisms by, for example, impairing movement as a result of reduced aerobic performance, associated with the onset of anaerobiosis (Pörtner, 2001; 2010).

Relative influences of maternal and developmental environments

The maternal environment

The timing of appearance of pre-oral cilia was driven by interactive effects between maternal and embryonic environment. Appearance was significantly delayed in those embryos reared under mild hypoxia, although maternal exposure to mild hypoxia resulted in a recovery of appearance time similar to control individuals. Additionally, appearance of the velum was found only to be influenced by maternal environment. Given that albumen diameter was not different across treatments and was not significantly correlated with development time of embryos, these differences in the timing of developmental events are unlikely to have resulted from differences in the quantity of nutrient provisioned. Instead, differences in developmental trajectories in this species may arise from some form of epigenetic mechanism (Jablonka and Raz, 2009; Munday, 2014), or through alterations to the nutritional quality, rather than quantity, of albumen supplied within the egg capsule (Fukazawa et al., 2005; Moran and McAlister, 2009). The recovery in appearance timing of pre-oral cilia arising from maternal pre-exposure may also indicate a form of 'anticipatory maternal effect', where the mother adjusts her phenotype in response to the environment she perceives (Simonini and Prevedelli, 2003; Marshall and Uller, 2007; Untersee and Pechenik, 2007; Parker et al., 2012). This accelerated appearance of pre-oral cilia in H–H individuals also probably explains the increase in T_1 pre-oral cilia length H–H individuals, as this trend fails to persist into other measured time points, notably into the T_3 timepoint.

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

Table 4. Statistically significant results of linear regression analyses of albumen diameter and aspects of embryo morphology

Correlation	Treatment	Time point	d.f., error d.f.	Slope	Intercept	R^2	P
AD×SL	N–H	T_2	1, 9	705	1932	0.4874	0.01689
AD×SL	N–H	T_3	1, 7	946	1888	3.556	0.04909
AD×VA	N–H	T_3	1, 6	-7.4×10^{-5}	2196	0.5319	0.04006

AD, albumen diameter (µm); SL, shell length (µm); VA, velum area (µm^2). Statistical significance at $P<0.05$.

Whilst no significant differences in albumen diameter were observed between mothers exposed to normoxia and mild hypoxia, albumen diameter was found to be linked with the sizes of embryonic traits. Albumen diameter was negatively correlated with velum area in embryos reared under mild hypoxia (N–H), and significantly positively correlated with shell length. Organic content is known to significantly influence offspring performance and aspects of morphology (Guisande and Harris, 1995), and there is good evidence to suggest that higher levels of maternal investment can act to recover aspects of morphology or physiology under environmental stressors, similar to levels of control individuals (Räsänen et al., 2005; Allen et al., 2006; Marshall and Bolton, 2007). Albumen diameter was also found to be significantly positively correlated with shell length at hatching. Increases in body size and sizes of specific morphological characters arising from greater levels of nutrient provisioning can be found in marine invertebrates with planktotrophic modes of development (Bertram and Strathmann, 1998; Emlet et al., 1987; Allen et al., 2006). Greater levels of nutrient provisioning in *L. littorea* reared under mild hypoxia may act to shift this trade-off, towards a greater shell size, at the expense of respiratory surface area, potentially reducing their vulnerability to predation in the water column once hatched (Bertram, 1993).

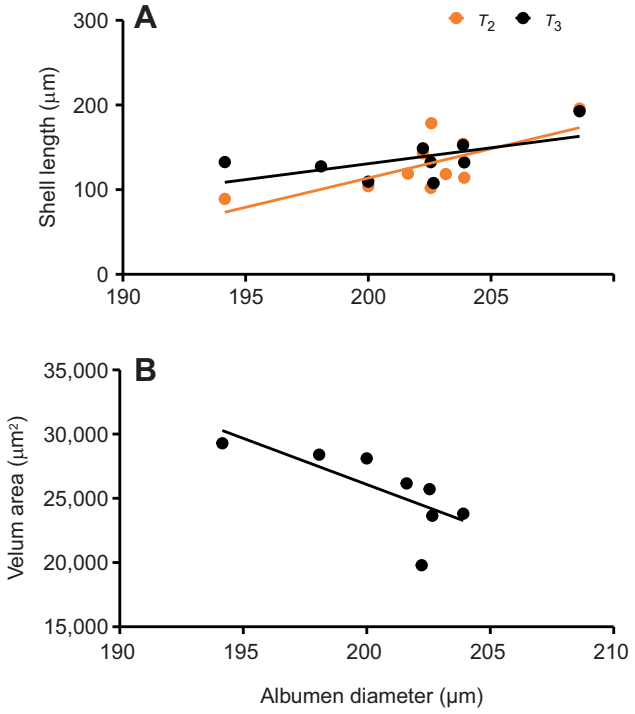

Fig. 5. Statistically significant correlations between albumen diameter and morphological traits in *L. littorea* embryos. Values are means±s.e.m.; statistical significance at $P<0.05$. (A) T_2 and T_3 shell length (µm) and albumen diameter (µm). (B) T_3 velum area (µm^2) and albumen diameter (µm) in N–H individuals.

The developmental environment

Whilst maternal environment was found to significantly influence the timings of appearance of the velum and its associated pre-oral cilia, only the developmental environment significantly influenced timing of appearance of the shell, and the growth of embryonic morphological traits.

The appearance of the shell was significantly delayed in those reared under mild hypoxia. Differences in the timing of developmental events driven by environmental perturbations, termed 'heterokairy' (Spicer and Burggren, 2003), may constitute a significant form of phenotypic plasticity, and have been proposed to act as a mechanism by which differences in developmental sequences between a species and its ancestor, known as 'heterochrony', may evolve (Spicer and Rundle, 2007).

The velum is thought to act as the main surface for gas exchange in planktotrophic veligers (Fioroni, 1966). Here, the velum was significantly larger in embryos developed under mild hypoxia. This hypertrophic response in respiratory surface area probably reflects an increased effort to enhance rates of oxygen uptake, and can be observed across multiple taxa, with the majority focusing on fish and amphibians (Bond, 1960; McDonald and McMahon, 1977; Burggren and Mwalukoma, 1983; Sollid et al., 2003; Chapman, 2007; Chan et al., 2008). Few studies focus on embryonic stages of gastropod molluscs (Chan et al., 2008; Rudin-Bitterli et al., 2016), and of these two studies, only one focuses on a species with a planktotrophic mode of development (*Nassarius festivus*). Whilst lower than the O_2 saturation used in the present study, Chan et al. (2008) reported a reduction in velum area under moderate hypoxia (57.5% air saturation) in planktotrophic *Reticunassa* (as *Nassarius*) *festivus*, contrasting with our results. Rudin-Bitterli et al. (2016) proposed that enhanced plasticity in encapsulated developers, relative to those with planktotrophic modes of development, could arise from greater fluctuations in P_{O_2} in the intertidal environment (Morris and Taylor, 1983). Here, planktotrophic *L. littorea* embryos, the focus of the present study, are shown to respond to relatively small reductions in dissolved oxygen, despite the supposed less extreme fluctuations these embryos are likely to experience in the water column (Agnew and Taylor, 1986).

The larger velum in individuals that developed under mild hypoxia was accompanied by a smaller relative shell size. Gastropod molluscs are known to invest relatively large amounts of energy into construction of the shell (Chow, 1987; Cheung et al., 2008). As aerobic performance declines under low oxygen, energy production becomes limited (De Zwaan et al., 1991; Roman et al., 2019). As a result, investment of energy into growth may become constrained, ultimately leading to the reductions in shell length observed in the present study. Additionally, the observed increases in velum size may result in a further reduction in energy allocated towards production of the shell, whereby energy is invested into structures that will assist survival under conditions larvae immediately find themselves in, at the expense of functionally unrelated structures (Schaack and Chapman, 2003). Stressor-induced changes in morphogenesis can be found in multiple marine species (Andraso, 1997; Bayne, 2004; Bagatto, 2005). For example, when reared

Journal of Experimental Biology (2020) 223, jeb221895. doi:10.1242/jeb.221895

under reduced food availability, larvae of the urchins *Strongylocentrotus purpuratus* and *S. franciscanus* exhibited a trade-off by increasing the size of their feeding apparatus to enhance rates of feeding, whilst reducing their stomach size (Miner, 2005). These reductions in shell length in those reared under mild hypoxia are likely to have consequences on the success of these individuals in the wild, given that greater larval size has been shown to significantly reduce mortality arising from predation (Webb, 1981; Bertram, 1993). For example, Gliwicz and Umana (1994) showed that increases in body size of the cladocerans *Daphnia* spp. and *Ceriodaphnia reticulata* resulted in significantly reduced predation by the freshwater copepod *Acanthocyclops robustus*. Whilst a greater velum area of *L. littorea* probably enhances the rates of gas exchange under mild hypoxia, survivability in the plankton may ultimately be compromised as a result of reduced body sizes.

Comparison of embryos that hatched or died under mild hypoxia

By comparing embryos that successfully hatched and died under mild hypoxia, inferences can be drawn on which morphological characters are linked with survival under mild hypoxia. Increased levels of nutrient provisioning in the form of a greater albumen diameter were linked with successful hatching under mild hypoxia. Greater levels of nutrient provisioning are known to increase survival (Bernardo, 1996; Moran and McAlister, 2009; Rudin-Bitterli et al., 2016), and are of particular importance for species with planktotrophic modes of development, given the relatively small amount of nutrients provided to the offspring by the mother (Gimenez and Anger, 2001). In the current study we also show that the sizes of aspects of embryo morphology are linked with survival under reduced oxygen. Those that successfully hatched possessed a significantly larger velum, suggesting that an increased surface area for gas exchange is linked with survival under mild hypoxia, and may confer a selective advantage under such conditions. Furthermore, we found that an increased length of pre-oral cilia was associated with those that successfully hatched. Cilia of molluscan larvae exert pressure on the egg capsule during hatching, probably weakened by the action of hatching enzymes (Boletsky, 1979; Pilkington, 1974; Boletsky, 1989; *Littorina littorea*: S. D. Rundle and O. Tills, unpublished observations), and are also involved in mixing of fluid within the egg

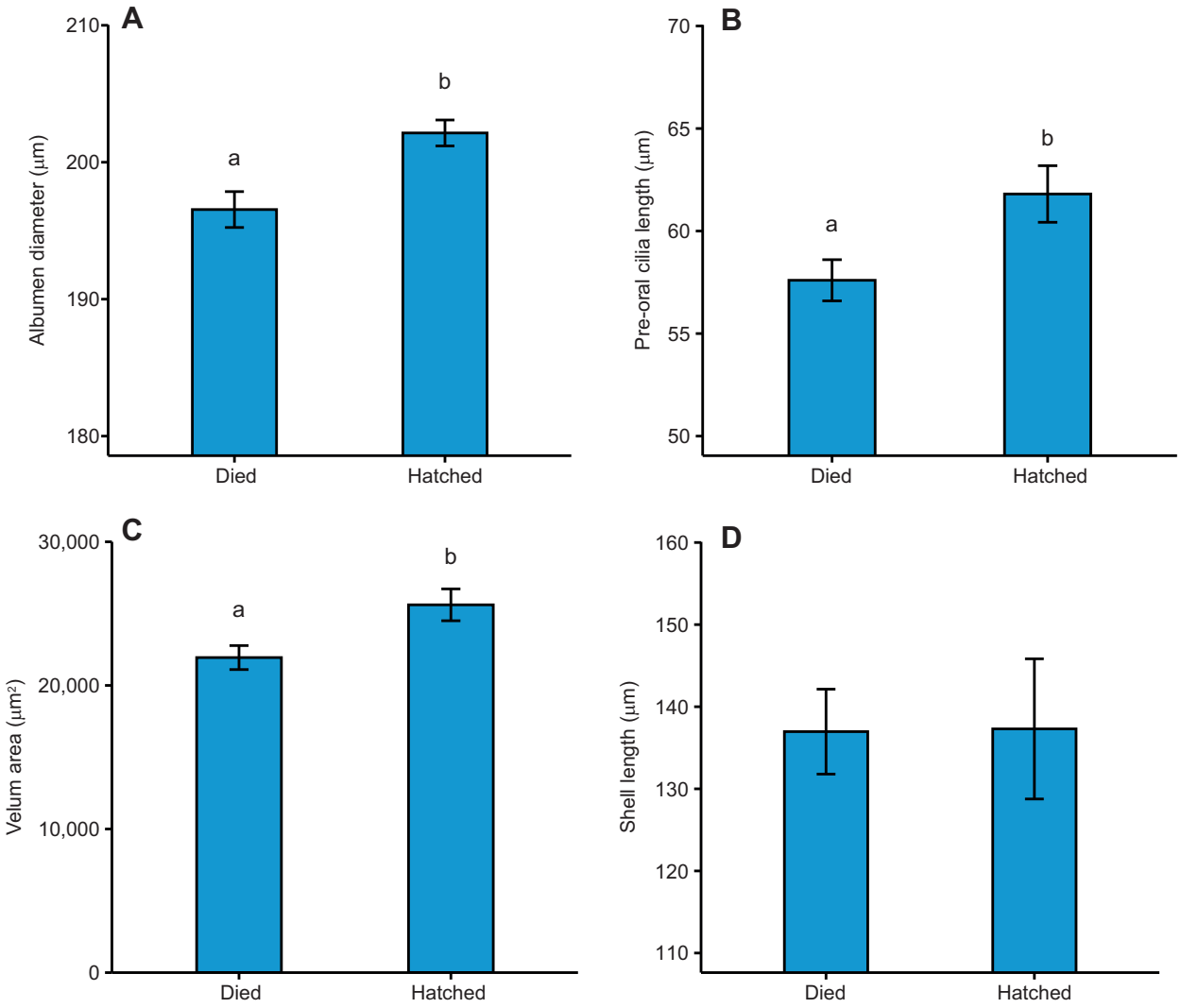

Fig. 6. Comparisons of morphological traits in *L. littorea* embryos that either survived or died within the N–H treatment. (A) Albumen diameter (µm) of successfully hatched (*N*=13) and dead (*N*=7) embryos. (B) Pre-oral cilia length (µm) of successfully hatched (*N*=10) and dead (*N*=6) embryos. (C) Velum area (µm^2) of successfully hatched (*N*=8) and dead (*N*=6) embryos. (D) Shell length (µm) of successfully hatched (*N*=9) and dead (*N*=5) embryos. Values are means±s.e.m. Pairwise significant differences are denoted by lower case letters above treatments (three-way ANOVA, $P<0.05$).

capsule (Goldberg et al., 2008). The increased pre-oral cilia length in successfully hatched individuals can be interpreted in one of two ways: (1) an increased capacity to disperse normoxic waters within the capsule, thereby enhancing mixing of oxygenated water (Widdows et al., 1989; Goldberg et al., 2008); and/or (2) increased mechanical action on the capsule during hatching by the larger pre-oral cilia.

Given the significantly improved hatching success and survivability of those individuals possessing a greater velum area and pre-oral cilia length, it is not unreasonable to question why these phenotypes are not present in all individuals, including those reared under control conditions. However, given that our study focused on the embryonic development stage of *L. littorea*, it is not possible to make inferences on the selective benefits of these traits in later stages of development. Hypertrophy in the velum and pre-oral cilia, accompanied by reductions in shell length may have consequences on the success of individuals during larval, juvenile and adult stages, despite being shown to be linked with survivability during embryonic development.

Conclusions

Here, both maternal and developmental exposure to mild hypoxia significantly influenced the embryonic development of *L. littorea*; however, the type of effect elicited by these treatments differed. Whilst the maternal environment and its interaction with the developmental environment significantly influenced the timings of appearance of developmental traits, only the developmental environment was found to significantly influence the size of morphological traits. Furthermore, embryos of *L. littorea* responded to reductions in oxygen saturation considerably lower than those of previous studies, indicating that embryos of species possessing planktotrophic stages of development may be more sensitive to reductions in oxygen levels than previously assumed. Hypertrophy in the velum probably reflects an increased effort to enhance rates of oxygen uptake and removal of carbon dioxide under mild hypoxia (Fioroni, 1966; Chan et al., 2008; Rudin-Bitterli et al., 2016). Reductions in shell length under mild hypoxia probably reflect a trade-off towards structures linked with survival under reduced oxygen tensions (Schaack and Chapman, 2003). The greater velum area and pre-oral cilia length in embryos that survived hypoxia compared with those that died, indicate that hypertrophy of these structures may be linked with survival and successful hatching under mild hypoxia. Despite showing no significant differences between treatments, levels of maternal nutrient provisioning in the form of albumen diameter are shown to be important in offspring survival and shaping phenotype under mild hypoxia (Mousseau and Fox, 1998).

We can infer that the consequences of maternal and developmental exposure to mild hypoxia on the embryonic development of *L. littorea* are complex, and interactive in some situations. Future studies should focus on how alterations to key aspects of embryo morphology such as velum size and shell size affect the success of later life stages, such as the larval veliger stage and the subsequent juvenile stage. In doing so, better inferences could be made on any adaptive significances of hypertrophy of structures implicated in respiratory gas exchange and locomotion, and the consequences reduced hatchling sizes of veliger larvae have for this species in areas of reduced oxygen tensions.

Acknowledgements

We thank technicians at the University of Plymouth for assistance with animal husbandry, Vun Wen Jie for assistance with animal collection, and Christopher Dwayne for his advice on image acquisition and analysis.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.C.S.M., O.T., J.I.S., S.D.R.; Methodology: J.C.S.M., O.T., J.I.S., S.D.R.; Formal analysis: J.C.S.M.; Investigation: J.C.S.M.; Writing – original draft: J.C.S.M.; Writing – review & editing: J.C.S.M., O.T., J.I.S., S.D.R.; Visualization: J.C.S.M.; Supervision: O.T., J.I.S., S.D.R.; Project administration: J.C.S.M.

Funding

This work was supported by the University of Plymouth School of Biological and Marine Sciences and the Marine Biological Association, and completed under receipt of funding from the Natural Environment Research Council and ARIES Doctoral Training Partnership (grant number NE/S007334/1).

References

Agnew, D. J. and Taylor, A. C. (1986). Seasonal and diel variations of some physico-chemical parameters of boulder shore habitats. *Ophelia* **25**, 83-95. doi:10.1080/00785326.1986.10429716

Allen, J. D., Zakas, C. and Podolsky, R. D. (2006). Effects of egg size reduction and larval feeding on juvenile quality for a species with facultative-feeding development. *J. Exp. Mar. Biol. Ecol.* **331**, 186-197. doi:10.1016/j.jembe.2005.10.020

Andraso, G. M. (1997). A comparison of startle response in two morphs of the brook stickleback (*Culaea inconstans*): further evidence for a trade-off between defensive morphology and swimming ability. *Evol. Ecol.* **11**, 83-90. doi:10.1023/A:1018487529938

Antonovics, J., Ellstrand, N. C. and Brandon, R. N. (1988). Genetic variation and environmental variation: expectations and experiments. In *Plant Evolutionary Biology* (ed. L.D. Gottlieb and S. K. Jain), pp. 275-303. Dordrecht: Springer.

Bagatto, B. (2005). Ontogeny of cardiovascular control in zebrafish (*Danio rerio*): effects of developmental environment. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **141**, 391-400. doi:10.1016/j.cbpb.2005.07.002

Bayne, B. L. (2004). Phenotypic flexibility and physiological trade-offs in the feeding and growth of marine bivalve molluscs. *Integr. Comp. Biol.* **44**, 425-432. doi:10.1093/icb/44.6.425

Bernardo, J. (1996). Maternal effects in animal ecology. *Am. Zool.* **36**, 83-105. doi:10.1093/icb/36.2.83

Bertram, D. F. (1993). Growth, development and mortality in metazoan early life histories with particular reference to marine flatfish. *PhD thesis*, McGill University.

Bertram, D. F. and Strathmann, R. R. (1998). Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. *Ecology* **79**, 315-327. doi:10.1890/0012-9658(1998)079[0315:EOMALN]2.0.CO;2

Blank, T. and Burggren, W. (2014). Hypoxia induced developmental plasticity of the gills and air-breathing organ of *Trichopodus trichopterus*. *J. Fish Biol.* **84**, 808-826. doi:10.1111/jfb.12319

Boletsky, S. V. (1979). Ciliary locomotion in squid hatching. *Experientia* **35**, 1051-1053. doi:10.1007/BF01949935

Boletsky, S. V. (1989). Recent studies on spawning, embryonic development, and hatching in the Cephalopoda. *Adv. Marine Biol.* **25**, 85-115. doi:10.1016/S0065-2881(08)60188-1

Bond, A. N. (1960). An analysis of the response of salamander gills to changes in the oxygen concentration of the medium. *Dev. Biol.* **2**, 1-20. doi:10.1016/0012-1606(60)90013-0

Brante, A., Fernandez, M. and Frederique, V. (2008). Effects of oxygen conditions on intracapsular development in two calyptreid species with different modes of larval development. *Mar. Ecol. Prog. Ser.* **368**, 197-207. doi:10.3354/meps07605

Burgess, S. C. and Marshall, D. J. (2014). Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos* **1233**, 769-776. doi:10.1111oik.01235

Burggren, W. and Mwalukoma, A. (1983). Respiration during chronic hypoxia and hyperoxia in larval and adult bullfrogs (*Rana catesbeiana*). I. Morphological responses of lungs, skin and gills. *J. Exp. Biol.* **105**, 191-203.

Calosi, P., Turner, L., Hawkins, M., Bertolini, C., Nightingale, G., Truebano, M. and Spicer, J. (2013). Multiple physiological responses to multiple environmental challenges: an individual approach. *Integr. Comp. Biol.* **53**, 660-670. doi:10.1093/icb/ict041

Cancino, J. M., Gallardo, J. A. and Torres, F. A. (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). *Mar. Biol.* **142**, 133-139. doi:10.1007/s00227-002-0925-3

Chan, H. Y., Xu, W. Z., Shin, P. K. S. and Cheung, S. G. (2008). Prolonged exposure to low dissolved oxygen affects early development and swimming behaviour in the gastropod *Nassarius festivus* (Nassariidae). *Mar. Biol.* **153**, 735-743. doi:10.1007/s00227-007-0850-6

Chapman, L. J. (2007). Morpho-physiological divergence across oxygen gradients in fishes. In *Fish Respiration and the Environment* (ed. M. N. Fernandes, F. T. Rantin, M. L. Glassand and B. G. Kapoor), pp. 14-29. Enfield: Science Publisher.
Cheung, S. G., Chan, H. Y., Liu, C. C. and Shin, P. K. S. (2008). Effect of prolonged hypoxia on food consumption, respiration, growth and reproduction in the marine scavenging gastropod *Nassarius festivus*. *Mar. Pollut. Bull.* **57**, 280-286. doi:10.1016/j.marpolbul.2008.03.039
Chevin, L.-M., Lande, R. and Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* **8**, e1000357. doi:10.1371/journal.pbio.1000357
Chow, V. (1987). Patterns of growth and energy allocation in northern California populations of *Littorina* (Gastropoda: Prosobranchia). *J. Exp. Mar. Biol. Ecol.* **110**, 69-89. doi:10.1016/0022-0981(87)90067-0
Cowden, C., Young, C. M. and Chia, F. S. (1984). Predation on marine invertebrate larvae by two benthic predators. *Mar. Ecol. Prog. Ser.* **14**, 145-149. doi:10.3354/meps014145
De Zwaan, A., Cortesi, P., van den Thillart, G., Roos, J. and Storey, K. B. (1991). Differential sensitivities to hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. *Mar. Biol.* **111**, 343-351. doi:10.1007/BF01319405
Díaz, R. J. (2001). Overview of hypoxia around the world. *J. Environ. Qual.* **30**, 275-281. doi:10.2134/jeq2001.302275x
Díaz, R. J. and Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science* **321**, 26-929.
Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N. et al. (2012). Climate change impacts on marine ecosystems. *Annu. Rev. Mar. Sci.* **4**, 11-37. doi:10.1146/annurev-marine-041911-111611
Emlet, R. B., McEdward, L. R. Strathmann, R. R. (1987). Echinoderm larval ecology viewed from the egg. In *Echinoderm Studies (ed. M.* Jangoux and J. M. Lawrence), pp. 55-136. Rotterdam: Ralkema.
Fioroni, P. (1966). Zur Morphologie und Embryogenese des Darmtraktes und der transitorischen Organe bei Prosobranchiern (Mollusca, Gastropoda). *Rev. Suisse Zool.* **73**, 621-876. doi:10.5962/bhl.part.75848
Fukazawa, H., Takami, H., Kawamura, T. and Watanabe, Y. (2005). The effect of egg quality on larval period and postlarval survival of an abalone *Haliotis discus hannai*. *J. Shellfish Res.* **24**, 1141-1147. doi:10.2983/0730-8000(2005)24[1141:TEOEQO]2.0.CO;2
Funch, P., Wang, T., Pertoldi, C. and Middelbo, A. D. (2016). Low oxygen levels slow embryonic development of *Limulus polyphemus*. *Biol. Bull.* **231**, 113-119. doi:10.1086/690091
Ghalambor, C. K., Mckay, J. K., Carroll, S. P. and Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* **21**, 394-407. doi:10.1111/j.1365-2435.2007.01283.x
Gimenez, I. and Anger, K. (2001). Relationships among salinity, egg size, embryonic development and larval biomass in the estuarine crab *Chasmagnathus granulata*, Dana 1851. *J. Exp. Mar. Biol. Ecol.* **260**, 241-257. doi:10.1016/S0022-0981(01)00258-1
Gliwicz, Z. M. and Umana, G. (1994). Cladoceran body size and vulnerability to copepod predation. *Limnol. Oceanogr.* **39**, 419-424. doi:10.4319/lo.1994.39.2.0419
Goldberg, J. I., Doran, S. A., Shartau, R. B., Pon, J. R., Ali, D. W., Tam, R. and Kuang, S. (2008). Integrative biology of an embryonic respiratory behaviour in pond snails: the 'embryo stir-bar hypothesis'. *J. Exp. Biol.* **211**, 1729-1736. doi:10.1242/jeb.016014
Guisande, C. and Harris, R. (1995). Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. *Limnol. Oceanogr.* **40**, 476-482. doi:10.4319/lo.1995.40.3.0476
Hassel, K. L., Coutin, P. C. and Nugegoda, D. (2008). Hypoxia impairs embryo development and survival in black bream (*Acanthopagarus butcheri*). *Mar. Pollut. Bull.* **57**, 302-306. doi:10.1016/j.marpolbul.2008.02.045
Hettinger, A., Sanford, E., Hill, T. M., Russell, A. D., Sato, K. N. S., Hoey, J., Forsch, M., Page, H. N. and Gaylord, B. N. (2012). Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. *Ecology* **93**, 2758-2768. doi:10.1890/12-0567.1
Huntington, K. M. and Miller, D. C. (1989). Effects of suspended sediment, hypoxia, and hyperoxia on larval *Mercenaria mercenaria* (Linnaeus, 1758). *J. Shellfish Res.* **8**, 37-42.
Jablonka, E. and Raz, G. (2009). Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q Rev. Biol.* **84**, 131-176. doi:10.1086/598822
Kennedy, J. J. and Keegan, B. F. (1992). The encapsular developmental sequence of the mesogastropod *Turritella communis* (Gastropoda: Turritellidae). *J. Mar. Biol. Assoc. U. K.* **72**, 783-805. doi:10.1017/S0025315400060045
Lebour, M. V. (1937). The eggs and larvae of the British prosobranchs with special reference to those living in the plankton. *J. Mar. Biol. Assoc. U. K.* **22**, 105-166. doi:10.1017/S0025315400011917
Leggett, W. C. and Deblois, E. (1994). Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? *Netherlands J. Sea Res.* **32**, 119-134. doi:10.1016/0077-7579(94)90036-1
Leung, J. Y. S., Cheung, S. G., Qiu, Q. W., Ang, P. O., Chiu, J. M. Y., Thiyagarajan, V. and Shin, P. K. S. (2013). Effect of parental hypoxic exposure on embryonic development of the offspring of two serpulid polychaetes: implication for transgenerational epigenetic effect. *Mar. Pollut. Bull.* **74**, 149-155. doi:10.1016/j.marpolbul.2013.07.014
Li, A. and Chiu, J. M. Y. (2013). Latent effects of hypoxia on the gastropod *Crepidula onyx*. *Mar. Ecol. Prog. Ser.* **480**, 145-154. doi:10.3354/meps10213
Mahaffey, C., Palmer, M., Greenwood, N. and Sharples, J. (2020). Impacts of climate change on dissolved oxygen concentration relevant to the coastal and marine environment around the UK. *MCCIP Sci. Rev.* **2020**, 31-53.
Marshall, D. J. and Bolton, T. F. (2007). Effects of egg size on the development time of non-feeding larvae. *Biol. Bull.* **212**, 6-11. doi:10.2307/25066575
Marshall, D. J. and Uller, T. (2007). When is a maternal effect adaptive? *Oikos* **116**, 1957-1963. doi:10.1111/j.2007.0030-1299.16203.x
McDonald, D. G. and McMahon, B. R. (1977). Respiratory development in Arctic char *Salvelinus alpinus* under conditions of normoxia and chronic hypoxia. *Can. J. Zool.* **55**, 1461-1467. doi:10.1139/z77-189
Merila, J. and Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Ecol. Appl.* **7**, 1-14.
Miner, B. J. (2005). Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. *J. Exp. Mar. Biol. Ecol.* **315**, 117-125. doi:10.1016/j.jembe.2004.09.011
Moran, A. L. (1999). Intracapsular feeding by embryos of the gastropod genus *Littorina*. *Biol. Bull.* **196**, 229-244. doi:10.2307/1542948
Moran, A. L. and McAlister, J. S. (2009). Egg size as a life history character of marine invertebrates: is it all it's cracked up to be? *Biol. Bull.* **216**, 226-242. doi:10.1086/BBLv216n3p226
Morris, S. and Taylor, S. C. (1983). Diurnal and seasonal variation in physico-chemical conditions within intertidal rockpools. *Estuar. Coast. Shelf Sci.* **17**, 339-355. doi:10.1016/0272-7714(83)90026-4
Morrison, G. (1971). Dissolved oxygen requirements for embryonic and larval development of the hardshell clam, *Mercenaria mercenaria*. *J. Fish. Res. Board Can.* **28**, 379-381. doi:10.1139/f71-050
Mousseau, T. A. and Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**, 403-407. doi:10.1016/S0169-5347(98)01472-4
Munday, P. L. (2014). Transgenerational acclimation of fishes to climate change and ocean acidification. *F1000Prime Rep.* **6**, 99. doi:10.12703/P6-99
Munday, P. L., Warner, R. R., Monro, L., Pandolfi, J. M. and Marshall, D. J. (2013). Predicting evolutionary responses to climate change in the sea. *Ecol. Lett.* **16**, 1488-1500. doi:10.1111/ele.12185
Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A. and Pörtner, H. O. (2012). Adult exposure influences offspring response to ocean acidification in oysters. *Glob. Change Biol.* **18**, 82-92. doi:10.1111/j.1365-2486.2011.02520.x
Pechenik, J. A. (1975). The escape of veligers from the egg capsules of *Nassarius obsoletus* and *Nassarius trivittatus* (Gastropoda, Prosobranchia). *Biol. Bull.* **149**, 580-589. doi:10.2307/1540388
Pechenik, J. A. (2006). Larval experience and latent effects – metamorphosis is not a new beginning. *Integr. Comp. Biol.* **46**, 323-333. doi:10.1093/icb/icj028
Pilkington, M. C. (1974). The eggs and hatching stages of some New Zealand prosobranch molluscs. *J. R. Soc. N. Z.* **4**, 411-431. doi:10.1080/03036758.1974.10419385
Pörtner, H.-O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Science* **88**, 137-146.
Pörtner, H.-O. (2010). Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* **213**, 881-893. doi:10.1242/jeb.037523
Price, T. D., Qvarnström, A. and Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proc. R. Soc. Lond.* **270**, 1433-1440. doi:10.1098/rspb.2003.2372
Räsänen, K., Laurila, A. and Merilä, J. (2005). Maternal investment in egg size: environment- and population-specific effects on offspring performance. *Oecologia* **142**, 546-553. doi:10.1007/s00442-004-1762-5
Roman, M. R., Brandt, S. B., Houde, E. D. and Pierson, J. J. (2019). Interactive effects of hypoxia and temperature on coastal pelagic zooplankton and fish. *Front. Mar. Sci.* **6**, 139. doi:10.3389/fmars.2019.00139
Rossiter, M. C. (1996). Incidence and consequences of inherited environmental effects. *Annu. Rev. Ecol. Syst.* **27**, 451-476. doi:10.1146/annurev.ecolsys.27.1.451
Rudin-Bitterli, T. S., Spicer, J. I. and Rundle, S. D. (2016). Differences in the timing of cardio-respiratory development determine whether marine gastropod embryos survive or die in hypoxia. *J. Exp. Biol.* **219**, 1076-1085. doi:10.1242/jeb.134411
Rundle, S. D. and Spicer, J. I. (2016). Heterokairy: a significant form of developmental plasticity? *Biol. Lett.* **12**, 5-9. doi:10.1098/rsbl.2016.0509
Schaack, S. and Chapman, L. J. (2003). Interdemic variation in the African cyprinid *Barbus neumayeri*: correlations among hypoxia, morphology, and feeding performance. *Can. J. Zool.* **81**, 430-440. doi:10.1139/z03-009
Schindelin, J., Arganda-Carreras, I. and Frise, E. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676-682. doi:10.1038/nmeth.2019

Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Climate Change* **5**, 61-66. doi:10.1038/nclimate2457

Segura, C. J., Chaparro, O. R., Pechenik, J. A., Paschke, K. A., Osores, S. J. A., Navarro, J. M. and Cubillos, V. M. (2014). Delayed effects of severe hypoxia experienced by marine gastropod embryos. *Mar. Ecol. Prog. Ser.* **510**, 59-71. doi:10.3354/meps10906

Shang, E. H. H. and Wu, S. S. R. (2004). Aquatic hypoxia is a teratogen and affects fish embryonic development. *Environ. Sci. Technol.* **38**, 4763-4767. doi:10.1021/es0496423

Simonini, R. and Prevedelli, D. (2003). Effects of temperature on two Mediterranean populations of *Dinophilus gyrociliatus* (Polychaeta: Dinophilidae). I. Effects on life history and sex ratio. *J. Exp. Mar. Biol. Ecol.* **291**, 79-93. doi:10.1016/S0022-0981(03)00099-6

Sollid, J., De Angelis, P., Gundersen, K. and Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *J. Exp. Biol.* **206**, 3667-3673. doi:10.1242/jeb.00594

Spicer, J. I. and Burggren, W. W. (2003). Development of physiological regulatory systems: altering the timing of crucial events. *Zoology* **106**, 91-99. doi:10.1078/0944-2006-00103

Spicer, J. I. and Rundle, S. D. (2007). Plasticity in the timing of physiological development: physiological heterokairy – what is it, how frequent is it, and does it matter? *Comp. Biochem. Physiol. A* **148**, 712-719. doi:10.1016/j.cbpa.2007.05.027

Strathmann, R. R. (1993). Hypotheses on the origins of marine larvae. *Annu. Rev. Ecol. Syst.* **24**, 89-117. doi:10.1146/annurev.es.24.110193.000513

Strathmann, R. R. (2007). Three functionally distinct kinds of pelagic development. *Bull. Mar. Sci.* **81**, 167-179.

Thorson, G. (1950). Reproduction and larval ecology of marine bottom invertebrates. *Biol. Rev.* **25**, 1-45. doi:10.1111/j.1469-185X.1950.tb00585.x

Travis, J. (1994). Evaluating the adaptive role of morphological plasticity. In *Ecological Morphology: Integrative Organismal Biology* (ed. P. C. Wainright and S. M. Reilly), pp. 99-101. Chicago: University of Chicago Press.

Truebano, M., Fenner, P., Tills, O., Rundle, S. D. and Rezende, E. L. (2018). Thermal strategies vary with life history. *J. Exp. Biol.* **221**, 171629. doi:10.1242/jeb.171629

UNEP (2011). *Hypoxia and nutrient reduction in the coastal zone: advice for prevention, remediation and research*. Nairobi: United Nations Environment Programme.

Untersee, S. and Pechenik, J. A. (2007). Local adaptation and maternal effects in two species of marine gastropod (genus *Crepidula*) that differ in dispersal potential. *Mar. Ecol. Prog. Ser.* **347**, 79-85. doi:10.3354/meps07063

Vasquez, M. C. (2013). Multiple stressor interactions and effects on embryo development of the American horseshoe crab, *Limulus polyphemus*. *PhD thesis*, University of Florida.

Vaughn, G. (1953). Effects of temperature on hatching and growth of *Lymnaea stagnalis appressa*. *Am. Midl. Nat.* **25**, 407-408.

Wang, W. X. and Widdows, J. (1991). Physiological responses of mussel larvae *Mytilus edulis* to environmental hypoxia and anoxia. *Mar. Ecol. Prog. Ser.* **70**, 223-236. doi:10.3354/meps070223

Webb, P. W. (1981). Responses of northern anchovy, *Engraulis mordax*, larvae to predation by a biting planktivore, *Amphiprion percula*. *Bull. US Fish Comm.* **79**, 726-736.

Widdows, J., Newell, R. I. E. and Mann, R. (1989). Effects of hypoxia and anoxia on survival, energy metabolism, and feeding of oyster larvae (*Crassostrea virginica*, Gmelin). *Biol. Bull.* **177**, 154-166. doi:10.2307/1541843

Wu, R. S. S., Zhou, B. S., Randall, D. J., Woo, N. Y. S. and Lam, P. K. S. (2003). Aquatic hypoxia is an endocrine disruptor and impairs fish reproduction. *Environ. Sci. Technol.* **37**, 1137-1141. doi:10.1021/es0258327

Wund, M. A. (2013). Assessing the impacts of phenotypic plasticity on evolution. *Integr. Comp. Biol.* **52**, 5-15. doi:10.1093/icb/ics050