RESEARCH ARTICLE

Near Future Ocean Acidification Increases Growth Rate of the Lecithotrophic Larvae and Juveniles of the Sea Star *Crossaster papposus*

JOURNAL OF EXPERIMENTAL ZOOLOGY
PART B
MOLECULAR AND DEVELOPMENTAL EVOLUTION

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ABSTRACT

Ocean acidification (OA) is believed to be a major threat for near-future marine ecosystems, and that the most sensitive organisms will be calcifying organisms and the free-living larval stages produced by most benthic marine species. In this respect, echinoderms are one of the taxa most at risk. Earlier research on the impact of near-future OA on echinoderm larval stages showed negative effects, such as a decreased growth rate, increased mortality, and developmental abnormalities. However, all the long-term studies were performed on planktotrophic larvae while alternative life-history strategies, such as nonfeeding lecithotrophy, were largely ignored. Here, we show that lecithotrophic echinoderm larvae and juveniles are positively impacted by ocean acidification. When cultured at low pH, larvae and juveniles of the sea star *Crossaster papposus* grow faster with no visible affects on survival or skeletogenesis. This suggests that in future oceans, lecithotrophic species may be better adapted to deal with the threat of OA compared with planktotrophic ones with potentially important consequences at the ecosystem level. For example, an increase in populations of the top predator *C. papposus* will likely have huge consequences for community structure. Our results also highlight the importance of taking varying life-history strategies into account when assessing the impacts of climate change, an approach that also provides insight into understanding the evolution of life-history strategies. *J. Exp. Zool. (Mol. Dev. Evol.) 314B, 2010.*

J. Exp. Zool. (Mol. Dev. Evol.) 314B, 2010.

How to cite this article: Dupont S, Lundve B, Thorndyke M. 2010. Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*. J. Exp. Zool. (Mol. Dev. Evol.) 314B:[page range].

Since the beginning of the industrial revolution, the burning of fossil fuels on a large scale has induced a 50% increase in atmospheric carbon dioxide (CO_2) with dramatic consequences for oceans. Because of these CO_2 emissions, a 0.4 pH unit decrease (a 2-fold increase in acidity) is expected in ocean surface waters in 2100, a rapid and dramatic change that will have widespread consequences for species and ecosystems (Caldeira and Wickett, 2003; Royal Society, 2005). These are rapid (100 times faster than anything seen in the past hundreds of millennia)

Grant Sponsors: K. & A. Wallenbergs Stiftelsen; Swedish Research Councils; Linnaeus initiative Adaptation to Changing Marine Environment (CeMEB); Royal Swedish Academy of Sciences.
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Received 10 September 2009; Revised 14 January 2010; Accepted 4 February 2010
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jezmde.21342

and unavoidable (global) stressors that will be more drastic and unpredictable in the near future.

Since the publication of the Royal Society report in 2005, the study of the impact of ocean acidification (OA) at the biological level has emerged as a new scientific field under the paradigm proposed by Orr et al. (2005) that calcifiers will be drastically affected. When CO_2 dissolves in seawater it forms carbonic acid, which dissociates to form equilibrium with hydrogen and bicarbonate ions and carbonate. This equilibrium is dominated by bicarbonate. Continued uptake of CO_2 by the oceans increases the concentration of hydrogen ions, thereby reducing pH. This pH shift changes the equilibrium between bicarbonate and carbonate, depleting the available carbonate pool. This process increases the rate of dissolution of deposited calcium carbonate ($CaCO_3$). The rate of dissolution depends on the crystalline form of the $CaCO_3$: aragonite (found in corals and molluscs) is twice as soluble as calcite (found in crustaceans). As a result, calcification is the primary target of many studies on the impact of CO_2-driven climate change in the oceans, because the calcium carbonate shells or skeletons of many planktonic organisms make them potentially susceptible to dissolution in acidic waters (Orr et al., 2005). However, this is also complicated by the fact that biogenic carbonates are stabilized by an organic matrix that alters dissolution kinetics, highlighting the importance of taking into account physiological processes to predict the impact of OA on marine calcifiers.

Echinoderms are among the most abundant and ecologically successful groups of marine animals (Micael et al., 2009), and are one of the key marine groups most likely to be impacted by predicted climate change events. For example, the larvae and/or adults of many species from this phylum form skeletal rods, plates, test, teeth, and spines from an amorphous calcite crystal precursor, magnesium calcite, which is 30 times more soluble than normal calcite (Politi et al., 2004).

Some of the information available on the impact of OA on echinoderms is in agreement with this hypothesis. For example, when juveniles of two species of shallow water sea urchins (*Hemicentrotus pulcherrimus* and *Echinometra mathaei*) were exposed to low pH (7.9 compared with 7.94 in the control) for 6 months, the mortality rate increased while growth rate (wet weight) decreased. Moreover, urchins from the low pH treatments had a thinner test making the skeleton more easily broken during handling (Shirayama and Thornton, 2005). Another example is the impact of OA on the larval development of the brittlestar *Ophiothrix fragilis*. When exposed to pH values expected in the year 2050 ($\Delta pH \approx 0.2$ units), 100% mortality is observed within 8 days postfertilization owing to abnormal development and disrupted skeletogenesis (Dupont et al., 2008).

However, a growing body of evidence shows that echinoderm calcification is less impacted than expected. Some species develop a normal skeleton, and both juveniles and adults can even grow faster at low pH, sometimes associated with a decreased (e.g. Wood et al., 2008; Clark et al., 2009; Gooding et al., 2009; Ries et al., 2009) or an increased calcification rate (Ries et al., 2009). Despite this, however, the impact of OA on echinoderm larvae is always neutral or negative according to data published so far, and the main affect is a delay in development leading to smaller larvae at a given time (Kurihara et al., 2004; Kurihara and Shirayama, 2004a, b; Dupont et al., 2008; Byrne et al., 2009; Clark et al., 2009; Dupont and Thorndyke, 2009a; O'Donnell et al., 2009). We have hypothesized that this delay in development is the consequence of the impact of low pH on feeding rate (Dupont and Thorndyke, 2009a).

As with many benthic invertebrates, echinoderms have developed a variety of life-history strategies that can be divided into modes based on larval nutritional pattern as well as whether they are released in the water column or not (Gillepsie and McClintock, 2007). Two main strategies are currently recognized: (i) planktotrophy, where a single parent invests energy in producing millions of small eggs that will develop into planktotrophic larvae feeding on exogenous sources, such as phytoplankton or dissolved organic matter; and (ii) lecithotrophy, where parents produce comparatively small numbers (usually thousands) of large, yolky eggs and lecithotrophic larvae that derive their nutrition from energy stored in the egg itself. Both strategies are the result of a trade-off between costs and benefits.

It is interesting to note that most studies on the impact of OA on echinoderm larvae have been performed on feeding planktotrophic larvae. The only exceptions being studies on *Heliocidaris erythrogramma* by Havenhand et al. (2008) and Byrne et al. (2009), but here only short-term experiments (till gastrula stage at 20–24 hr postfertilization) were performed with no information provided on longer term viability and settlement success.

Our aim was to fill this knowledge gap and study the impact of near-future OA ($\Delta pH \approx 0.4$ units) on the whole larval development, from fertilization to metamorphosis of the lecithotrophic sea star species *Crossaster papposus*.

C. papposus develops via a simple nonfeeding pelagic brachiolaria larvae ("yolky brachiolaria"; McEdward and Miner, 2001). Each female produces only 2,000–6,000 eggs each year (Gemmil, '20). These yolky eggs are large (>0.8 mm) and develop into nonfeeding pelagic larvae that settle and metamorphose into 1 mm juveniles with 9 arms in less than 2 months (Strathmann, '87). As adults, sea stars have flexible skeletons comprising small calcite ossicles embedded in a connective tissue matrix. In *C. papposus*, the dorsal surface is covered with large groups of bristly spines. Skeletal rods are present in the larval stages of brittle stars and sea urchins, but are largely absent from the comparable stages in sea stars, sea cucumbers, and feather stars, despite the adults of these groups often being calcified. However, in *C. papposus*, calcification of the adult rudiment starts early during development and thus within the larval body (22d; Gemmil, '20). Thus, under the current paradigm that

calcification will be the major biological process impacted by near-future OA, we can hypothesize that the sea star *C. papposus* (both larval and juvenile stages) will be negatively impacted (hypothesis 1).

However, an alternative and complementary hypothesis suggests that calcification may not be the major threat for marine species and that the main impact of OA will be on other physiological processes, such as respiration, metabolism, and feeding (Pörtner, 2008; Dupont and Thorndyke, 2009a). Regarding earlier observations on sea urchin larvae that show a negative effect of low pH on growth and feeding (see Dupont and Thorndyke, 2009a, b for reviews) in larvae, we can hypothesize that the nonfeeding lecithotrophic larvae of *C. papposus* may be more resilient to environmental changes (hypothesis 2).

METHODS

This study was conducted on the common sun star *C. papposus* L. (Fig. 1A). Nine individuals were collected in shallow water in the vicinity of the Sven Lovén Centre for Marine Sciences, Kristineberg (Sweden), between January and April 2009. They were kept in flowing natural deep seawater under natural temperature and light regime (pH 8.06 ± 0.07 units (control), salinity 32‰, and alkalinity of 2.18 ± 0.02 mM as measured following Sarazin et al. '99). Other parameters (pCO_2, Ωca, and Ωar) were calculated from pH and alkalinity using $SWCO_2$, with dissociation constants from Mehrbach et al. ('73) and refitted by Dickson and Millero ('87). Animals were fed with living sea stars *Asterias rubens*. *C. papposus* spawns mainly in March and each individual may spawn several times during this period, at intervals of 2–10 days (Gemmil, '20). We were unable to induce spawning using the classical methods for sea star (e.g. by intracoelomic injection with 2 mL of 0.1 mM 1-methyladenine). Fertilized eggs were, thus, only collected after natural spawning in an experimental aquarium on March 10 (2 females and 1 male, 9,000 eggs) and April 3 (1 female and 1 male, 2,000 eggs). In *C. papposus*, sexes are separate and the male always releases sperm first, thereby inducing spawning in the female (Gemmil, '20). Large yolky red eggs were slowly released through the numerous gonoducts opening on the external epithelium (Gemmil, '11; Fig. 1B) and started floating on the surface of the aquarium where they were collected after fertilization, rinsed, and transferred into 5 L culture aquariums with filtered seawater (FSW) at 12°C. One-third of the water was replaced every 4 days. For the experimental manipulation, we selected a seawater pH predicted to occur by the year 2100 ($\Delta pH \approx -0.4$ units; Caldeira and Wickett, 2003). This was regulated by manipulation of environmental CO_2 levels. The treatments were: control/natural seawater (pH = 8.1; $pCO_2 = 372$ ppm; Ωca = 3.2; Ωar = 2.0) and pH 7.7 ($pCO_2 = 930$ ppm; Ωca = 1.5; Ωar = 1.0). pH was maintained in each 5 L aquarium using a computerized control system (AquaMedic) that regulated pH, by the addition of pure gaseous CO_2 directly into the experimental tank. The pH never deviated for more than 0.04 units from the programmed value (7.7 units). The complete experiment was repeated twice, once in March (density of larvae of 9 eggs mL^{-1}) and once in April (density of larvae of 2 eggs mL^{-1}). Similar trends were observed in both repetitions and all data pooled for further analysis. Density of the culture and stage of development were measured several times a week until the end of the experiment when more than 90% of the larvae settled and metamorphosed into juveniles. Settlement success was calculated as the ratio between settled larvae (all larvae fixed to the aquarium walls) and the total number of free swimming larvae (from the density estimated by subsamples of the culture). Larval length and/or rudiment/juvenile diameter were measured on 20 larvae/juveniles on days 0, 7, 13, 18, 24, and 38. Each mean value was expressed with its standard error of mean (mean ± SEM). Qualitative impact on calcification was assessed on days 18, 24, and 38 on embryos cultured in 50 µg/mL calcein in FSW for 1–7 days (Yajima and Kiyomoto, 2006). Specimens were mounted in PBS and examined using a Leica confocal microscope, and were analyzed by collecting stacks of images and then projecting them in the Y-axis. Growth rates and changes in larval density were calculated as linear regression of size over time. Growth rates and changes in density were compared using analysis of covariance using a covariable time. The Shapiro–Wilk statistic W (Shapiro and Wilk, '65) was used to check the data for normality of distribution. When data were not normally distributed or showed heteroscedasticity, a logarithmic transformation was carried out following Sokal and Rohlf ('95). Analyses were performed using SAS/STAT (SAS Institute, '90).

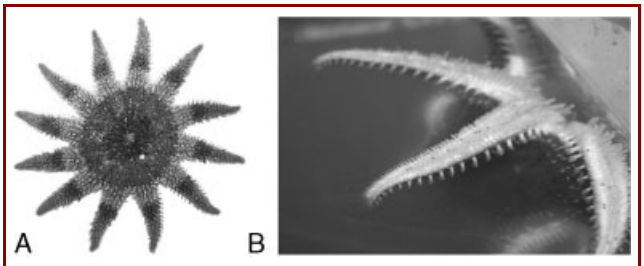

Figure 1. (A) Specimen of *Crossaster papposus*. (B) Female naturally spawning in the aquarium. The large yolky red eggs are visible floating on the surface of the aquarium.

RESULTS

The development of *C. papposus* originally described by Gemmil ('20) was followed. In the control (pH 8.1), a swimming blastula appeared 3 days postfertilization; gastrulation occurred after 5 days followed by elongation of the larvae (10d; Fig. 2A and B) and formation of 3 brachiolaria arms (15d; Fig. 2C). Around day 20, the brachiolaria arms started to show adhesive properties and the larvae fixed on the walls of the culture aquaria and progressively formed a rudiment (Fig. 2D). A 6-lobed juvenile

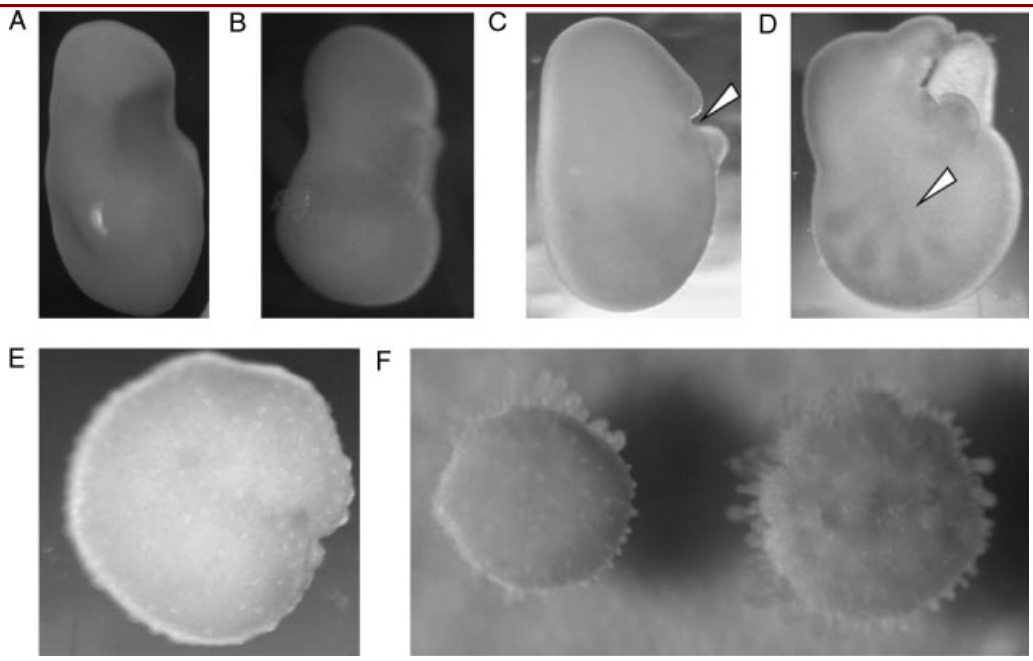

Figure 2. Development of *Crossaster papposus*. (A) (pH 7.7, 7d) and (B) (pH 7.7, 13d): elongated larvae; (C) (pH 8.1, 18d): emergence of the three brachiolaria arms (arrowhead); (D) (pH 8.1, 28d): formation of the rudiment (arrowhead); (E) (pH 7.7, 28d): early juvenile; (F) (d38, pH 8.1 left, pH 7.7right): the juveniles raised at low pH are larger than the one raised under controlled conditions.

started to appear around day 32, with the first tube feet visible (Fig. 2E). On day 38, 95% of the larvae reached the juvenile stage (Fig. 2F). The first signs of calcification were already visible on day 7 in the superficial layer of the dermis as a few small crystals on the aboral surface (Fig. 3A), and then were more abundant and uniformly distributed over the aboral surface through time (Figs. 2E, 3B and C). The first spines were visible on day 38 (Fig. 3E).

Larval survival rate was high and more than 21% of the fertilized eggs reached the juvenile stage (38 days). No significant difference was observed in density between the two pH treatments (ANCOCA, $F = 1.02$, $P < 0.3258$; Fig. 4). However, there was a significant difference in the rate of both growth and developmental progression between the two pH treatments. Larvae at low pH (7.7 units) followed a similar pattern of development as in the control (8.1), but the timing was faster in acidic conditions. For example, on day 28, 95% of the larvae were settled and had developed their rudiment in controls while in the low pH, 50% of the larvae had passed through this stage and were already transformed as juveniles. Thus, at a given time, larvae and/or rudiment/juveniles were larger in low pH water compared with the control (Fig. 5). For example, on day 38, juveniles were 11% larger at low pH than in control (Fig. 2F) and were more advanced in development (e.g. well formed spines were already visible at low pH but not in the control) (Fig. 3D and E). Growth rates were significantly faster at low pH (Fig. 5) where the larvae were growing approximately 2.8 times faster (ANCOVA, $F = 6.92$, $P < 0.0391$) and the rudiments/juveniles were growing 2.2 times faster (ANCOVA, $F = 11.2$, $P < 0.0406$).

DISCUSSION

As far as we know, this is the first evidence of a positive effect of OA on growth rate in any invertebrate larva. In *C. papposus*, larvae and juveniles raised at low pH grow and develop faster, with no negative effect on survival or skeletogenesis within the time frame of the experiment (38 days). However, it is important to remember that other negative effects may appear in the longer term or on parameters not measured in this study (e.g. calcification rate). For example, Gooding et al. (2009) showed increase in growth rates in sea star juveniles under acidic conditions associated with a decreased calcification.

Our two working hypotheses were either: (1) that owing to their calcite skeleton, *C. papposus* will be negatively affected or (2) that owing to their lecithotrophic larvae, they may be more resistant to environmental changes. Our findings, of a positive effect on growth and developmental rate, allow us to reject the first hypothesis. However, *C. papposus* seem to be not only more than simply resistant to OA, but are also performing better. We interpret this as a generally positive direct effect on metabolism. However, we cannot rule out the possibility that this is a short-term positive effect, with negative effects only appearing in the

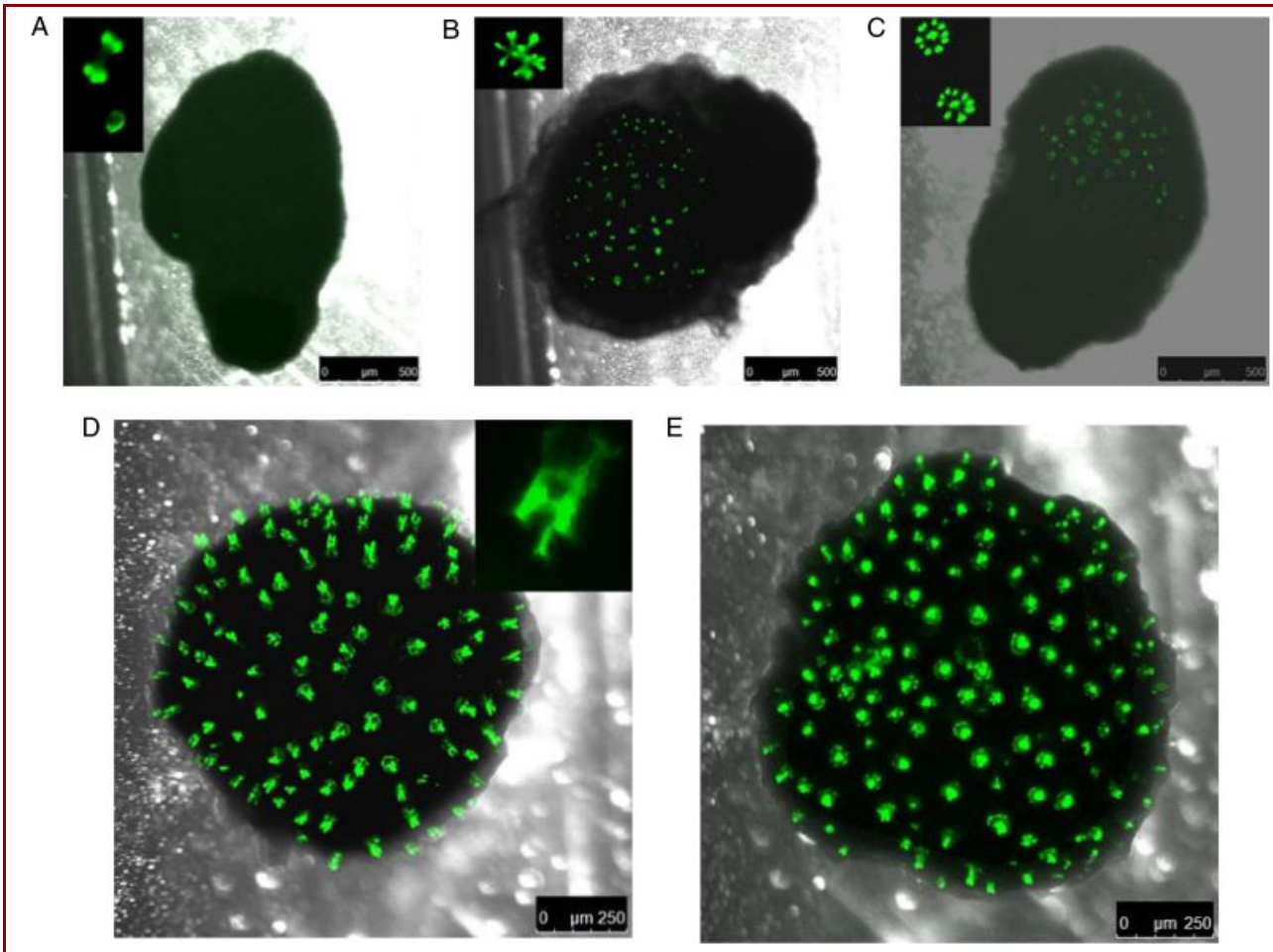

Figure 3. Confocal image of calcein-labeled skeleton during development of *C. papposus*. (A) (pH 8.1,d7): small isolated crystals are the first sign of calcification; (B) (pH 7.7, d7) and (C) (pH 8.1, d24) crystals are more complex and widespread on all the aboral surface; (D) (pH 7.7, d28) and (E) (pH 8.1, d38): well-developed spines are visible after 28d in acidic conditions and more developed than in the control after 38 days.

longer term, later in juvenile or adult life, a phenomenon known as hormesis (Calabrese, 2005).

Earlier work has demonstrated that the impact of OA in echinoderms is highly species specific (Dupont and Thorndyke, 2009a). Our current data confirm this observation and highlight the fact that it is dangerous to extrapolate generalities from a few species, and that it is essential to include different life-history strategies (e.g. planktotrophy vs. lecithotrophy) in any global prediction of impact of OA on species and ecosystems.

Consequences for the Species and Ecosystem

C. papposus is a long lived, slow growing species (at least 20 years) living in stable populations. As a result of the lack of predators, adults have a high survivorship (Carlston and Pfister, '99). This species is found in all northern seas, from subtidal to oceanic depths (D'yakanov, '68) and mainly on rocky bottoms (McConnaughey and McConnaughey, '85). It has 8–16 arms and can reach 34 cm in diameter (Lambert, '81). It is also a highly mobile and dominant predator, feeding mainly on other echinoderms and molluscs (Mauzey et al., '68; Mortensen, '77; Coleman, '91; Himmelman and Dutil, '91). This species is considered to be the dominant predator in its food web and can, therefore, influence the distribution of many other species, determining community structure (Sloan, '79; Himmelman and Dutil, '91). As a result, any change in species fitness could have profound impacts on its ecosystem (Grush, '99).

Our data suggest that in the future ocean, the direct impact of OA on growth and development potentially will produce an increase in *C. papposus* reproductive success. A decrease in developmental time will be associated with a shorter pelagic period and a higher proportion of eggs reaching settlement. It is frequently assumed that selection to shorter larval duration

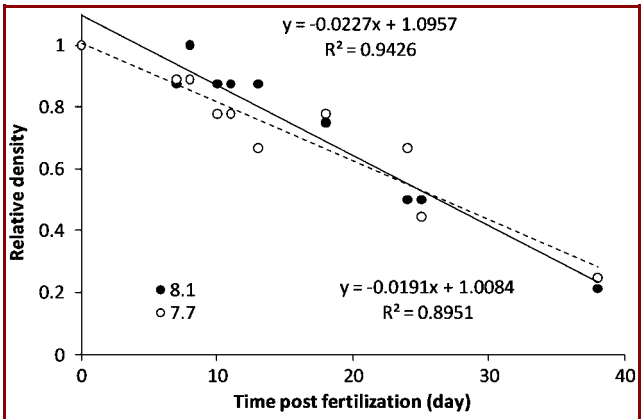

Figure 4. Density of larvae and juveniles of the starfish *C. papposus* under control (pH 8.1) and near-future acidic conditions (pH 7.7 expected for 2100). No difference was observed between the treatments.

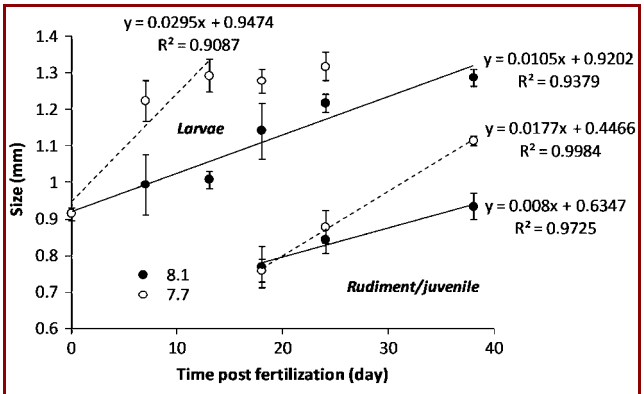

Figure 5. Growth of larvae and juveniles of the starfish *C. papposus* under controlled (pH 8.1) and near-future acidic conditions (pH 7.7 expected for 2100). Under acidic conditions, growth rates are two times higher.

drives evolution of reproductive strategies (Reitzel et al., 2004). The cost of planktonic life can be severe because of mortality risks, such as predation, starvation, offshore transport, and exposure to intolerable environmental conditions. For example, field reports for sea urchin larvae show mortality of more than 15% d^{-1} (Lamare and Barker, '99).

However, other parameters, such as synchrony with food, are also critical for planktotrophic larvae, and synchrony with optimal environmental temperature is vital for both planktotrophic and lecithotrophic larvae. Food and temperature are known as the two primary environmental variables that influence duration of larval life. Our results, together with analyses of the available literature, demonstrate that pH has a direct consequence for growth and developmental rate independently of temperature or food intake (this study; see Dupont and Thorndyke, 2009a, b, for review). This may lead to metamorphosis at an inappropriate time and, therefore, potentially into a disadvantageous environment (poor abiotic conditions, density of predators, etc.; Reitzel et al., 2004). A change in growth rate, either positive or negative, may then be detrimental for both planktotrophic and lecithotrophic larvae.

Other potential indirect effects should also be considered. For example, *C. papposus* is a predatory species. In the future ocean, its prey is also likely to be affected. For example, it is very likely that the sea star, *A. rubens*, will be negatively impacted (Hernroth, Baden, Dupont, and Thorndyke, personal communication). As a result, the positive direct effect observed on the growth and development may be countered by a decrease in food abundance.

Evolutionary Consequences

Many benthic marine invertebrates develop by means of a free-living dispersive larval stage. Larvae are morphologically and ecologically distinct from the adult until they reach the juvenile stage at metamorphosis. It is estimated that 60–90% of all benthic marine invertebrates produce planktotrophic larvae and about 10% produce lecithotrophic larvae (see Pechenik, '99,for review). The ecological and functional demands on larvae impose limits on developmental evolution and developmental patterns (McEdward and Miner, 2001). The question of the factors responsible for this present distribution of larval development among benthic invertebrates is still under debate, as well as the potential for human activities to influence the direction of future evolutionary change in reproductive patterns (Pechenik, '99). It has been suggested that selective pressures, such as decreased larval predation and increased recruitment success, should be considered in addition to the reduction in developmental time (Reitzel et al., 2004). For example, planktotrophic larvae will have the advantage of wide dispersal capacities, but they will also experience high mortality owing to a variety of biotic and abiotic factors. On the other hand, although production of yolky eggs is energetically costly, the probability of successful metamorphosis of lecithotrophic larvae into juveniles is significantly higher, owing to a decrease in developmental time and independence of some highly variable environmental parameters, such as food availability (see Pechenik, '99; McEdward and Miner, 2001 for review).

Based on our data, it is tempting to speculate that planktotrophic and lecithotrophic larvae may have differing relative tolerances to environmental stressors, such as OA. The marine environment is highly variable in space and time and the level of variation is increasing owing to anthropogenic perturbation, such as climate change. Life-history strategies enable species to persist in a variable environment; the predictability of change determining developmental success (Jennings, '97; Hamdoun and Epel, 2007). Changes in environmental conditions and variability acts as a selective pressure, and

our results suggest that life-history strategy is a key factor for this selection. Based on our findings, we hypothesize that lecithotrophic larvae may be better competitors in an unpredictable environment (e.g. near-future OA) than planktotrophic larvae. This is supported by data from lecithotrophic larvae of the cephalopod *Sepia officinalis* that can successfully develop and calcify under low pH condition (Gutowska and Melzner, 2009). Melzner et al. (2009) speculate that the demand to cope with low pH during embryogenesis in *S. officinalis* and perhaps also other lecitothrophic larval stages selects for tolerant physiotypes. To understand this difference in sensitivity, we urgently need further information and robust experimental data. For example, the development of the sea urchin, *Strongylocentrotus purpuratus*, is one of the best described. Available information (e.g. gene regulatory networks [GRNs]) and tools should be transposed to a lecithotrophic species for a comparison of the developmental process and the potential buffering system. One factor that should be considered in any future analysis is the role played by changes in the temporal expression profile of GRNs or their individual components, known to occur in direct developers (Kauffman and Raff, 2003) and the sharing of GRNs between adults and larval stages. For example, heat shock proteins (HSPs) are known to play a number of critical regulatory roles in both development and organismal cellular responses to a variety of environmental stressors. (Gunter and Degnan, 2008). Little is known about any potential changes in temporal expression of HSPs in direct developers that might parallel such changes known to occur in other GRNs (Kaufman and Raff, 2003). However, it is an interesting possibility to predict that such changes that might take place could be responsible, at least partially, for the positive response to OA stress, seen here in the sun star.

It is also relevant to note that lecithotrophy has evolved independently and repeatedly in each echinoderm class (McEdward and Miner, 2001). The main hypothesis is that this evolution is a consequence of selective pressures in a food limiting environment. A new alternative and complementary hypothesis could be erected from our results. Lecithotrophy may be an advantage in unpredictable and extreme environment.

ACKNOWLEDGMENTS

The authors are grateful to Professors Carol Turley and Ulf Riebesell for advice and comments on this article. This article is a contribution to the "European Project on Ocean Acidification" (EPOCA) which received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°211384.

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