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ORIGINAL PAPER

Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*

S. Dupont · N. Dorey · M. Stumpp · F. Melzner · M. Thorndyke

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Abstract Anthropogenic CO_2 emissions are acidifying the world's oceans. A growing body of evidence demonstrates that ocean acidification can impact survival, growth, development and physiology of marine invertebrates. Here, we tested the impact of long-term (up to 16 months) and trans-life-cycle (adult, embryo/larvae and juvenile) exposure to elevated pCO_2 (1,200 µatm, compared to control 400 µatm) on the green sea urchin *Strongylocentrotus droebachiensis*. Female fecundity was decreased 4.5-fold when acclimated to elevated pCO_2 for 4 months during reproductive conditioning, while no difference was observed in females acclimated for 16 months. Moreover, adult pre-exposure for 4 months to elevated pCO_2 had a direct negative impact on subsequent larval settlement success. Five to nine times fewer offspring reached the juvenile stage in cultures using gametes collected from adults previously acclimated to high pCO_2 for 4 months. However, no difference in larval survival was observed when adults were pre-exposed for 16 months to elevated pCO_2. pCO_2 had no direct negative impact on juvenile survival except when both larvae and juveniles were raised in elevated pCO_2. These negative effects on settlement success and juvenile survival can be attributed to carry-over effects from adults to larvae and from larvae to juveniles. Our results support the contention that adult sea urchins can acclimate to moderately elevated pCO_2 in a matter of a few months and that carry-over effects can exacerbate the negative impact of ocean acidification on larvae and juveniles.

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S. Dupont (✉) · N. Dorey · M. Stumpp
Department of Biological and Environmental Sciences, The Sven Lovén Centre for Marine Sciences, University of Gothenburg, Kristineberg, Fiskebäckskil, 45178 Gothenburg, Sweden
e-mail: sam.dupont@bioenv.gu.se

F. Melzner
Biological Oceanography, Leibniz Institute of Marine Sciences (IFM-GEOMAR), 24105 Kiel, Germany

M. Thorndyke
The Royal Swedish Academy of Sciences, The Sven Lovén Centre for Marine Sciences, Kristineberg, Fiskebäckskil, 45178 Gothenburg, Sweden

Introduction

Anthropogenic CO_2 emissions are acidifying the world's oceans. A growing body of evidence is showing that ocean acidification—a decrease in ocean pH due to an increase in pCO_2—impacts survival, development, physiology and growth of marine invertebrates. In all phyla examined so far, responses to near-future ocean acidification appears to be highly species-specific ranging from negative to positive effects on fitness-related parameters (Doney et al. 2009). For example, in echinoderms—a marine calcifying phyla including sea urchins, sea stars and brittle stars—61 % of all studied species (data updated from the study by Dupont et al. 2010a) showed a negative response to high pCO_2 in fitness-related parameters (including potential species extinction in 7 % of echinoderm species, for example the brittle star *Ophiothrix fragilis*, Dupont et al. 2008), while 34 % showed no measurable response and 5 % positive effects (e.g., the sea star *Crossaster papposus*, Dupont et al. 2010b). Explaining this diversity in response to high pCO_2 is one of the challenges in ocean acidification research. It is further complicated by the fact that most experiments focus on a single life-history stage and are short term, ignoring

processes such as acclimation, selection and carryover between successive and different life-history stages.

The literature on the impact of ocean acidification on marine invertebrates is mostly based on the short-term perturbation experiments. For example, 66 % of all studies exploring the impact of ocean acidification on echinoderms (45 papers do date) consider exposure times <2 weeks (data updated from Dupont et al. 2010a). However, it has been shown that longer acclimation times (>10 weeks) to high pCO_2 trigger negative effects on survival on such long-lived adult invertebrates (Langenbuch and Pörtner 2004; Shirayama and Thornton 2005; Kurihara et al. 2008). Moreover, short exposure times are not sufficient to allow (or take into account) potential acclimation to a new environment. Sea urchins lack fast escape and are able to acclimate to a new environment by modulating their morphology and physiology. For example, it was shown that *Strongylocentrotus purpuratus* is able to reshape its skeleton and change its behavior in a matter of 8–20 weeks when exposed to a different habitat structure (Hernández and Russell 2010). As a consequence, short-term exposure may both over- (no acclimation) and underestimate (not enough time to induce lethal effects) the real impacts of a chronic exposure to high pCO_2.

Not only have most experiments on the impact of ocean acidification on invertebrates been short-term, but also they traditionally have had a focus on one life-history stage (but see Mayor et al. 2007; Kurihara et al. 2004, 2008; Kurihara and Ishimatsu 2008; McDonald et al. 2009; Dupont et al. 2010a; Byrne et al. 2008). In echinoderms, only three papers of the 45 published to date on the impact of ocean acidification considered two successive life-history stages (larvae and juveniles: Dupont et al. 2010b, Byrne et al. 2008; adult and larvae: Uthicke et al. submitted). However, a majority of marine invertebrates develop by means of free-living larval stages, often going through several ontogenetic and ecological transitions including gastrulation, molting, metamorphosis, settlement and sexual maturation (Fig. 1). Each stage can differ in the form and function with various degrees of autonomy and sensitivity to environmental stressors. These life stages form a continuum and an environmental change leading to a disturbance in one stage can *carry over* into the following stage (Podolsky and Moran 2006). Both positive and negative carry-over effects have been documented (Pechenik 2006). In a recent paper, Parker et al. (2012) pre-exposed adult oysters to high pCO_2 (856 µatm) for 5 weeks during reproductive conditioning and showed a positive carry-over effect on larval growth. This was interpreted as an increased maternal energetic investment per offspring (e.g., increased egg size) induced when females are exposed to an environmental stressor, a trait beneficial for the offspring (Podolsky and Moran 2006). On the other hand,

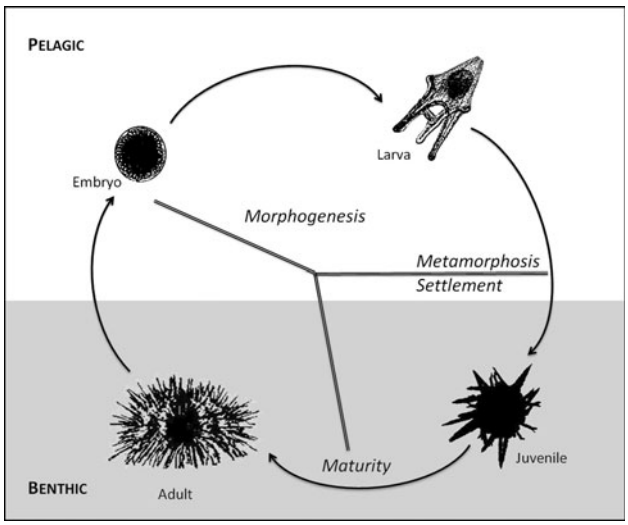

Fig. 1 Life cycle of the green sea urchin *Strongylocentrotus droebachiensis*. Adults live on rocky substratum, and three major transitions occur during the life cycle: (1) a first ecological transition occurs in early spring when gametes (eggs or sperm) are synchronously released into the water column to ensure fertilization. This transition between benthic and pelagic environment is also associated with complex developmental changes (fertilization, embryo and larval development); (2) the second ecological transition (settlement from pelagic to benthic environment) occurs at about the same time as anatomical changes during the metamorphosis of the larva into a young juvenile; and (3) the post-metamorphic anatomical transition between the juvenile and the adult

exposure to an environmental stressor also can induce negative carry-over effects that persist into later stages. For example, exposure to an environmental stressor during the pelagic phase can reduce juvenile performance and be exacerbated if stressful conditions persist (Emlet and Sadro 2006). In conclusion, considering impacts on a single life-history stage can also lead to misinterpretation of the impact of high pCO_2 on a given species.

The aim of this paper is to investigate the contribution of these neglected aspects of responses to ocean acidification. Long-term (up to 16 months) and trans-life-cycle impacts of high pCO_2 (1,200 µatm) were tested on the green sea urchin *S. droebachiensis,* a widely distributed calcifier that plays a key ecological and economical role in boreal coastal ecosystems. For example, on European coasts, grazing by the green sea urchin is central for structuring marine benthic communities leading to two stable states: abundant kelp forests with a high biodiversity and species-depleted sea urchin barrens (Norderhaug and Christie 2009). In some areas, the green sea urchin has been considered a pest as intensive grazing destroyed kelp habitats and limited the production of commercial species such as cod (Kålås et al. 2006). On the other hand, it is fished and now cultured for roes in the Northwest Atlantic and Northeast Pacific (Vadas et al. 2000).

Our hypotheses based on increased energetic costs under high pCO_2 (e.g., Stumpp et al. 2011, 2012) were that (1) chronic exposure of adults to high pCO_2 will have a negative effect on fertility and gamete quality and (2) juveniles from larvae exposed to high pCO_2 will be more sensitive to exposure to high pCO_2 than juveniles from larvae exposed to control pCO_2.

Materials and methods

Animal collection and maintenance

Adult green sea urchins *S. droebachiensis* with a diameter ranging between 4.5 and 5 cm were collected in December 2008 (Experiment 1) and March 2010 (Experiment 2) from a sea urchin dominated barren in the vicinity of Hammerfest (Barent Sea, Norway) and a stone reef at Anholt (Denmark). Individuals from both locations were pooled and crossed to maximize genetic variability. They were transferred to the Sven Lovén Centre for Marine Sciences—Kristineberg (Fiskebäckskil, Sweden) and maintained in 400-L basins with natural flowing seawater with a seawater replacement of 1 L min^{-1}, allowing a minimum volume of 10 L per individual. Sea urchins were kept at 12 °C and $pH_{NBS} = 8.1$ on a diet of *Ulva* spp. for 2 weeks prior to experiments.

Experimental conditions and seawater chemistry

Adult sea urchins were either used immediately to collect gametes ($n = 20$, experiment 2 in 2010) or acclimated to two different pCO_2 seawater levels (401 vs 1,217 µatm) for a total period of 4 or 16 months before collection of gametes (experiment 1; $n = 24$ in 2009; $n = 8$ in 2010). During long-term acclimation, sea urchins were maintained in 4 × 400 L basins (two basins per *p*CO2) with natural flowing seawater with a seawater replacement of 1 L min^{-1}, allowing a minimum volume of 10 L per individual (maximum of 40 individual per basin). Sea urchins were kept at 12 °C and fed ad libitum with *Ulva* spp.

pH and alkalinity (A_T) were measured twice a week. pH was measured with a Metrohm (827 pH lab) pH electrode calibrated with NIST or total (T) scale buffers following Dickson et al. (2007) and A_T following Sarazin et al. (1999). The carbonate system speciation (pCO_2, Ω_{Ca} and Ω_{Ar}) was calculated from pH and A_T using CO2SYS (Lewis and Wallace 1998) with dissociation constants from the study by Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Our treatments for adult cultures were control/natural seawater ($pH_{NBS} = 8.1$, $pCO_2 = 401$ µatm, $\Omega_{Ca} = 2.96$, $\Omega_{Ar} = 1.88$) and high seawater pCO_2 ($pH_{NBS} = 7.7$, $pCO_2 = 1{,}217$ µatm, $\Omega_{Ca} = 1.18$, $\Omega_{Ar} = 0.75$) for a temperature of 12 °C and $A_T = 2.16$ mM. pH was maintained in each aquarium using a computerized feedback system (AquaMedic) that regulates pH (NBS scale) by addition of pure gaseous CO_2 directly into the seawater (±0.02 pH units) mixed by Eheim 600 submersible pumps at a rate of 300 L h^{-1}.

Larval and juvenile cultures

Spawning was induced by intracoelomic injection of 1 mL of 0.5 M KCl in filtered seawater (FSW). Eggs were collected in FSW, and sperm was collected dry and kept on ice until use. Fecundity was measured as the total number of eggs per female and estimated as the average of five subsamples of 50 µL of a 1 L egg dilution. Sperm stock solution in FSW (four males for experiment 1; eight males for experiment 2) was added to an egg dilution (four females for both experiments) to a final concentration of ~1,000 sperm mL^{-1}, allowing a fertilization success >95 %. After fertilization (15 min), eggs were rinsed with FSW. Cleaving embryos (two-cell stage) were placed in 5-L aquaria filled with FSW at a density of 10 embryos per mL, a temperature of 12 °C and the relevant pCO_2 (361 vs 941 µatm). The FSW was continuously aerated to maintain oxygen concentrations close to air saturation and mixed by the slow convective current of a stream of single bubbles (approx. 100 bubbles per min). After 5 days, larvae were fed daily with the cryptophyte algae *Rhodomonas* sp., which were raised in B1 medium (Guillard and Ryther 1962) at 20 °C under a 12:12 h light:dark cycle. Algal strains were provided by the Marine Algal Culture Centre at Gothenburg University (GUMACC). The carbon content of the algae was estimated based on biovolume measurements as equivalent spherical diameter (ESD) with an electronic particle analyzer (Elzone 5380, Micrometrics, Aachen, Germany) and equations provided by Mullin et al. (1966). To prevent changes in food concentration, algae concentration and size were checked daily using a Coulter counter (Elzone 5380, Micrometrics, Aachen, Germany) and then adjusted in the experimental bottles to the maximum concentration of 150 µg C L^{-1} (~3,000–6,000 cells mL^{-1} for diameters ranging between 6 and 8 µm). At the chosen algae concentration (150 µg C L^{-1}), time of exposure (24 h, algae added daily), seawater pCO_2 treatment levels and temperature had no impact on algal growth and survival. This was demonstrated by testing the impact of *p*CO2 (400 vs 1,200 µatm) on growth of *Rhodomonas* (initial concentration of 150 µg C L^{-1}) over 24 h in the absence of sea urchin larvae (four replicates). After 24 h, no significant difference (ANOVA, $F = 1.22$, $p < 0.35$) in algae concentration was observed between control (189.8 ± 0.9 µg C L^{-1}) and elevated pCO_2 (190.5 ± 0.7 µg C L^{-1}). Juveniles (30–300 individuals per aquarium) were

kept in the same 5-L aquaria and were fed with natural biofilm and ad libitum with small fragments of *Ulva* spp. for later stages. Larval and juvenile cultures were maintained at salinity of 32 ‰, temperature of 12 °C and A_T of 2.17 mM. Our treatments were control/natural seawater ($pH_T = 8.07$, $pCO_2 = 361$ µatm, $\Omega_{Ca} = 3.09$, $\Omega_{Ar} = 1.96$) and high pCO_2 levels ($pH_T = 7.69$, $pCO_2 = 942$ µatm, $\Omega_{Ca} = 1.46$, $\Omega_{Ar} = 0.92$).

Experiment 1: effect of adult acclimation on fertility and settlement success

Female fecundity was measured on 4–11 females after 4 and 16 months of acclimation at the two tested pCO_2 (control vs high). Many temperate sea urchins show a seasonal reproductive pattern. In the studied area, the breeding season of the green sea urchin *S. droebachiensis* is a narrow time window between March and April (Falk-Petersen and Lønning 1983). This experiment focused on the impact of elevated pCO_2 on survival, fertility and gamete quality. As a consequence, only two sampling times were considered corresponding to the peak of the breeding season. The impact of a 4-months acclimation period on subsequent larval settlement was assessed in a 2 × 2 experimental design: 2 adult acclimation pCO_2 treatments (control vs high) × 2 larval culture pCO_2 treatments (control vs high). The experiment was replicated four times using the same batch of parental animals. Quality of the gametes was assessed using several parameters: (1) Egg size: After spawning (4 and 16 months), 50 eggs per female were photographed with a digital camera mounted on a dissecting microscope and measured using Image J software. (2) Settlement success (4 months) was measured after 28-day post-fertilization as the number of juvenile in the culture. As a consequence of a power failure in our culture room, it was not possible to measure settlement success in larval cultures from adults acclimated for 4 months. Daily mortality rate was then calculated as another proxy for gamete quality. (3) Daily mortality rate (16 months): Larval cultures were monitored daily for the first 15-day post-fertilization. Each day, a subsample of >50 larvae was counted. Density at time t (Nt, number of larvae per liter) was estimated by dividing the number of larvae by the corresponding volume needed to collect this number of individuals. Daily mortality was calculated as: $Mt = 1 - (Nt/Nt–1)$.

Experiment 2: effect of larval environment on juvenile growth and survival

A large number of juveniles were needed to study the potential carryover from planktonic larval to post-settlement juvenile stages. As a consequence, a second experiment was designed using a large number of larval cultures (64). This was studied using a 2 × 2 experimental design: 2 larval culture pCO_2 treatments (control vs high) × 2 juvenile culture pCO_2 treatments (control vs high). The experiment was fully replicated 2 times using the same batch of juveniles pooled from 32 individual larval cultures (families from 8 males × 4 females at each pCO_2 to maximize genetic diversity). After 3 months post-settlement, juvenile survival (%) and growth (mm month^{-1}, calculated as the difference between average test diameter after 3 months of exposure and initial size) were measured. It is important to notice that because of the high mortality in one of the treatments, juvenile growth may be biased due to size-dependent sensitivity.

Statistical analyses

Each mean value is expressed with its standard error of mean (mean ± SEM). One-way analysis of variance (ANOVA) was used to test the impact of the treatment (pCO_2) on fecundity. Two- and three-factor mixed model ANOVA with the treatment (fixed, pCO_2 treatment and acclimation) and replicate (random) were used to test the impact of pCO_2 on settlement success. The Shapiro–Wilk test (1965) was used to confirm that the data were normally distributed, and the Levene test was used to confirm that variances were homogenous. When data were not normally distributed or when heteroscedasticity occurred, a logarithmic or arcsine transformation of data was performed as indicated by Sokal and Rohlf (1995). All data were analyzed using SAS/STAT software.

Results

Experiment 1: effect of adult acclimation on fertility and settlement success

All adult sea urchins survived the 4- and 16-month acclimation period. Fecundity—the number of eggs per female—was 4.5 times lower in females acclimated to high pCO_2 for 4 months compared to controls (Fig. 2, ANOVA, $F = 29.53$, $p < 0.0001$). In contrast, no difference between pCO_2 treatments was observed for fecundity in females acclimated to high pCO_2 for 16 months compared to controls (Fig. 2, ANOVA, $F = 0.77$, $p < 0.77$).

There was no significant difference in the diameter of eggs (average size = 0.163 ± 0.007 mm) from females acclimated to high pCO_2 compared to control whatever the acclimation duration (4 vs 16 months; Fig. 3, ANOVA III, female, time, pH; $F = 1.79$, $p < 0.06$).

Five to nine times less fertilized eggs reached the juvenile stage in cultures derived from gametes obtained

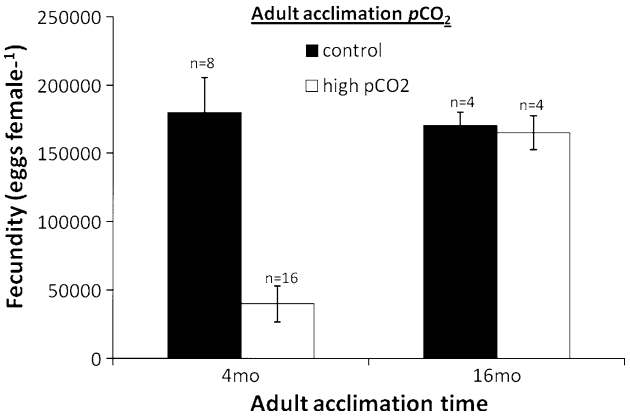

Fig. 2 Fecundity (number of eggs per female) of females acclimated for either 4 or 16 months to control (400 µatm) or high pCO_2 (1,200 µatm) environments

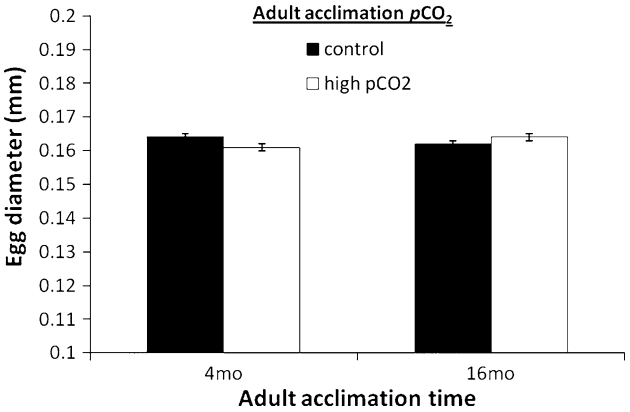

Fig. 3 Diameter of eggs collected from females acclimated for either 4 or 16 months to control (400 µatm) or high pCO_2 (1,200 µatm) environments

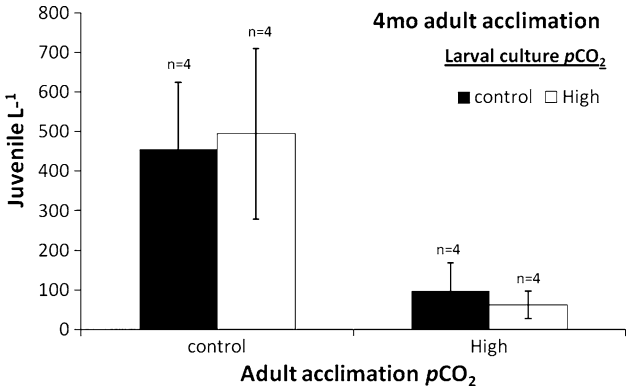

Fig. 4 Impact of pCO_2 (control vs high) during adult acclimation for 4 months and subsequent larval development on juvenile settlement success (number of juvenile per culture 28-day post-fertilization)

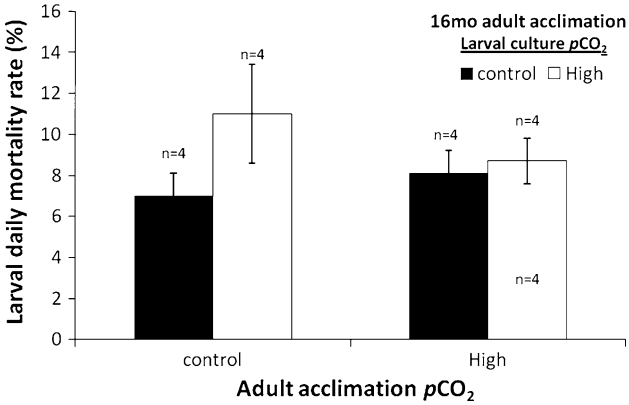

Fig. 5 Impact of pCO_2 (control vs high) during adult acclimation for 16 months and subsequent larval development on larval daily mortality rate (first 2 weeks of development)

from adults acclimated in a high pCO_2 environment for 4 months (ANOVA II, $F = 4.87$, $p < 0.031$, log-transformed data; acclimation pCO_2: $F = 9.71$, $p < 0.0098$), but pCO_2 during larval development had no significant effect on the settlement success (Fig. 4; larval culture pCO_2: $F = 0.03$, $p < 0.88$).

In contrast, there was no significant difference in larval daily mortality rate (average 8.76 ± 0.76 % for the first 2 weeks of post-fertilization) in cultures derived from gametes obtained from adults acclimated in a high pCO_2 environment for 16 months (Fig. 5, ANOVA II, $F = 1.36$, $p < 0.26$).

Experiment 2: effect of larval exposure on juvenile growth and survival

46 to 67 % of the juveniles survived 3 months after metamorphosis and settlement with the exception of juveniles from larvae raised at high pCO_2 and maintained at high pCO_2. These juveniles suffered a mortality of 95 % (Fig. 6a).

pCO_2 during larval development did not have any significant impact on juvenile growth rate (Fig. 6b, ANOVA II: $F = 29.27$, $p < 0.0001$; larval development pCO_2: $F = 3.72$, $p < 0.054$). However, growth of surviving juveniles was significantly higher when animals were kept at the same pCO_2 during both larval and juvenile development (juvenile growth pCO_2: $F = 6.89$, $p < 0.0088$; interaction larval development pCO_2 x juvenile growth pCO_2: $F = 58.13$, $p < 0.0001$).

Discussion

Our results highlight the importance of long-term chronic exposure and of examining trans-life-history stages in experiments assessing organismal responses to elevated pCO_2.

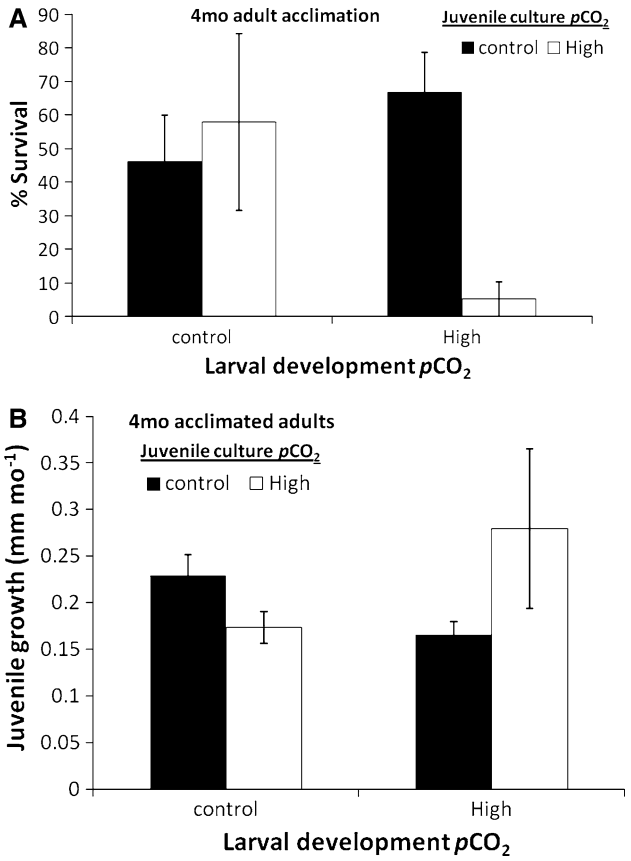

Fig. 6 Impact of pCO_2 (control vs high) during larval and juvenile growth on juvenile (**a**) survival (% of surviving juvenile 3 months after settlement) and (**b**) growth (mm month^{-1})

Chronic exposure

No mortality was observed during chronic exposure of adult sea urchins *S. droebachiensis* to high pCO_2 for up to 16 months. This is consistent with previous long-term exposures to high pCO_2 on adult echinoderm species (40-day exposure on brittle stars, Wood et al. 2008, 2010; Findlay et al. 2011; 49-day exposure on sea urchins, Dashfield et al. 2008, Uthicke et al. submitted; 6-month exposure on sea stars, Hernroth et al. 2011) including *S. droebachiensis* (45 days, Stumpp et al. 2012; 56 days, Siikavuopio et al. 2007), with increased mortality being only documented in juvenile sea urchins (6 months, Shirayama and Thornton 2005). However, high pCO_2 induced a 4.5-fold decrease in fecundity in females exposed for 4 months during the reproductive conditioning period. A decrease in fecundity under high pCO_2 was also documented for shrimps (Kurihara et al. 2008), but no effect of adult exposure on egg production was observed in copepods (Mayor et al. 2007; Kurihara et al. 2004; Kurihara and Ishimatsu 2008) or barnacles (McDonald et al. 2009). This is also consistent with the recent study by Uthicke et al. (submitted), showing a decrease in the volume of sperm produced in males acclimated for 49 days to $pCO_2 > 800$ µatm. Gonads can be considered the most plastic body compartment in adult echinoderms and are often used as an energy storage compartment that can be filled or depleted depending on conditions (Russell 1998). For example, increased energetic costs led to significantly reduced gonadal growth in *S. droebachiensis* adults exposed for 45 days to high pCO_2 (2,840 µatm, Stumpp et al. 2012). As a consequence, decreased fecundity is likely to reflect the increased energy costs needed for survival in a challenging new environment. Recent physiological studies highlight the importance of energy in a species' response to ocean acidification (Stumpp et al. 2011, 2012). Melzner et al. (2011) show that integrity of the inner shell surface in blue mussel is tightly coupled to the animal's energy budget under high pCO_2 and negative impacts observed at high pCO_2 are exacerbated in low food conditions. In adult green sea urchins, it has been shown that a reduced scope for growth under high pCO_2 (45 days exposure) is caused primarily by decreased feeding rates and elevated ammonium excretion rates (Stumpp et al. 2012). This may reflect the increased energy needed to regulate pH in the peri-visceral coelomic fluid during exposure to high pCO_2. An uncompensated respiratory acidosis is observed in *S. droebachiensis* during short-term (5 days) exposure to high pCO_2 (Spicer et al. 2011), but full pH compensation accomplished by significant bicarbonate accumulation could be observed within 10 days and up to 45 days at 1,000–1,400 µatm CO_2 (Stumpp et al. 2012). The observed decreased fecundity could be a consequence of these increased energetic costs.

In contrast, no impact on fecundity was observed after 16-month exposure to elevated pCO_2. This suggests that adults were fully acclimated to their new environmental conditions and were able to replenish their energy stores for successful reproduction. Sea urchins are well known to exhibit a high degree of plasticity toward a great range of stressors (e.g., Levitan 1991, Lau et al. 2009, Selden et al. 2009, Hernandez and Russell 2010) and required up to 20 weeks to fully exhibit new morphological and physiological changes (Hernandez and Russell 2010). These changes also require energy with accompanying potential negative effects on energy stores. As a consequence, 4 months of exposure may not be sufficient enough of a time interval for the green sea urchin to fully acclimate (e.g., more efficient pH regulatory processes in place) and/or replenish their energy stores. It is also important to notice that adult sea urchins were acclimated under optimal food conditions. Acclimation potential under limiting food condition would deserve additional investigations.

Trans-life-stage exposure and carry-over effects

Our results revealed strong carry-over effects from adults to planktonic larvae and from larvae to settled juveniles. Five to nine times fewer eggs reached the juvenile stage in cultures produced from gametes collected from adults previously acclimated to high pCO_2 for 4 months, and pCO_2 concentration had no direct negative impact on juvenile survival except when both larvae and juveniles were raised in elevated pCO_2. To our knowledge, only two other published experiments were designed to study carry-over effects related to ocean acidification. Parker et al. (2012) pre-exposed adult oysters to high pCO_2 for 5 weeks and showed a positive carry-over effect on larval growth. This was interpreted as an increase in maternal energy investment per offspring; a phenomenon previously described when adults were exposed to environmental challenges (Podolsky and Moran 2006). Exposure of adult sea urchin *Echinometra mathaei* to elevated pCO_2 for 7 weeks had no significant effect on egg diameter and 3-day larval response to elevated pCO_2 (Uthicke et al. submitted). Few other studies on the impact of ocean acidification on marine invertebrates have considered more than one life-history stage. Mayor et al. (2007) showed that pre-exposure of adults to high pCO_2 (8,000 µatm) had no effect on egg production of the copepod *Calanus finmarchicus*. However, only 4 % of the eggs successfully yielded nauplii after 72 h in the experimental treatment. Constant exposure to high pCO_2 throughout the whole life cycle had no increased negative nor positive impacts compared to the observed impact on single life stages in copepods (Kurihara et al. 2004; Kurihara and Ishimatsu 2008), barnacles (McDonald et al. 2009) and echinoderms (Dupont et al. 2010a, b; Byrne et al. 2008).

The negative carry-over effects observed in this study could be attributed to the increased energy demands associated with exposure to high pCO_2 at one stage. This increased energy cost is leading to decreased energy availability for later stages and/or during transition processes. Transitions such as gamete production or ontogenetic shifts are energetically expensive (Gosselin 1997). For example, 60 % of the energy stores accumulated by oyster larvae are expended during metamorphosis (Videla et al. 1998). The barnacle *Balanus balanoides* increases its oxygen consumption when the metamorphosing cyprid forms its first calcified shell (Lucas et al. 1979). It was shown that during the larval phase of the sea urchin *Heliocidaris erythrogramma*, approximately 50 % of the energy provided by aerobic metabolism is channeled into the development of the future juvenile (Hoegh-Guldberg and Emlet 1997). The sea urchin *Tripneustes gratilla* accumulates lipids in stomach cells during larval development, which are later incorporated into the juvenile during metamorphosis (Byrne et al. 2008). In sea urchin larvae, it was recently demonstrated that exposure to high pCO_2 is associated with increased energetic demands for maintenance (Stumpp et al. 2011). This shift in the larval energy budget then influences metamorphose success and juvenile fitness.

Juvenile mortality was high (95 % in high pCO_2 compared to 33–54 % in the control) when both larvae and juveniles were exposed to elevated pCO_2. This suggests that energetic disadvantages at the larval stage amplify sensitivity to stressors in juveniles. Several studies have reported such latent effects defined as effects that have their origin in early development but that are first exhibited in juveniles or adults (Pechenik 2006). Growth of surviving juveniles was significantly higher when maintained at high pCO_2 during both larval and juvenile development. Because of the high juvenile mortality, it is not possible to discriminate between a direct positive impact of high pCO_2 on growth (faster growth at elevated pCO_2 as previously documented for sea stars; Gooding et al. 2009), or a size-dependent mortality in response to elevated pCO_2 (better survival of the fast growing individuals at elevated pCO_2 leading to a biased population average size in elevated pCO_2 cultures compared to control). Size-dependent mortality in response to elevated pCO_2 was previously documented in bivalves (e.g., Green et al. 2004). For example, it was shown that selectively bred fast growing oysters were more resilient to ocean acidification (Parker et al. 2011). However, additional experiments are required to investigate the potential relationship between size, growth and survival in juvenile urchins exposed to elevated pCO_2.

No negative carry-over effect between adult and larvae was observed when adults were pre-exposed to elevated pCO_2 for 16 months. No difference in daily mortality rate was observed between combinations of adult acclimation and larval culture pCO_2. This also suggests that adults were fully acclimated to their new environmental conditions and were able to replenish their energy stores for successful reproduction. This is consistent with the results from Uthicke et al. (submitted) showing no carry-over between adult sea urchin *E. mathaei* pre-exposed to elevated pCO_2 for 7 weeks and 3-day-old larval response to elevated pCO_2. However, this is somewhat contrasting with the negative carry-over effect observed in *S. droebachiensis* acclimated for 4 months. This could be an indication that time needed for full acclimation in tropical species such as *E. mathaei* may be shorter (<7 weeks) compared to temperate species such as *S. droebachiensis* (>4 months). On the other hand, it is also possible that the time exposure and parameters used in Uthicke et al. (submitted) failed to detect any negative effect. Carry-over effect between adult and larvae was assessed through two parameters: egg diameter and 3-day larval morphology. Egg size is a classic

proxy for egg quality. For example, smaller eggs can have reduced lipid content (Emlet and Hoegh-Guldberg 1997) with negative consequences for larval fitness by increasing age at metamorphosis and reducing juvenile quality (Alcorn and Allen 2009; Bertram et al. 2009). However, in our study, egg size appeared to be a poor predictor of the further negative impacts observed on settlement success (no effect of adult acclimation and larval culture pCO_2 was observed on egg diameter). As a consequence, egg size also appeared to be a poor predictor of the negative effect observed on further larval and juvenile stages. This carry-over effect between adult and larvae was egg size independent and may not be directly related to energy/lipid content but rather to other parental effects (e.g., epigenetic processes, Gilbert and Epel 2008). Similarly, using impacts on 3-day larvae as a proxy of larval fitness may lead to an underestimation of the real impact as demonstrated by several recent studies showing that negative impacts of elevated pCO_2 on larval survival and morphology only appeared later in the development (e.g., when the larval start to feed, Dupont et al. 2008; Stumpp et al. 2011).

Ecological consequences

These experiments were not designed to assess the ecological consequences of near-future environmental conditions on the green sea urchin *S. droebachiensis*. For example, only one stressor was considered (pCO_2) ignoring other stressors such as temperature and their natural variability. As a single-species perturbation experiment, ecological interactions and complex evolutionary processes were not assessed. It is thus dangerous to draw conclusions or predictions on the near-future consequences for this species. However, it is clear from our results that experiments considering short-term (e.g., <4 months for long-lived sea urchins) adult exposure and neglecting acclimation may overestimate the severity of their responses and that taking only a single life-history stage into account can lead to an underestimation of the real impact. Taken individually, each life-history stage (larval, juvenile and adult) appeared to be quite resilient to high pCO_2. However, carry-over effects (adult to larvae and larvae to juvenile) led to a negative effect of high pCO_2 with overall 100 times fewer eggs reaching the late juvenile stage (3 months). As a consequence, in the absence of rapid evolutionary adaptation (but see Sunday et al. 2011 showing potential for rapid adaptation in the larval sea urchin *S. franciscanus* exposed to short-term exposure to high pCO_2), the green sea urchin *S. droebachiensis* is likely to be negatively impacted in the near-future oceans.

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