Vol. 373: 285–294, 2008
doi: 10.3354/meps07800

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published December 23

Contribution to the Theme Section 'Effects of ocean acidification on marine ecosystems'

OPEN ACCESS

Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*

Sam Dupont[1,*], Jon Havenhand[2], William Thorndyke[1], Lloyd Peck[3], Michael Thorndyke[1,4]

[1]Department of Marine Ecology, Göteborg University, The Sven Lovén Centre for Marine Sciences, Kristineberg, Sweden
[2]Department of Marine Ecology, Göteborg University, The Sven Lovén Centre for Marine Sciences, Tjärnö, Sweden
[3]British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 0ET, UK
[4]Royal Swedish Academy of Science, The Sven Lovén Centre for Marine Sciences, Kristineberg, Sweden

ABSTRACT: The world's oceans are slowly becoming more acidic. In the last 150 yr, the pH of the oceans has dropped by ~0.1 units, which is equivalent to a 25% increase in acidity. Modelling predicts the pH of the oceans to fall by 0.2 to 0.4 units by the year 2100. These changes will have significant effects on marine organisms, especially those with calcareous skeletons such as echinoderms. Little is known about the possible long-term impact of predicted pH changes on marine invertebrate larval development. Here we predict the consequences of increased CO_2 (corresponding to pH drops of 0.2 and 0.4 units) on the larval development of the brittlestar *Ophiothrix fragilis*, which is a keystone species occurring in high densities and stable populations throughout the shelf seas of northwestern Europe (eastern Atlantic). Acidification by 0.2 units induced 100% larval mortality within 8 d while control larvae showed 70% survival over the same period. Exposure to low pH also resulted in a temporal decrease in larval size as well as abnormal development and skeletogenesis (abnormalities, asymmetry, altered skeletal proportions). If oceans continue to acidify as expected, ecosystems of the Atlantic dominated by this keystone species will be seriously threatened with major changes in many key benthic and pelagic ecosystems. Thus, it may be useful to monitor *O. fragilis* populations and initiate conservation if needed.

KEY WORDS: Climate change · Ocean acidification · Echinoderms · Larval development · CO_2 · Brittlestar · Calcification · Skeletogenesis

INTRODUCTION

Recent global models predict that pH at the ocean surface will fall by an estimated 0.2 to 0.4 units by the year 2100 largely due to human-driven emissions of CO_2 (Caldeira & Wickett 2003, 2005, Royal Society 2005, Cao et al. 2007); work by Doney et al. (2007) suggests that this may be exacerbated by anthropogenically released sulphur and nitrogen, especially in coastal waters. These predicted changes in ocean pH are greater, and far more rapid, than any that have been experienced in the past 300 million yr, and the ability of marine organisms, populations and ecosystems to adapt to this unprecedented environmental modification is largely unknown.

Available estimates suggest that rates of calcification in marine organisms have decreased by 11 to 44% since pre-industrial times (Andersson et al. 2005), and will fall to 60% during the 21st century (Kleypas et al. 2006). The calcium carbonate shells or skeletons of many planktonic organisms make them susceptible to dissolution in acidic waters, their degree of susceptibility being dependent not only on pH and carbonate saturation, but also on the crystalline form of calcium carbonate used (aragonite being ~2× more soluble than calcite; Royal Society 2005. Experiments on organisms

*Email: sam.dupont@marecol.gu.se

as varied as corals, coralline algae, molluscs, foraminiferans and coccolithophorids have all documented reduced capacity for biomineralization at high pCO_2 and its associated low pH (e.g. Kleypas et al. 2006).

Despite this recent work, the impacts of CO_2-driven acidification on the delicate embryonic and larval stages that are essential for recruitment and population maintenance of many marine invertebrate taxa have been largely ignored. To date, only the works of Kurihara and others (Kurihara & Shirayama 2004, Kurihara et al. 2004, 2007, Kurihara & Ishimatsu 2008) have focussed on these early life-history stages. These authors have shown significant deleterious effects of CO_2-induced acidification on larval development and survival in echinoderms, crustaceans and molluscs. In 2 sea urchin species, fertilization rate decreased with pH but significant effects were only observed when the acidification was severe (pH 6.95 and 7.13 depending on the species). Acidification also induced a decrease in body length at Day 3, with significant effect at pH 7.6 to 7.7 (Kurihara & Shirayama 2004, Kurihara et al. 2004).

Echinoderms are appropriate model organisms as they play major roles in ecosystems as keystone predators and grazers (Paine 1966, Estes & Palmisano 1974), as bioturbators and remineralizers (Ambrose et al. 2001), and as food sources for commercial fish (e.g. *Limanda limanda*; Duineveld & Noort 1986, Mattson 1992) and crustaceans (e.g. *Nephrops norvegicus*; Baden et al. 1990). Critically for this study, echinoderm larvae have been shown to form skeletal rods from an amorphous calcite crystal precursor, which is 30× more soluble than normal calcite (Politi et al. 2004). It is therefore likely that echinoderm larvae will be particularly susceptible to CO_2-induced decreases in ocean pH, and that this may result in compromised larval development and survival, possibly leading to developmental and/or recruitment failure.

Here we report the first detailed assessment of the effects of increased CO_2 on embryonic and larval stages of the echinoderm *Ophiothrix fragilis*, which is a keystone brittlestar species that occurs in high densities and stable populations throughout the shelf seas of northwestern Europe (Morgan & Jangoux 2005).

MATERIALS AND METHODS

Specimens of *Ophiothrix fragilis* were collected using an Agassi trawl from a rocky substratum in the Gullmarsfjord in the vicinity of the Sven Lovén Centre for Marine Sciences, Kristineberg, Sweden, and were subsequently maintained in natural flowing seawater at 14°C. Individuals were collected during the period of sexual maturity between May and August 2007. Ripe individuals were identified by their obvious gonads (white testes; orange ovaries) visible through the extended walls of the bursae. Two males and 10 females were used for each fertilization. All 12 ind. were placed in a container of seawater, and males were slightly agitated by hand for a few seconds until the release of sperm, which subsequently induced the females to spawn (Morgan & Jangoux 2005).

Cleaving embryos (two-cell stage) were placed in 5 l aquaria filled with filtered seawater (FSW, taken from the sampling site) at a density of 10 ml^{-1}. The FSW was continuously aerated, and a 1 l volume was replaced every 3 d.

Ophiothrix fragilis gonads are most developed in May to July (George & Warwick 1985), with highest gonadal index in June and July (Lefebvre et al. 1999). The gametes are released from June to September depending on locality (Davoult et al. 1990, Lefebvre & Davoult 2000), although individuals can breed throughout the year in some populations (Ball et al. 1995). Larvae are affected by environmental and physical factors that are independent of the benthic environment experienced by adults. Adults are located at depths between 20 and 80 m, while larval life is pelagic. The planktonic larval phase lasts ~26 d and the larvae metamorphose into juveniles while still in the plankton (MacBride 1907). Larvae are present in the plankton over several months (Lefebvre & Davoult 2000), with the main recruitment occurring between the end of August and beginning of September (Davoult et al. 1990). Larvae are concentrated near the surface and are more abundant in the upper 15 m (Lefebvre & Davoult 1998, 2001).

During the period May to September, the pH in Gullmarsfjord decreases with depth (ranging between 8.33 and 7.97), but never falls below 8.07 in the upper 30 m where *Ophiothrix fragilis* larvae are concentrated (data from SMHI Database Svenskt Havrarkiv). Based on these data, we selected a range of seawater pH predicted to occur by the year 2100 ($\Delta pH \approx -0.2$ to -0.4 units; Caldeira & Wickett 2003, 2005), which we regulated by manipulation of environmental CO_2 levels. These treatments were control/natural seawater (pH = 8.1), pH 7.9 and pH 7.7. One 5 l aquarium was used for each of the 3 treatments. Cultures were maintained at 14°C, a salinity of 32‰ and alkalinity of 2.12 ± 0.02 mM as measured following Sarazin et al. 1999. After Day 2, larvae were fed daily with the red alga *Rhodomonas* sp. at a concentration of 150 µg C l^{-1}. Food concentration was checked using an Elzone 5380 particle sizing and counting analysis system and corrected daily (at this concentration, the pH had no impact on algal growth and/or survival). The entire experiment was repeated 3× (n = 3) using different batches of parental animals. pH was maintained in each aquarium using a computerised control system (AquaMedic) that regulated pH by the addition of pure gaseous CO_2 directly into the water to a resolution of 0.04 pH units.

Larval cultures were monitored daily. Each day, a subsample of 50 larvae was removed and fixed in 4% paraformaldehyde in FSW for later analysis. Density at time t (N_t, larvae l^{-1}) was estimated by dividing the number of larvae (50) by the corresponding volume needed to collect this number of individuals. Instantaneous mortality was calculated as: $M_t = 1 - (N_t/N_{t-1})$. Larvae were photographed with a digital camera mounted on a dissecting microscope using polarised light to visualise the skeleton. Six morphometric parameters (see Fig. 2) were measured for each larva using LAS software (Leica). In addition, a symmetry index (SI = ratio of left to right overall length) was calculated. Images were processed using Adobe Photoshop.

Data were analysed using 1- and 2-way ANOVA, Scheffé's and Dunnett's tests, with Bonferroni correction. Canonical discriminant analysis was used to assess the impacts of pH and/or exposure time on morphometric parameters. The Shapiro-Wilk statistic W (Shapiro & Wilk 1965) was used to check the data for normality of distribution. When data were not normally distributed or showed heteroscedasticity, a logarithmic transformation was done following Sokal & Rohlf (1995). Analyses were performed using SAS/STAT (SAS Institute 1990). Percentages of abnormal larvae through time were analysed using the Bhattacharya (1967) method in order to estimate means and SEs, using FISAT II software (FAO-ICLARM Stock Assessment Tools).

RESULTS

Effects on survival

Survival in the controls (pH 8.1) was 29.5 ± 5.5% after 8 d (average equivalent mortality rate of 20% d^{-1}), in comparison to <0.1% in both low pH treatments (average equivalent mortality rate of 35 ± 10.8% d^{-1} at pH 7.9 and 50.4 ± 10.5% d^{-1} at pH 7.7). A significant mortality increase in the low pH treatments versus controls was first observed after 7 d at pH 7.9, and after 5 d at pH 7.7 (Fig. 1). After 25 d, control larvae still showed an overall survival rate of 10% (equivalent to a mortality rate of 9.1% d^{-1}).

Effects on growth

Under our 'control' rearing conditions (pH 8.1, 14°C), larval development was complete after 25 d. The chronology of development followed the pattern described by Morgan & Jangoux (2005). After 24 h, 72% of the larvae had reached the 2-arm (posterolateral) stage (Figs. 2 & 3). The second pair of arms (post-oral) started to develop on the second day. By Day 3, the larvae had begun to feed on the supplied *Rhodomonas* microalgae. The 6-arm stage (anterolateral arms) was completed after 5 d and the 8-arm stage (post-oral arms) started on Day 7. A similar developmental series was observed at low pH but with 3 notable differences: (1) none of the larvae in the low-pH treatments reached the 8-arm pluteus stage, (2) a high proportion of the larvae raised at low pH were either abnormal or asymmetric (see 'Results; Effects on development'), and (3) despite similarity, the temporal dynamics of development was delayed at low pH, with larvae taking longer to reach the same developmental stage. Thus, 50% of the larvae in the control cultures were 4-armed after 1.83 d compared to 2.07 and 2.25 d at pH 7.9 and 7.7, respectively. Similarly, 50% of the control larvae were 6-armed after 5.42 d compared to 5.73 and 5.71 d at pH 7.9 and 7.7, respectively.

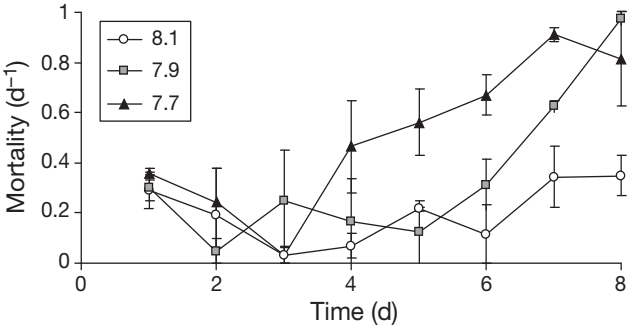

Fig. 1. *Ophiothrix fragilis.* Daily instantaneous mortality rates over time at the 3 tested pH values (n = 2). ANOVA showed significant effects of pH (df = 2, F = 14.66, $p < 0.001$), time (df = 7, F = 11.12, $p < 0.001$) and pH × time (df = 14, F = 2.3, $p < 0.035$)

The impact of ocean acidification on larval and skeletal growth was assessed by measuring and comparing 7 morphometric parameters against 'normal'

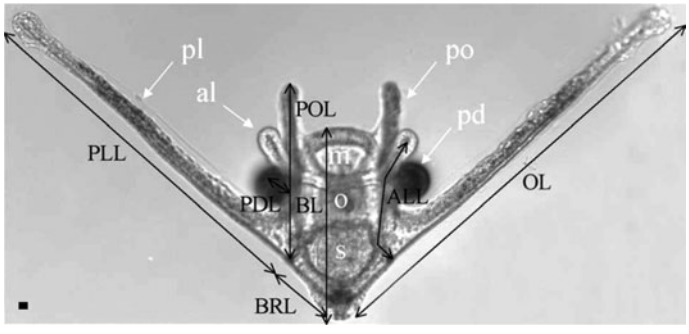

Fig. 2. *Ophiothrix fragilis.* Morphometric coordinates and morphology of the control 8-arm pluteus (Day 8, pH 8.1): al, anterolateral arm; ALL, anterolateral rod length; BL, body length; BRL, body rod length; pd, post-dorsal arm; m, mouth; o, oesophagus; PDL, post-dorsal rod length; pl, posterolateral arm; PLL, posterolateral rod length; po, post-oral arm; POL, post-oral rod length; OL, overall length; s, stomach. Scale bar = 10 µm

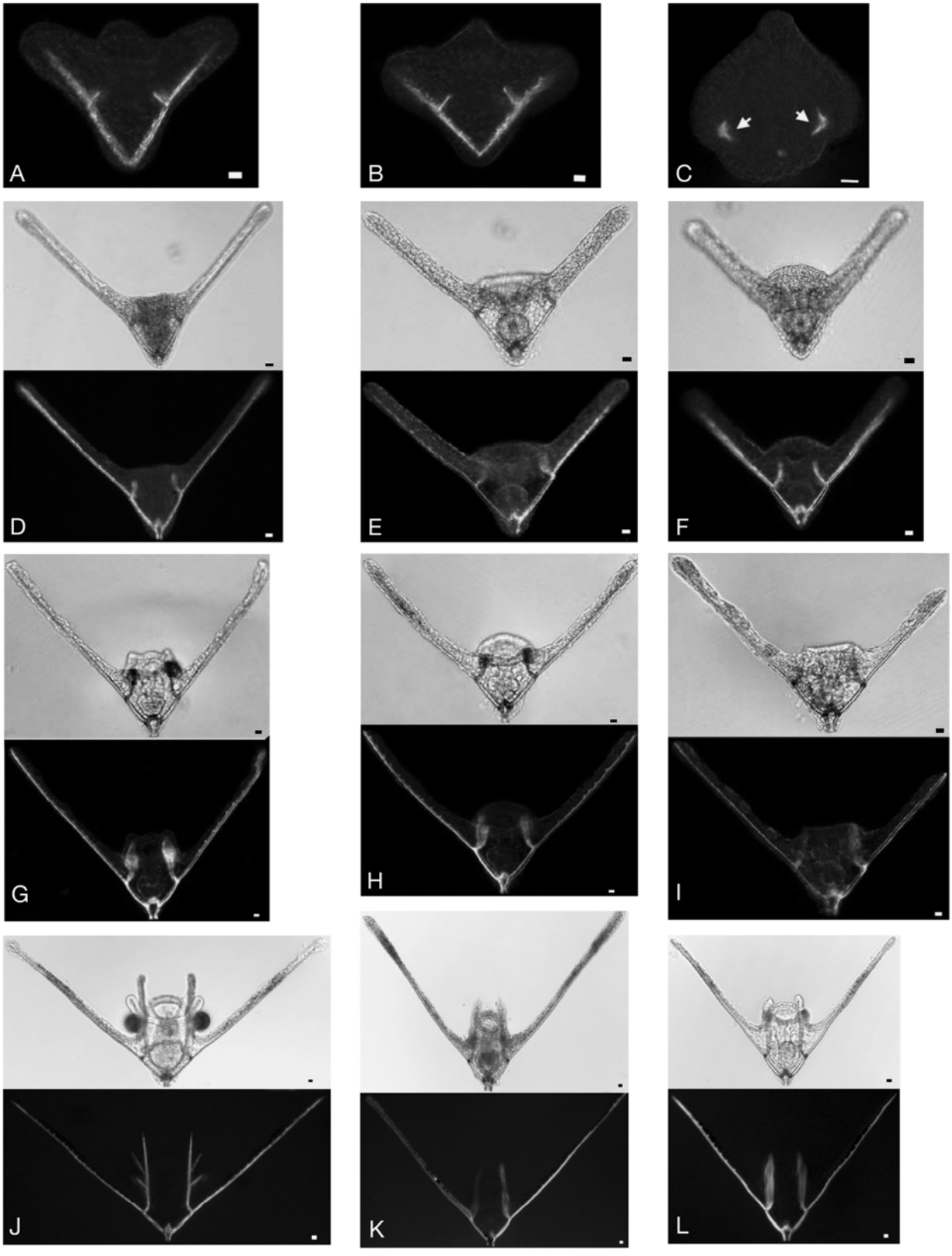

Fig. 3. *Ophiothrix fragilis*. Larval development at the 3 pHs used. First column (A, D, G, J), pH 8.1 (control); second column (B, E, H, K), pH 7.9; third column (C, F, I, L), pH 7.7. (A to C) Day 1, (D to F) Day 2, (G to I) Day 5, (J to L) Day 8. Dark panels: normal transmitted light; light panels: polarized light. Scale bars = 10 µm

larvae (Fig. 2). Abnormal and asymmetric larvae were excluded from this analysis. pH had no significant effect on anterolateral rod length (ALL, Fig. 4E) while differences were observed for other parameters. The most consistent differences were observed after Day 2 for body rod length (BRL, Fig. 4B), the rod being longer in the control than at pH 7.7. From Days 2 to 5, the larvae were more symmetric in the control than in those at low pH, even if the most asymmetric larvae (SI < 0.83) were not taken into account in this analysis. For the other parameters, some individual differences were observed, rods in the control being generally longer than at low pH (Fig. 4). Canonical discriminant analyses were performed on the morphometric para-

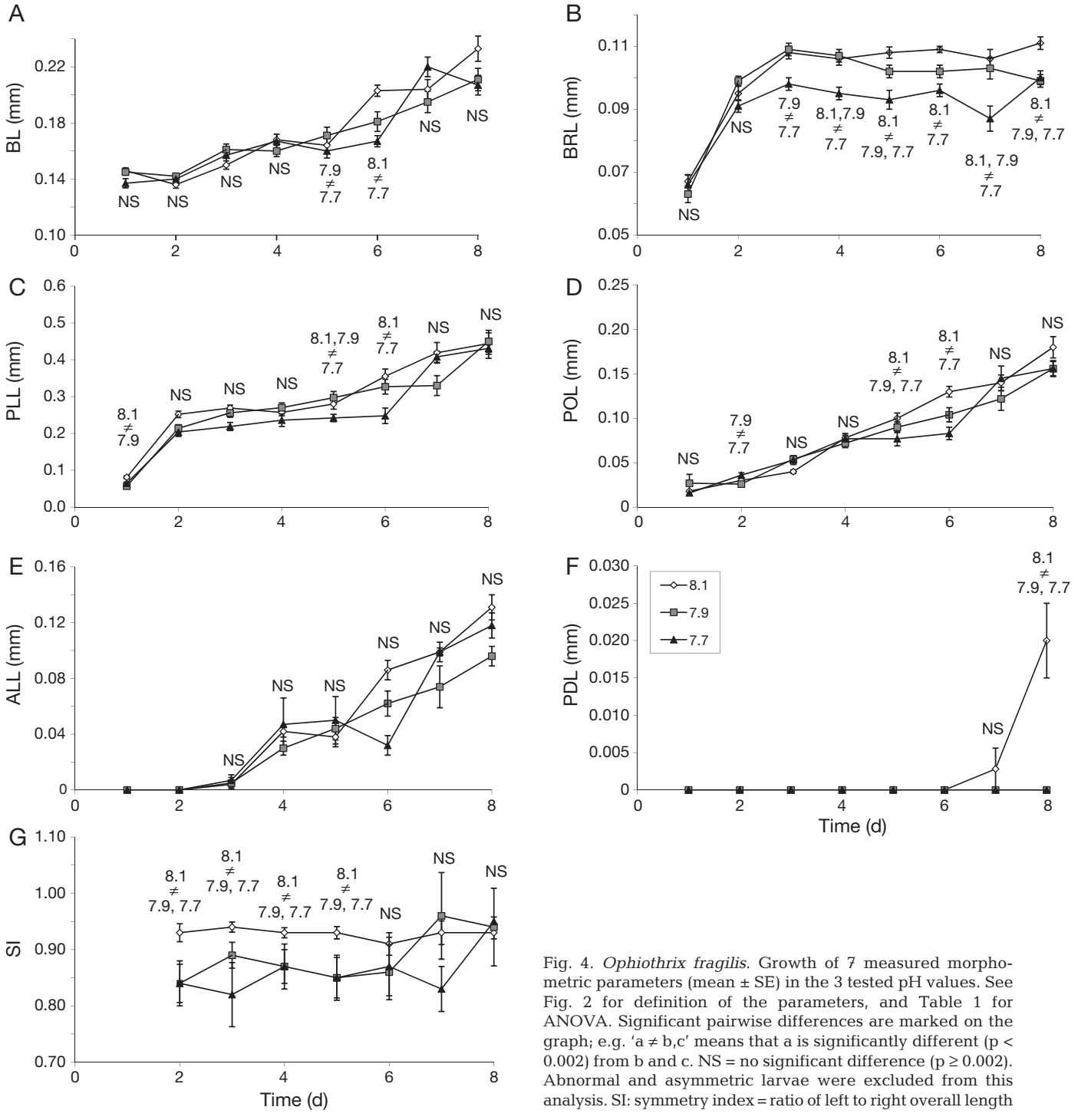

Fig. 4. *Ophiothrix fragilis.* Growth of 7 measured morphometric parameters (mean ± SE) in the 3 tested pH values. See Fig. 2 for definition of the parameters, and Table 1 for ANOVA. Significant pairwise differences are marked on the graph; e.g. 'a ≠ b,c' means that a is significantly different ($p < 0.002$) from b and c. NS = no significant difference ($p \geq 0.002$). Abnormal and asymmetric larvae were excluded from this analysis. SI: symmetry index = ratio of left to right overall length

Table 1. *Ophiothrix fragilis.* ANOVA of morphometric parameters as a function of pH and time. See Fig. 2 for definition of morphometric parameters. SI: symmetry index = ratio of left to right overall length

Parameter	Source	df	*F*	p
BL	pH	2	15.96	<0.0001
	Time	7	72.44	<0.0001
	pH × Time	14	4.76	<0.0001
BRL	pH	2	30.34	<0.0001
	Time	7	10.31	<0.0001
	pH × Time	14	2.93	0.0003
PLL	pH	2	25.99	<0.0001
	Time	7	102.82	<0.0001
	pH × Time	14	3.07	0.0001
POL	pH	2	13.40	<0.0001
	Time	7	152.15	<0.0001
	pH × Time	14	5.34	<0.0001
ALL	pH	2	1.04	0.36
	Time	7	0.81	0.58
	pH × Time	14	0.84	0.63
PDL	pH	2	10.51	<0.0001
	Time	7	7.82	<0.0001
	pH × Time	14	8.59	<0.0001
SI	pH	2	10.35	<0.0001
	Time	6	1.28	0.26
	pH × Time	12	1.05	0.40

meters to assess individual variation within the treatments at Days 1 and 4 (Fig. 5). At Day 1, all the larvae from the 3 pH treatments are clustered together indicating that they share similar body proportions. At Day 4, larvae raised at pH 7.7 are discriminated from those growing at pH 8.1 (all days) and pH 7.9 (Day 4). Larvae raised at pH 7.7 possessed proportions that were never observed in those raised at normal pH.

Effects on development

A high proportion of the larvae raised at low pH were either abnormal (unable to develop into normal pluteus larvae; Fig. 6A) or asymmetric (Fig. 6B). The frequency of abnormal larvae through time followed a normal distribution (Fig. 7A). The highest proportion of abnormal larvae (calculated as the maximum of the normal distribution using the method of Battacharya [1967]) was observed after 3.7 ± 0.09 d at pH 7.7 and after 4.92 ± 0.07 d at pH 7.9. Abnormalities were completely absent in the control larvae.

A significant proportion of larvae showed asymmetry at low pH (Fig. 7B): after 2 d, 25 and 32% of normal larvae (i.e. no abnormalities) were asymmetric at pH 7.9 and 7.7 respectively. These percentages decreased throughout the larval period until the last 2 d, by which time very few individuals remained in culture.

DISCUSSION

CO_2-driven acidification had a dramatic impact on survival and development of *Ophiothrix fragilis* larvae. After only 8 d, all larvae at reduced pH (7.9 and 7.7) were dead, whereas control larvae (pH 8.1) showed only 30% mortality (Fig. 1). This corresponds to a 12-fold increase in larval mortality rate, caused by CO_2-induced acidification. Even allowing for the possibility that our treatments may have elevated the sensitivity of larvae (due to stress, suboptimal feeds, laboratory conditions, etc.), our results imply that the levels of CO_2-induced acidification predicted to occur within the next 50 to 100 yr ($\Delta pH \approx -0.2$ to -0.4 units; Caldeira & Wickett 2003, 2005) could, at the very least, cause severe reductions in larval survival, and quite possibly completely eradicate *O. fragilis* populations with little potential for acclimation and/or adaptation.

If our oceans continue to acidify as expected, *Ophiothrix fragilis* larvae will not be able to escape from these deleterious conditions. Ophiopluteus larvae have low swimming capabilities (Mileikovsky 1971) and act as passive particles without diel vertical migration (Lefebvre & Davoult 1998, 2001). Adult populations show little interannual variability in density and partly act as metapopulations. While some populations are mainly self-sustaining, larval supply from neighbouring populations (larvae can disperse within 70 to 100 km by water displacement; Davoult et al. 1990) can exceed local retention in other populations (Lefebvre et al. 2003). Thus, even a local acidification event could impact *O. fragilis* populations on a wider scale.

Ophiothrix fragilis is a widely distributed species in the eastern Atlantic, from northern Norway to the Cape of Good Hope. It is a keystone and dominant species in many coastal communities (Lefebvre & Davoult 1997). It is also an essential component of the epibenthos that feed on phytoplankton and provide coupling between benthic and pelagic ecosystems in the English Channel. It can reach very high densities of up to 7000 ind. m^{-2} (Davoult 1989, Migné & Davoult 1997, Davoult & Migné 2001) forming beds of considerable physical complexity with many crevices and shelters. In some beds where *O. fragilis* represents half of the biomass, up to 78 other species have been recorded (Warner 1971). *O. fragilis* also has a dominant role in nutrient exchanges between estuarine and coastal ecosystems (Lefebvre & Davoult 1997). For example, precipitation of calcium carbonate in skeletal ossicles is a source of carbon; for the English Channel community, *O. fragilis* provides as much as 35% of the phytoplankton carbon requirement (Migné & Davoult 1997, Migné et al. 1998). Stomach contents of most common predators also show that *O. fragilis* is an important food for many species (Warner 1971). If *O. fragilis* is threat-

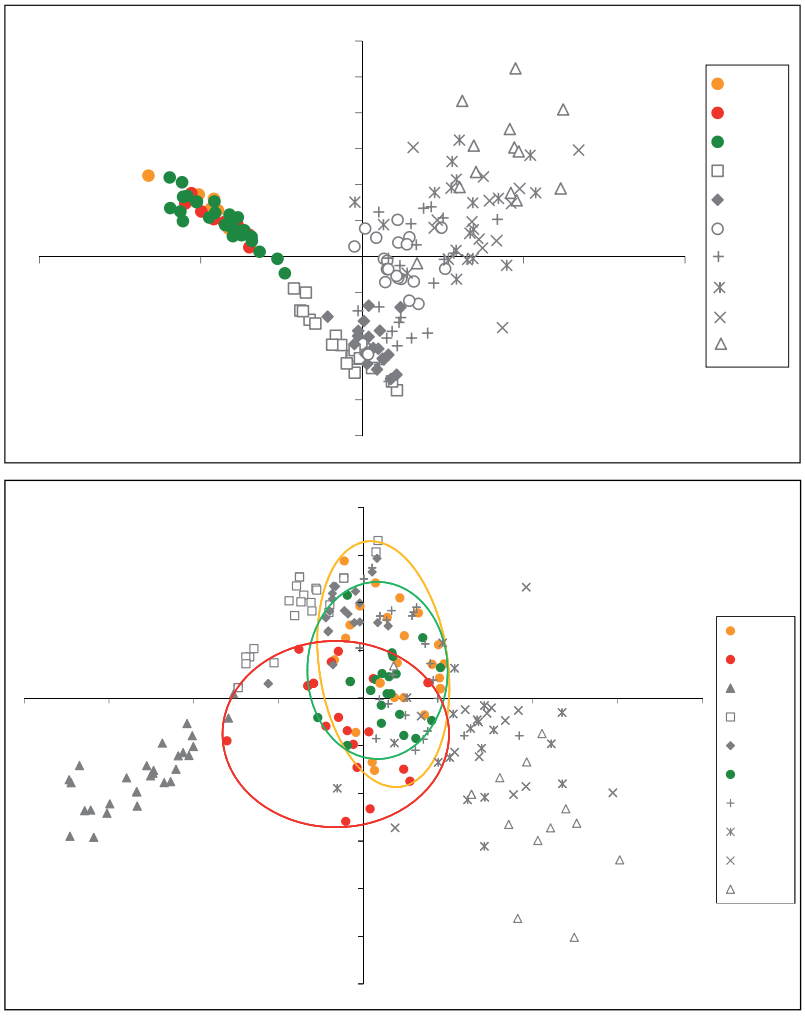

Fig. 5. *Ophiothrix fragilis.* Canonical discriminant analysis of the morphometric parameters used to separate the different pH treatments and time post fertilization (d). The data from Days 1 to 8 were used for the control pH 8.1 (8.1_1 to 8.1_8 in grey) when only the data from Day 1 (A) or Day 4 (B) were used for lower pH. (A) Day 1, no difference between the 3 treatments; (B) Day 4, larvae from pH 7.7 are discriminated from the other treatments and from larvae in the control. (A): can 1 = 0.73BL + 0.88BRL + 0.94PLL + 0.86POL + 0.8ALL + 0.3PDL; can 2 = 0.57BL + 0.31BRL – 0.01PLL + 0.48POL + 0.56ALL + 0.37PDL. (B): can 1 = 0.69BL + 0.82BRL + 0.89PLL + 0.85POL + 0.7ALL + 0.3PDL; can 2 = 0.58BL – 0.35BRL + 0.01PLL + 0.5POL + 0.54ALL + 0.41PDL

ened in the near future as suggested by our results, major changes in many key benthic and pelagic ecosystems of the Atlantic will likely occur. Thus, it may be useful to monitor *O. fragilis* populations and initiate conservation if needed.

Very few other workers have reported the impacts of CO_2-driven pH change on larval performance. The few studies available in the literature used ΔpH values much greater than those used here (Kurihara & Shirayama 2004, Kurihara et al. 2004, 2007). For example, in the oyster *Crassostrea gigas*, a reduction in pH of 0.7 units induced major morphological abnormalities in larvae (only 4 to 5% developed normally), and a significant decrease in the calcification rate (Kurihara et al. 2007). In the sea urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei*, ΔpH of −1.0 to −1.4 had significant negative impacts on fertilization rate, cleavage rate, developmental speed and larval size (Kurihara & Shirayama 2004, Kurihara et al. 2004). The high ΔpH values used in these studies correspond to much higher levels of acidification than predicted for the coming 2 centuries (Caldeira & Wickett 2005, Cao et al. 2007). Kurihara and colleagues also detected negative impacts on larvae at lower ΔpH values, although these effects were smaller possibly due to the shorter duration of their experiments (3 d, Kurihara & Shirayama 2004; 2 d, Kurihara et al. 2007). After comparable periods, our own experiments also showed nonsignificant declines in larval performance (Fig. 1). We can thus speculate statistically significant effects at smaller ΔpH values if Kurihara and co-workers had run their experiments for longer periods.

A key function of development is to put the right cells in the right places at the right time while simultaneously ensuring function and survival (Strathmann 2000). The calcite skeleton of larval brittlestars (and of echinopluteus larvae of sea urchins) has been proposed to confer several adaptive developmental benefits including maintenance of body shape (aids morphogenesis and feeding; Hörstadius 1939, Okazaki 1956, Pennington & Strathmann 1990); passive larval orientation (aids feeding and vertical migration; Pennington & Strathmann 1990); and defence against predators (Emlet 1983, but see Pennington & Strathmann 1990). Abnormal development of the skeleton would therefore be expected to have dramatic consequences for fitness, consistent with the results obtained here.

Ophiothrix fragilis larvae raised at low pH exhibited several developmental problems. A high proportion (>50% of the culture at Days 5 to 6, Fig. 7A) of abnormal larvae with none of the features of normal pluteus larvae (see Fig. 7 for examples) and a high proportion (Fig. 7B) of asymmetric larvae would result in problems with maintenance of normal larval orientation.

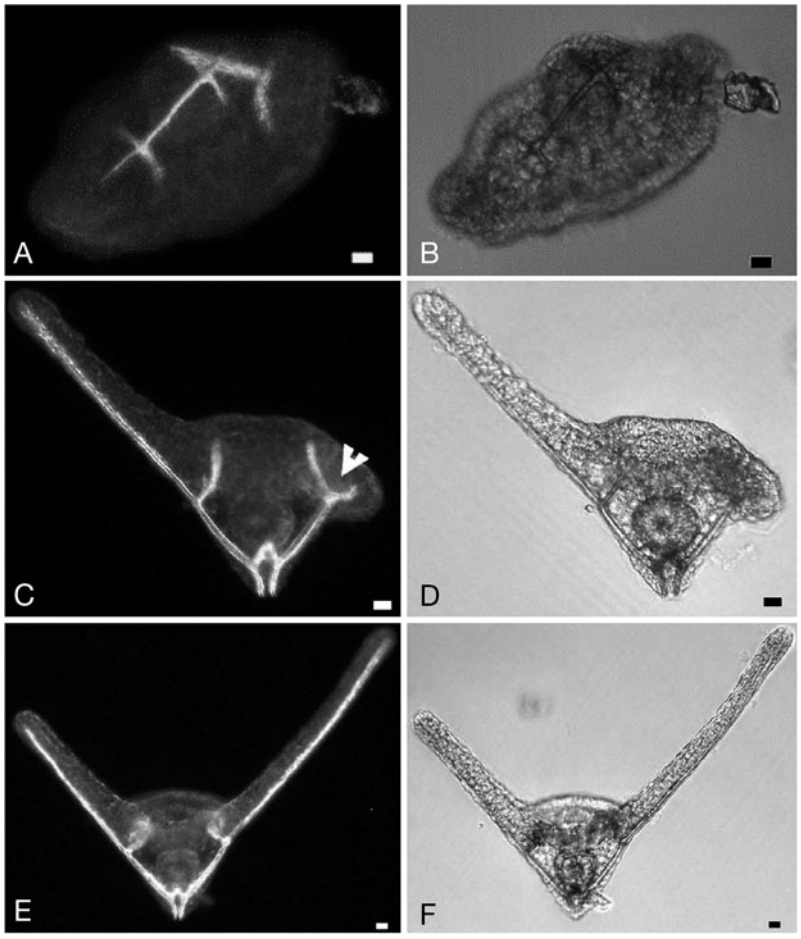

Fig. 6. *Ophiothrix fragilis.* Examples of abnormal (A,B) and asymmetric (C–F) larvae: (A,B) Day 2 larva at pH 7.7; (C,D) Day 2 pluteus at pH 7.7 with a reduced posterolateral rod (arrowhead); (E,F) Day 2 asymmetric pluteus at pH 7.9. (A,C,E) Under normal transmitted light; (B,D,F) under polarized light. Scale bars = 10 µm

Moreover, even larvae with normal shape (not abnormal or asymmetric) raised at low pH have different morphometric proportions than those raised at normal pH (Fig. 5). This may also have consequences for larval orientation and thus fitness and survival.

Our results showed marked increases in mortality in low pH treatments after 4 to 6 d (Fig. 1) — the stage at which the larvae started to feed — suggesting that mortality may be a consequence of compromised larval feeding performance at reduced pH as we observed in other species (S. Dupont & M. Thorndyke unpubl. data). Interestingly, the percentage of abnormalities decreased after Days 4 to 5 (Fig. 7), reflecting selective mortality.

It seems highly probable that pH-induced changes in skeletogenesis (abnormalities, asymmetry, morphometric changes) such as those observed here (Fig. 6) were due to the disruption of one or more molecular mechanisms involved in calcification (Livingston et al. 2006), in addition to interference with the basic chemistry of calcification. Moreover, ion transport mechanisms control asymmetry in sea urchins as they do in several vertebrate species (Hibino et al. 2006); these processes are highly sensitive to variations in pH (Mignen & Shuttleworth 2000).

Some authors have used guidelines published by the US Environmental Protection Agency to argue that a change of 0.2 pH units will be essentially unimportant for marine species (Loáiciga 2006). Even larger ΔpH ranges have been suggested to be 'environmentally safe' (Knutzen 1981). Yet few, if any, of the studies on which those conclusions were based had manipulated seawater pH (and carbonate saturation levels) by controlling pCO_2. There is still a critical shortage of environmentally relevant observations of the likely impacts of ocean acidification on marine species (Harley et al. 2006); however, current understanding of the relevant processes, in combination with experimental results (Langdon et al. 2000, Riebesell et al. 2000, Feely et al. 2004, Kurihara & Shirayama 2004, Shirayama & Thornton 2005, Berge et al. 2006, Kurihara et al. 2007, Miles et al. 2007) lends strong support to the assertion that such relatively small ranges of pH change should be considered as potentially harmful for marine biota (Caldeira & Wickett 2005).

Our data show that small changes in pH as low as the 0.2 unit decrease predicted for the coming few decades (Caldeira & Wickett 2003, 2005) can have dramatic consequences for larval development and survival of key species. Our results for the brittlestar *Ophiothrix fragilis* clearly show that such changes could threaten the long-term viability of the species. Whether other species of marine invertebrates are equally sensitive to such small pH shifts is unknown; there are no other strictly comparable data, although we argue above that the results of Kurihara & Shirayama 2004 are consistent with the results obtained here. Taxa from habitats that experience large natural pH shifts (e.g. algal bloom specialists such as planktonic copepods, or burrowing crustaceans and worms) are certainly likely to be better adapted to such changes. It has been suggested that this variability in sensitivity could have considerable implications for the diversity and functioning of communities as ocean pH declines (Royal Society 2005), placing some ecosystems more 'at risk' than others. If

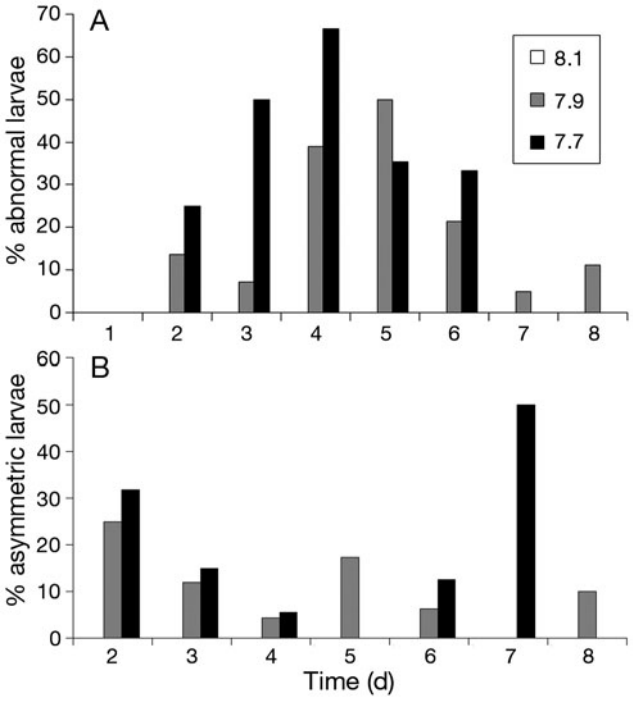

Fig. 7. *Ophiothrix fragilis.* Percentage of (A) abnormalities and (B) asymmetry in larvae raised at pH 8.1, 7.9 and 7.7 (data pooled from the different repetitions). By definition, no larva was asymmetric at pH 8.1 (controls). Note the absence of abnormalities at pH 8.1 (controls)

the pH continues to decrease as suggested by current models, we can then expect a strong selection for the more tolerant species and a major reorganisation at the ecosystem level.

We strongly echo the comments of Harley et al. (2006) that 'more research on the ecological implications of pH change is desperately needed' (Harley et al. 2006, p. 233). Experiments testing the impact of long-term exposure to small and environmentally relevant CO_2-induced decreases in pH should be conducted on other potential high-risk species such as echinoderms, molluscs and corals; more importantly, these experiments should be conducted on all life stages. Extinction does not require the instantaneous death of all individuals in a species. A decrease of as little as 1% per generation may reduce many animal populations to unsustainable densities in a little more than a century. Sublethal impacts of ocean acidification on egg production, fertilization success, larval development, larval dynamics and feeding, settlement success, metamorphic success and post-metamorphic survivorship will all influence the fitness and resilience of marine populations. Consequently, it is vital that future studies 'close the loop' by analysing the effects of acidification on all aspects of the life cycle, and over several generations, to assess acclimation, adaptive potential and adaptation of key species.

Acknowledgements. We thank Formas; the Network of Excellence, Marine Genomics Europe (GOCE-04-505403); the Royal Swedish Academy of Sciences; Göteborg University GRIP platform; K. & A. Wallenbergs Stiftelsen, BAS Q4 BIOREACH/BIOFLAME core programmes and Linnéstöd och Berzelius Centre Grant to Göteborg University, 'ACME' (Adaptation to changing marine environments) for financial support. We also thank B. Petersson and K. Alexandersson, expert skippers of RV 'Arne Tiselius' and RV 'Oscar von Sydow'; and P. Andersson from SMHI for the data on pH in Gullmarsford.

LITERATURE CITED

Ambrose WG, Clogh LM, Tilney PR, Beer L (2001) Role of echinoderms in benthic remineralisation in the Chukchi Sea. Mar Biol 139:937–949

Andersson AJ, Mackenzie FT, Lerman A (2005) Coastal ocean and carbonate systems in the high CO_2 world of the anthropocene. Am J Sci 305:875–918

Baden SP, Pihl L, Rosenberg R (1990) Effects of oxygen depletion on the ecology, blood physiology and fishery of the Norway lobster *Nephrops norvegicus.* Mar Ecol Prog Ser 67:141–155

Ball BJ, Costelloe J, Könnecker G, Keegan BF (1995) The rocky subtidal assemblages of Kinsale Harbour (south coast of Ireland). In: Eleftheriou A, Ansell AD, Smith CJ (eds) Proc 28th Eur Mar Biol Symp. Olsen & Olsen, Fredensborg, p 293–302

Berge JA, Bjerkeng B, Pettersen O, Schaanning MT, Oxnevad S (2006) Effects of increased seawater concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L. Chemosphere 62:681–687

Bhattacharya CG (1967) A simple method of resolution of a distribution into Gaussian components. Biometrics 23:115–135

Caldeira K, Wickett ME (2003) Oceanography: anthropogenic carbon and ocean pH. Nature 425:365

Caldeira K, Wickett ME (2005) Ocean model prediction of chemistry changes from carbon dioxide emission to the atmosphere and ocean. Geophys Res Lett 110, C09S04, doi:10.1029/2004JC002671

Cao L, Caldeira K, Jain AK (2007) Effects of carbon dioxide and climate change on ocean acidification and carbonate mineral saturation. Geophys Res Lett 34, L05607, doi: 10.1029/2006GL028605

Davoult D (1989) Structure démographique et production de la population d'*Ophiothrix fragilis* (Abildgaard) du détroit du Pas de Calais (France). Vie Mar 10:116–127

Davoult D, Migné A (2001) Respiration and excretion of a dense *Ophiothrix fragilis* population in the Bay of Seine (English Channel, France). In: Barker M (ed) Echinoderms 2000. Swets & Zeitlinger, Lisse, p 243–248

Davoult D, Gounin F, Richard A (1990) Dynamique et reproduction de la population d'*Ophiothrix fragilis* (Abildgaard) du détroit du Pas de Calais (Manche orientale). J Exp Mar Biol Ecol 138:201–216

Doney SC, Mahowald N, Lima I, Feely RA, Mackenzie FT, Lamarque JF, Rasch PJ (2007) Impact of anthropogenic atmospheric nitrogen and sulfur deposition on ocean acidification and the inorganic carbon system. Proc Natl Acad Sci USA 104:14580–14585

Duineveld GCA, Noort GJV (1986) Observations on the population dynamics of *Amphiura filiformis* (Ophiuroidea: Echinodermata) in the southern North Sea and its exploitation by the dab *Limanda limanda.* Neth J Sea Res 20:85–94

Emlet RB (1982) Echinoderm calcite: a mechanical analysis from larval spicules. Biol Bull 163:264–275

Estes JA, Palmisano JF (1974) Sea otters: their role in structuring nearshore communities. Science 185:1058–1060

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) The impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305:362–366

George CL, Warwick RM (1985) Annual macrofauna production in a hard-bottom reef community. J Mar Biol Assoc UK 65:713–735

Harley CDG, Hughes AR, Hultgren KM, Miner BJ and others (2006) The impact of climate change in coastal marine systems. Ecol Lett 9:228–241

Hibino T, Ishii Y, Levin M, Nishino A (2006) Ion flow regulate left-right asymmetry in sea urchin development. Dev Genes Evol 216:265–276

Hörstadius S (1939) The mechanics of sea urchin development, studied by operative methods. Biol Rev Camb Phil Soc 14:132–179

Kleypas JA, Feely RA, Fabry VJ, Langdon C, Sabine CL, Robbins LL (2006) Impact of ocean acidification on coral reefs and other marine calcifiers: a guide for future research, report of a workshop held 18–20 April 2005, St. Petersburg, FL, sponsored by NSF, NOAA, and the US Geological Survey

Knutzen J (1981) Effects of decreased pH on marine organisms. Mar Pollut Bull 12:25–29

Kurihara H, Ishimatsu A (2008) Effects of high-CO_2 seawater on the copepod *Acartia tsuensi* through all life stages and subsequent generations. Mar Pollut Bull 56:1086–1090

Kurihara H, Shirayama Y (2004) Effects of increased atmospheric CO_2 on sea urchin early development. Mar Ecol Prog Ser 274:161–169

Kurihara H, Shimode S, Shirayama Y (2004) Sub-lethal effects of elevated concentration of CO_2 on planktonic copepods and sea urchins. J Oceanogr 60:743–750

Kurihara H, Kato S, Ishimatsu A (2007) Effects of increased seawater pCO_2 on early development of the oyster *Crassotrea gigas.* Aquat Biol 1:91–98

Langdon C, Takahashi T, Sweeney C, Chipman D and others (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. Global Biogeochem Cycles 14:639–654

Lefebvre A, Davoult D (1997) Recrutement d'*Ophiothrix fragilis* (Echinoderme: ophiuride) en Manche orientale: Etude biométrique. J Res Oceanogr 22:109–116

Lefebvre A, Davoult D (1998) Vertical distribution of the ophioplutei of *Ophiothrix fragilis* (Echinodermata: Ophiuroidea) in the Dover Strait (eastern English Channel). In: Candia MD and Bonasoro F (eds) Echinoderm research. Balkema, Rotterdam, p 505–509

Lefebvre A, Davoult D (2000) Larval distribution of *Ophiothrix fragilis* (Echinodermata: Ophiuroidea) in a macrotidal area, the Dover strait (eastern English Channel, France). J Mar Biol Assoc UK 80:567–568

Lefebvre A, Davoult D (2001) Horizontal distribution of *Ophiothrix fragilis* planktonic larvae associated with a tidal front in the open coastal sea. In: Barker M (ed) Echinoderms 2000. Swets & Zeitlinger, Lisse, p 293–297

Lefebvre A, Davoult D, Gentil F, Janquin MA (1999) Spatiotemporal variability in the gonad growth of *Ophiothrix fragilis* (Echinodermata: Ophiuroidea) in the English Channel and estimation of carbon and nitrogen output toward the pelagic system. Hydrobiologia 414:25–34

Lefebvre A, Ellien C, Davoult D, Thiébault E, Salomon JC (2003) Pelagic dispersal of the brittlestar *Ophiothrix fragilis* larvae in a megatidal area (English Channel, France) examined using an advection/diffusion model. Estuar Coast Shelf Sci 57:421–433

Livingston BT, Killian CE, Wilt F, Cameron A and others (2006) A genome-wide analysis of biomineralization-related proteins in the sea urchin *Strongylocentrotus purpuratus.* Dev Biol 300:335–348

Loáiciga HA (2006) Modern-age buildup of CO_2 and its effects on seawater acidity and salinity. Geophys Res Lett 33, L10605, doi:10.1029/2006GL026305

MacBride EW (1907) Development of *Ophiothrix fragilis.* Q J Microsc Sci 51:557–606

Mattson S (1992) Food and feeding habits of fish species over a sublittoral bottom in the northeast Atlantic, 3. Haddock *Melanogrammus aeglefinus* (L.) (Gadidae). Sarsia 77: 33–45

Migné A, Davoult D (1997) Carbon dioxide production and metabolic parameters in the ophiuroid *Ophiothrix fragilis.* Mar Biol 127:699–704

Migné A, Davoult D, Gattuso JP (1998) Calcium carbonate production of a dense population of the brittlestar *Ophiothrix fragilis* (Echinodermata: Ophiuroidea): role of the carbon cycle of a temperate coastal ecosystem. Mar Ecol Prog Ser 173:305–308

Mignen O, Shuttleworth TJ (2000) I_{ARC}, a novel arachidonate-regulated, noncapacitative Ca^{2+} entry channel. J Biol Chem 275:9114–9119

Mileikovsky SA (1971) Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Mar Biol 10:193–213

Miles H, Widdicombe S, Spicer JI, Hall-Spencer J (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris.* Mar Pollut Bull 54:89–96

Morgan R, Jangoux M (2005) Larval morphometrics and influence of adults on settlement in the gregarious ophiuroid *Ophiothrix fragilis* (Echinodermata). Biol Bull 208:92–99

Okazaki K (1956) Skeleton formation of sea urchin larvae, I. Effect of Ca concentration of the medium. Biol Bull 110:320–333

Paine RT (1966) Food web complexity and species diversity. Am Nat 100:65–75

Pennington JT, Strathmann RR (1990) Consequences of the calcite skeletons of planktonic echinoderm larvae for orientation, swimming, and shape. Biol Bull 179:121–133

Politi Y, Arod T, Klein E, Weiner S, Addadi L (2004) Sea urchin spine calcite forms via a transient amorphous calcite carbonate phase. Science 306:1161–1164

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature 407: 364–367

Royal Society (2005) Ocean acidification due to increasing atmospheric carbon dioxide. Policy Document 12/05, The Royal Society, London

Sarazin G, Michard G, Prevot F (1999) A rapid and accurate spectroscopic method for alkalinity measurement in seawater samples. Water Res 33:290–294

SAS Institute (1990) SAS/STAT user's guide, version 6, 4th edn. SAS Institute, Cary, NC

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality. Biometrika 52:591–599

Shirayama Y, Thornton H (2005) Effect of increased atmospheric CO_2 on shallow water marine benthos. J Geophys Res 110, C09S08, doi:10.1029/2004JC002618

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. Freeman, San Francisco

Strathmann RR (2000) Functional design in the evolution of embryos and larvae. Cell Dev Biol 11:395–402

Warner GF (1971) On the ecology of a dense bed of the brittlestar *Ophiothrix fragilis.* J Mar Biol Assoc UK 55:199–210

Submitted: November 27, 2007; Accepted: October 26, 2008 *Proofs received from author(s): December 15, 2008*