Polar Biol (2012) 35:455–461
DOI 10.1007/s00300-011-1074-2

SHORT NOTE

# Sea urchin *Arbacia dufresnei* (Blainville 1825) larvae response to ocean acidification

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Received: 16 December 2010 / Revised: 13 July 2011 / Accepted: 15 July 2011 / Published online: 31 July 2011


**Abstract** Increased atmospheric $CO_2$ emissions are inducing changes in seawater carbon chemistry, lowering its pH, decreasing carbonate ion availability and reducing calcium carbonate saturation state. This phenomenon, known as ocean acidification, is happening at a faster rate in cold regions, i.e., polar and sub-polar waters. The larval development of *Arbacia dufresnei* from a sub-Antarctic population was studied at high (8.0), medium (7.7) and low (7.4) pH waters. The results show that the offspring from sub-Antarctic populations of *A. dufresnei* are susceptible to a development delay at low pH, with no significant increase in abnormal forms. Larvae were isometric between pH treatments. Even at calcium carbonate ($CaCO_3$) saturation states (of both calcite and aragonite, used as proxies of the magnesium calcite) <1, skeleton deposition occurred. Polar and sub-polar sea urchin larvae can show a certain degree of resilience to acidification, also emphasizing *A. dufresnei* potential to poleward migrate and further colonize southern regions.

**Keywords** Ocean acidification · Sea urchin · Sub-Antarctic population · *Arbacia dufresnei* · Larvae

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## Introduction

Increased atmospheric $CO_2$ emissions are inducing changes in seawater carbon chemistry, lowering its pH, a phenomenon known as ocean acidification. The average surface seawater pH has reduced by approximately 0.1 units since the industrial revolution and future reductions are expected to be around 0.3–0.5 units by 2100 and 0.7–0.8 units or more by 2300 (Caldeira and Wickett 2003, 2005; IPCC 2007). The result is a decrease in the concentration of the carbonate ion ($CO_3^{2-}$) that reduces the saturation state of calcium carbonate ($\Omega_{CaCO3}$) minerals (Feely et al. 2004; Orr et al. 2005). These shifts in seawater chemical and pH levels are occurring at a rate not experienced by marine organisms in the last 20 or more millions of years (Turley et al. 2006; Ridgwell and Schmidt 2010), challenging the potential of species to acclimatize and/or to adapt. Ocean acidification is happening at a faster rate in high-latitude regions such as Antarctica, as a result of a higher $CO_2$ solubility due to cold temperatures and because of $CO_2$ enriched waters supplied by an active upwelling system (Feely et al. 2004; Fabry et al. 2009). In fact, most models of ocean-carbon cycles predict that the shallowing of the $CaCO_3$ saturation horizons will be more significant and will occur earlier at higher latitudes, such as in polar and sub-polar waters (Feely et al. 2004; Orr et al. 2005; Andersson et al. 2008; Fabry et al. 2009).

These changes are believed to affect several physiological functions of marine organisms, namely growth, reproduction and behaviour (Pörtner 2008). A reduction in calcification rates has broadly been reported and associated with a decrease in $CO_3^{2-}$ availability and consequent low $CaCO_3$ saturation states (Fabry et al. 2009; Hofmann et al. 2010; Hofmann and Todgham 2010). Furthermore, early developmental stages of marine invertebrates can be

particularly sensitive to environmental stresses, such as ocean acidification (Pörtner and Farrell 2008; Dupont et al. 2010). Echinoids could be vulnerable to seawater acidification due to the nature of their magnesium calcite (Ca–Mg–$CO_3$) skeleton, the solubility of which is similar or higher than that of aragonite (Morse and Mackenzie 1990; Andersson et al. 2008). Indeed, the development of early stages of some sea urchin species has revealed vulnerabilities when raised in lower pH seawaters such as decreased cleavage rates, a reduction in the pluteus larva size and/or a delay in their development (Kurihara and Shirayama 2004; Havenhand et al. 2008; Kurihara 2008; Clark et al. 2009; Dupont et al. 2010; Sheppard Brennand et al. 2010; Moulin et al. 2011). Natural selection can play an important role in defining species responses as some echinoids coming from contrasting environments present distinct larval development when reared at low pH waters, possibly due to acclimatization (phenotypic plasticity at an individual level) and/or adaptation (linked to genetic variability at a population level) (Clark et al. 2009; Moulin et al. 2011). Early stages of *Heliocidaris erythrogramma* seem not to be affected by a pH decrease (until 7.6), but rather by temperature (Byrne et al. 2009a, b). The only Antarctic species investigated in this matter, *Sterechinus neumayeri*, (Clark et al. 2009; Ericson et al. 2010) is thought to be more robust to ocean acidification than tropical and temperate sub-tidal species, but less than a temperate one (Clark et al. 2009; Moulin et al. 2011). Overall, the response of echinoid early life-history stages appears to be highly species-specific (Dupont et al. 2010; Moulin et al. 2011). More studies on different species will, therefore, help to clarify the response of polar and subpolar sea urchin larvae in an acidified ocean.

The sea urchin *Arbacia dufresnei* can be found from Argentina (Atlantic 35°S) and Chile (Pacific 42°S) to the southern tip of South America and in the Antarctic Peninsula (southern limit 65°S, 64°W) (Bernasconi 1953; David et al. 2005; Mutschke and Ríos 2006). Adults are highly mobile and inhabit kelp forests and rocky shores from the surface to 300 m (Bernasconi 1953; Jara and Céspedes 1994; David et al. 2005). This species has an important role in its habitats: it regulates other species distributions, not only because of its voracious grazing behaviour but also due to a significant carnivorous activity (Vasquez et al. 1984; Jara and Céspedes 1994; Penchaszadeh and Lawrence 1999; Zaixso 2004). However, no information is available on the impact of low pH on this species. Consequently, the goal of the present work was to increase the knowledge on the effects of ocean acidification on the early development of this high-latitude sea urchin larvae, assessing this species response to low pH waters (before its exotrophic stage).

## Materials and methods

Adult specimens of *Arbacia dufresnei* (ambital diameter exceeding 3.5 cm) were collected in the Magellan Strait at 53°37′S, 70°56′W in Punta Santa Ana/Fuerte Bulnes (Punta Arenas, Chile) in November 2008, corresponding to this species reproduction season (Brögger et al. 2004). The Magellan Strait waters, during austral spring, have temperatures varying essentially between 6–8°C and pH between 7.8–8.0. These values are considered to be quite homogenous for the first 50 m depth (Valdenegro and Silva 2003). Animals were collected at the base of a kelp forest of *Macrocystis pyrifera* by scuba diving at around 6 m depth and transported immediately to the aquaculture facilities of the *Centro de Cultivos Marinos Bahía Laredo* (Universidad de Magallanes, Punta Arenas) until further use. Seawater supplying the aquaculture centre and used in the present experiment was pumped in from the front bay, filtered and re-filtered (0.22 μm) for this experiment. The mean $pH_T$ (pH in total scale), measured between October and December 2008, was ca. 8.0, while the mean temperature was ca. 9.3°C and salinity was 30.7 (Catarino, unpublished data).

Adults were injected with 1–1.5 ml 0.5 M KCl into their perivisceral cavity to induce spawning. Eggs were obtained from 5 females and checked to ensure that these had a normal round shape. Sperm was obtained from 5 males. Gametes of the same sex were gently mixed in order to have a homogeneous batch and to average individual variations. Eggs were suspended as a homogeneous layer in a Petri dish containing natural ambient seawater (filtered at 0.22 μm, 8.5°C, $pH_T$ 7.95), and a drop of diluted sperm was added. Around 2 h later, the presence of the fertilization membrane was checked. The fertilization rate was higher than 95%. Four hours after insemination, replicates of around 30 embryos $ml^{-1}$, at the 1st cleavage stage, were transferred into three 125 ml vials, filled with filtered seawater at each selected nominal pH: high 8.0, medium 7.7 and low 7.4 (3 replicates per pH). Medium and low pH were adjusted by bubbling $CO_2$ (AGA, Sweden) in previously highly aerated seawater with a salinity of 30.7. Vials were completely filled to avoid air spaces, preventing gas exchanges and placed in a bath of running water at constant temperature (9.6°C ± 0.48, $n = 5$). These values corresponded to field conditions, i.e., temperatures that vary between 6–8°C and salinity between 30.0–32.5 (Valdenegro and Silva 2003). Larvae were reared for 5 days in the dark (to prevent algal growth) until early pluteus stage, with most larvae in high pH treatments having 2 arms. One of the high pH replicates had a drastic pH drop at the end of the experiment (data not shown), and its results were not included in the analysis.

Larvae were fixed in ethanol 70% (v/v). One hundred pluteus larvae were observed in each replicate using an optical microscope and the number of normal, abnormal and larvae with delayed development was recorded. The morphological criteria to evaluate larvae were adapted from Warnau and Pagano (1994): abnormality was considered in the presence of abnormal arm shape, complete abnormal shape or when embryos were unable to differentiate, i.e., arrested development at gastrula or blastula stage. Normal larvae (Brögger et al. 2004) were distinguished into pluteus (2 and 4 arms) and prism stages.

Twenty-five larvae in each replicate were observed in a phase contrast inverted microscope and photographed using a digital camera (QImaging, Micropublisher, software Qcapture, Canada). Morphometric measurements were performed as described by Lamare and Barker (1999). The postoral arm length (PL), i.e., the linear distance between the tip of the postoral arm and its base, and the overall length (OL), that is the linear distance between the tip of the postoral arm and the ventral end of the larval body, were measured using the freeware ImageJ (NIH, USA) (see Lamare and Barker 1999 and Kurihara and Shirayama 2004 for measurements illustration).

Physicochemical parameters were measured at the beginning and at the end of the experiment: temperature, salinity and $pH_{NIST}$ (National Institute of Standards and Technology). The temperature and $pH_{NIST}$ were measured using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor) calibrated with $pH_{NIST}$ buffers 4 and 7 (Merck CertiPUR®, Germany). The electromotive force (e.m.f.) values were further measured and applied to the calculation of the pH expressed in total scale using standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS), (DOE 1994; Del Valls and Dickson 1998; Dickson et al. 2007). The salinity was measured using a conductivity meter pH/Cond 340i WTW (USA). Seawater samples were collected at the beginning and end of the experiment, immediately filtered (0.22 μm) and used to determine total alkalinity (TA). This was carried out by a potentiometric titration with HCl 0.1 M using a Titrino 718 STAT Metrohm (Switzerland) and calculated using the Gran function (Gran 1952). Our measurements had a deviation of 0.65% of the standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control laboratory. Aragonite and calcite saturation values ($\Omega_{ar}$ and $\Omega_{cal}$, respectively) and $pCO_2$ were determined from TA, $pH_T$, temperature and salinity data using the software CO2SYS (Pierrot et al. 2006) and by using the dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and from Dickson (1990) for K of $SO_4$.

To test differences in seawater pH in the vials between the beginning and the end of the experiment (5 days), a repeated measures ANOVA was done. Absolute frequencies of the different morphological types of larvae (normal, abnormal and delayed) were summed for each treatment, gathered in a contingency table and analysed using a G-Test (model II with one fixed margin and 4 degrees of freedom). The null hypothesis was the independence of variables, in this case of 'larval stage' and 'pH treatment'. The proportion of abnormal larvae (arcsine transformed) in each treatment was analysed using a one-way ANOVA, fixed factor 'pH treatment'. The PL data were analysed using a model III nested ANOVA: fixed factor 'pH treatment' and random factor 'replicate vial' nested in 'pH treatment'. The PL data were further analysed using a model III nested ANCOVA (factors as stated before and 'OL' as the covariate). A regression analysis was done for the variables 'PL' (dependent) and 'OL' (independent) for each pH treatment, and the homogeneity of slopes was tested using ANCOVA. An appropriate sum of squares was used in unbalanced factorial ANOVA, i.e., with unequal $n$ (Type III, Statistica©), as high pH treatment had only 2 replicates, while the others had 3. The level of significance $\alpha$ was set at 0.05 for all tests.

## Results and discussion

The pH of seawater in which larvae were raised decreased between the beginning and the end of the experiment ($F = 808$, df $= 2$, $P_{ANOVA} < 10^{-6}$, Table 1) since water was not changed and there was $CO_2$ production by larvae, bacteria and other biotics in the vials. However, all 3 tested pH conditions (high, medium and low pH) remained different between each other ($P_{Tukey} < 2.9 \times 10^{-4}$). Furthermore, the observed differences between the mean starting pH and the final one, 0.17, 0.13 and 0.05 for high, medium and low pH, respectively, were similar to those reported in previous works where sea urchin larval development was studied (Kurihara and Shirayama 2004; Moulin et al. 2011).

After 5 days, most *Arbacia dufresnei* echinopluteus were at 2 arms and the few larvae where the second pair of arms was beginning to develop were only observed in high pH replicates. This larval growth rate is slightly slower than that reported for *A. dufresnei* Argentinean specimens reared at 11–13°C (adults collected at 42°46′S; 65°02′W) (Brögger et al. 2004), most likely due to temperature differences.

The proportion of the different morphological categories differed according to the pH ($G = 35.66$, df $= 4$, $P = 3.40 \times 10^{-7}$; Table 2). Nevertheless, the proportion of abnormal larvae did not differ according to treatment

**Table 1** Seawater physicochemical parameters (mean ± SD) measured and calculated (CO2SYS) at the beginning and at the end (5 days) of the larval development experiment

| Treatment pH | Initial values | | | Final values | | |
|---|---|---|---|---|---|---|
| | High pH ($n = 2$) | Medium pH ($n = 3$) | Low pH ($n = 3$) | High pH ($n = 2$) | Medium pH ($n = 3$) | Low pH ($n = 3$) |
| $pH_T$ | 7.95 | 7.69 ± 0.002 | 7.40 ± 0.006 | 7.78 | 7.56 ± 0.007 | 7.35 ± 0.036 |
| TA (μmol $kg^{-1}$) | 2.05* | 2.05* | 2.05* | 2.05 | 2.06 ± 0.007 | 2.05 ± 0.023 |
| DIC (μmol $kg^{-1}$) | 1.92 | 2.00 ± 0.001 | 2.08 ± 0.002 | 1.99 | 2.06 ± 0.009 | 2.11 ± 0.030 |
| $pCO_2$ (μatm) | 479 | 940 ± 4.4 | 1882 ± 26.2 | 722 | 1255 ± 23.7 | 2062 ± 182.3 |
| $\Omega_{Calcite}$ | 2.54 | 1.45 ± 0.006 | 0.79 ± 0.012 | 1.54 | 0.96 ± 0.014 | 0.61 ± 0.053 |
| $\Omega_{Aragonite}$ | 1.61 | 0.92 ± 0.004 | 0.50 ± 0.008 | 0.97 | 0.60 ± 0.009 | 0.38 ± 0.033 |

Effective ($n$) was of 2 or 3, with the exception of the initial TA values (*), where sampling was done from the original seawater batch. Temperature was of 9.6°C ± 0.49 ($n = 5$), and salinity was constant at 30.7

**Table 2** Percentage (mean ± SD) of pluteus, prism and abnormal larvae ($n = 200$ for high pH, $n = 300$ for each of the other treatments) and postoral arm length (PL; mean ± SD; $n = 50$ for high pH, $n = 75$ for each of the other treatments) observed in each treatment

| pH treatment | High pH | Medium pH | Low pH |
|---|---|---|---|
| Pluteus (%) | 66 ± 3.5 | 46 ± 3.5 | 44 ± 12.7 |
| Prism (%) | 9 ± 3.5 | 25 ± 6.0 | 21 ± 7.1 |
| Abnormal (%) | 26 ± 7.1 | 29 ± 5.2 | 35 ± 16.0 |
| PL size (mm) | $0.11^{a}$ ± 0.017 | $0.086^{a,b}$ ± 0.025 | $0.067^{b}$ ± 0.015 |

The PL data with different superscripts differed significantly ($P_{Tukey} < 0.05$)

($F = 0.5$, df = 2, $P_{ANOVA} = 0.66$). So, lower pH induced a delay in development, but did not increase abnormality. The proportion of abnormal larvae was rather high. However, this does not seem to have been due to oxygen reduction in the vials. For instance, Marsh et al. (1999) reported maximum oxygen consumption rates per individual of *Sterechinus neumayeri* larvae, before feeding stage, of 17 pmol $O_2$ $h^{-1}$ (overall length 0.35 mm). In a worst-case scenario, had embryos and larvae consumed oxygen at these maximal rates during 5 days and at the same larval density as in our study (30 larvae $ml^{-1}$), this would only have had represented a reduction until 83% of oxygen saturation in experimental seawater. However, individual oxygen consumption rate increases with the age of the embryo and larvae and that earlier stages uptake oxygen at lower rates (Marsh et al. 1999). Taking this example into account, oxygen was most likely not restrictive in the present work and it did not constrain larval development. Indeed, by the end the experiment, *A. dufresnei* larvae were actively swimming (personal observation) and developmental arrest occurred at a time where $O_2$ would not have been limiting. The cause for the proportion of abnormal embryos/larvae was probably related to the natural variability of the mature state of the gametes within or among progenitors that can have influenced development (Brögger 2005; Kino and Agatsuma 2007).

Postoral arm length (PL) was significantly smaller at lower pH ($F = 16.5$, df = 2, $P_{ANOVA} = 0.0063$) (Table 2), with larvae exposed to the low pH significantly differing from those observed at high pH ($P_{Tukey} = 0.006$). The mean PL had a ~20% size reduction when early stages were raised at medium pH, compared to those raised at high pH values, and ~40% when raised at low pH. This is in accordance with increasing evidence that ocean acidification slows down growth of sea urchin larvae, affecting their development rates (Dupont et al. 2010). The overall length (OL) observed values in high pH treatments (Fig. 1) correspond to normal development sizes previously reported for *A. dufresnei* echinopluteus at 2/4 arms stage (0.19–0.34 mm) by Brögger et al. (2004) (larvae raised at 11–13°C during 4–5 days).

The PL was strongly related to the OL in all treatments (Fig. 1), and even though the larvae at medium and low pH were smaller than the high pH treatments, their size was always isometric as the slopes did not differ significantly for the 3 regressions ($P_{pH*OL} = 0.3$, Fig. 1). When taking OL into account, the pH effect on PL was no longer evident ($P_{ANCOVA\ model\ III} = 0.7$). One of the most frequently stated impacts of reduced larval size caused by ocean acidification is a decrease in feeding activity due to reduction in arm ciliated band, i.e., the food capture apparatus (Kurihara and Shirayama 2004; Kurihara 2008; Sheppard Brennand et al. 2010). Longer arms/ciliated bands increase food uptake (Hart and Strathmann 1994; Miner 2005; Soars et al. 2009). However, as larvae are facing an isometric growth delay, it is expected that their

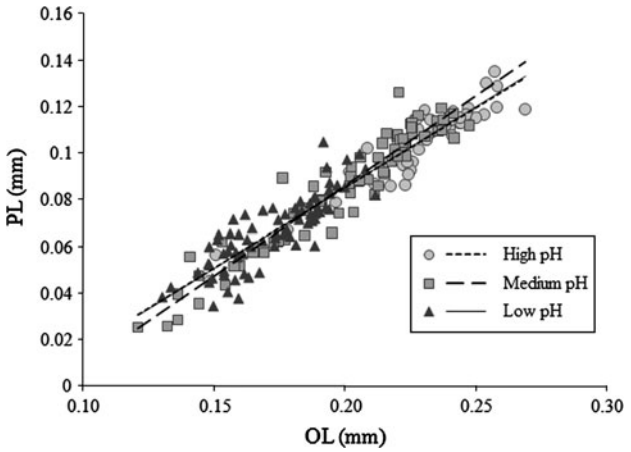


**Fig. 1** Postoral arm length (PL) according to overall larva length (OL) in all 3 pH treatments (total $n = 225$). Linear model equations were for high pH: $y = 0.69x - 0.52$, $R^2 = 0.86$; medium pH: $y = 0.77x - 0.69$, $R^2 = 0.91$ and low pH: $y = 0.69x - 0.53$, $R^2 = 0.70$; all $P < 10^{-6}$. Slopes were homogeneous ($P_{pH*OL} = 0.3$)

relative foraging capacity will not be globally affected. Isometric size reduction per se should not result in an insufficient food gathering. No studies have yet reported if or how food acquisition by echinoid larvae will respond to ocean acidification (for a review see Dupont et al. 2010), and so, it is not yet known whether a smaller size can be linked to an altered feeding behaviour (e.g. due to modified ciliary activity). Furthermore, pH effects on physiological processes starting with feeding are also not yet investigated.

Size reduction can be a result of a combination of factors, such as developmental delay and metabolic depression. Nevertheless, depending on the intensity and/or variability of the effect, it can have serious consequences by increasing vulnerability to predation and possibly reducing recruitment success (Pedrotti and Fenaux 1992; Pedrotti 1993; Balch and Scheibling 2001). In marine invertebrates with complex life cycles, larval experience can also affect juvenile performance due to latent effects, i.e., events that are experienced in one stage but are only manifested later in the life cycle, such as a larval size reduction or a metamorphosis delay (Pechenik 2006; Allen and Marshall 2010; Giménez 2010). Juvenile mortality in benthic marine invertebrates is known to be high, and the success of the first days of the benthic life can partly depend on the history of the premetamorphic larvae (Gosselin and Qian 1997; Vaïtilingon et al. 2001).

Although existing literature often emphasizes the impact of ocean acidification on calcification, this is just one of the physiological functions that can potentially be affected (Pörtner 2008). Calcification in echinoids occurs in a calcium carbonate supersaturated closed compartment, the calcification vacuole (Dubois and Chen 1989). In addition, the carbonate ($CO_3^{2-}$) ion, essential for calcification and considered to be problematic due to its reduced availability caused by ocean acidification, hardly ever crosses biological membranes, entering cells via $CO_2$ diffusion or by bicarbonate ($HCO_3^-$) transport (Hofmann and Todgham 2010). In the present study, even if rod size decreased, it is noteworthy that at $pCO_2$ values as high as ~2,000 µatm corresponding to saturation states (Ω) of both calcite and aragonite lower than 1 and, therefore, also of magnesium calcite, skeleton deposition still occurred in *A. dufresnei* larvae. The same was observed in other echinoid species whose larvae were raised in very low pH (for reviews see Kurihara 2008; Dupont et al. 2010; Moulin et al. 2011). Thus, the influence of seawater physicochemistry in calcification, if existing, will instead be an indirect one, most likely due to low acid–base regulation and/or metabolic depression (Pörtner 2008; Hofmann and Todgham 2010).

The larval size decrease was very significant at low pH (7.4). This is a higher pH than that reported for other species and lower than that for *S. neumayeri* (7.6) (Clark et al. 2009; Moulin et al. 2011). Furthermore, larval morphology was not significantly affected at low pH (7.4), a pH at which temperate and tropical species showed a higher proportion of abnormal larvae (Kurihara and Shirayama 2004; Kurihara and Shirayama 2004; Moulin et al. 2011). Studies have reported that the pH in the Magellan Strait can be quite low, varying between 7.8 and 8.0 during spring (Valdenegro and Silva 2003), and these populations can be either acclimatized or adapted to already low pH conditions. The present results suggest that at medium pH, approximately the surface seawater pH predicted to occur in the near future, *A. dufresnei,* just as the Antarctic sea urchin *S. neumayeri* (Clark et al. 2009; Ericson et al. 2010), would not be more sensitive than other low latitude species, reinforcing the idea that polar species might not be especially at a higher risk in front of acidification. *A. dufresnei* can clearly cross the Antarctic convergence, a natural boundary for many species distributions, an indication of the resilience of its larvae (as the adult distribution limit is 300 m depth, these are unable to cross the Drake passage), and is present in the Antarctic Peninsula (Bernasconi 1953; David et al. 2005), a place considered to be a hotspot of anthropogenic-induced changes (Barnes and Peck 2008). Even if the synergistic effects of global warming and ocean acidification on the larval development of this species are unknown, we can predict that global change will promote a shift on *A. dufresnei* distribution. Should the sea surface warming continue and extend further in the water column, this invasive potential could be amplified by breaking the physiological barrier of water temperature. Furthermore, its adult opportunistic feeding behaviour can be highly competitive, promoting a potential invasion of other Antarctic ecosystems besides the Antarctic Peninsula.

**Acknowledgments** A. I. Catarino holds a FCT grant (SFRH/BD/27947/2006, Portugal). Ph. Dubois is a Senior Research Associate of the NFSR (Belgium). Work supported by FRFC contract 2.4532.07 and Belspo contract SD/BA/02B. The authors are grateful to the Laredo Centre personnel for technical support, to E. Newcombe and C. Cardenas (INACH) for diving support and technical advice, to C. Gimpel and G. Asencio (INACH) for assistance, to P. Gosselin for technical advice. The authors are also thankful to N. Khatri, the reviewers S. Dupont and M. Thorndyke (Sven Lovén Centre for Marine Sciences) and to an anonymous reviewer for helping improving the quality of this work.

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