Argonauta at risk: dissolution and carbonate mineralogy of egg cases

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Abstract. Cephalopods are champion mineralisers. Nautilids produce robust external shells and internal mineralised tissues; *Spirula* makes an internal chambered spiral; sepiids produce flat “cuttlebone;” some squids and octopus produce beaks and statoliths. Most cephalopod carbonate is aragonite, but one octopus is an exception: the female *Argonauta* secretes a fragile calcitic spiral egg-case. Three argonaut cases were collected in NSW, Australia. Four replicate pieces from each were immersed in seawater of varying pH: 8.1 (ambient), 7.8, 7.6, 7.4, 7.1 and 6.7. Weight loss was measured after 14 days. Dissolution rate increased with decreasing pH, with less than 1% loss in 14 days at pH 7.8, 5% loss at pH 7.4, and 20% loss at pH 6.5. Carbonate from all treatments was analysed using x-ray diffractometry, showing no significant changes in mineralogy as shells dissolved. The pelagic life-habit of these cephalopods makes them particularly vulnerable to ocean acidification. Unlike an internal skeleton, which can be protected from seawater while still needed, the *Argonauta* egg case is exposed to sea water from inception. These egg cases, unprotected by mucous or epithelium, with high surface-area and low volume, and presumably without the capacity to adjust to a less soluble carbonate mineral, are exceptionally vulnerable to dissolution as ocean pH decreases.

Key words: Argonauta, calcite, ocean acidification, Cephalopoda

Introduction

Cephalopods are champion mineralisers. Members of the family Nautilidae produce robust external shells as well as many internal mineralised tissues incorporating at least seven different biominerals (Lowenstam et al., 1984; Crick et al, 1985). Most other cephalopod mineralisation is internal and aragonitic: *Spirula* makes an internal but chambered spiral shell (Bandal & Boletzky, 1979). Sepiids produce “cuttlebone,” and various squids and octopus mineralise only their radular teeth and vestigial chitinous structures (e.g., “lateral stylets”). The genus *Argonauta* is, however, an exception; this female octopus, known as the paper nautilus, secretes a calcitic egg-case (Figure 1).

Calcification in argonauts is unique, with a delicate spiral egg case secreted by specialised glands on the dorsal arms of the female. Intermediate-Mg (mean = 5.1 wt% $MgCO_3$, SE ± 0.14; N = 9) prismatic calcite is precipitated on both the inside and outside of the shell, radiating outwards from a thin organic matrix core (Mitchell et al., 1994). Unlike most invertebrate skeletal carbonate, the argonaut egg case floats in sea-water unprotected by membranes and is presumably unable to repair or modify its mineral structure. There is considerable potential for sea- water chemistry to affect this biomineral construction, with consequent effects on the early life-stages of the species.

The uptake of anthropogenic CO_2 by global oceans is driving a world-wide lowering of ocean pH and availability of carbonate ions (e.g., Raven et al, 2005; Doney et al., 2009). The effects of this ‘ocean acidification’ on marine invertebrates are gradually becoming clear, with many calcifiers adjusting mineralogy, shell thickness, or lifestyle in response (e.g., Orr et al., 2005; Byrne, 2011). The pelagic, unprotected, calcitic egg cases of argonauts must be among the most susceptible to ocean acidification.

Here we investigate the response of carbonate in egg cases of *Argonauta nodosa* to lowered pH and any related changes in skeletal carbonate mineralogy.

Material and Methods

Argonauta nodosa egg cases were collected from Bateman’s Bay, New South Wales, Australia (35° 44’S, 150° 11’ E) in late 2010 following a mass beaching event. Three egg cases were cleaned, photographed, and sectioned into fragments of about 2 cm^2. Four weighed fragments from each egg case were immersed in each of six seawater treatments: pH 8.1 (control), 7.8, 7.6, 7.4 7.1 and 6.7, achieved by bubbling CO_2 and air into FSW and monitored throughout the experiment, at a constant temperature of 19°C. Fragments were removed, rinsed, dried and weighed after 14 days. Change in dry weight was recalculated to percentage weight loss. Three fragments from each treatment were then ground to a fine powder with 0.1 g NaCl and examined using X-ray diffractometry for carbonate minerals present using methods described in Smith et al. (1998) and calibration equations as in Gray and Smith (2004). Calcite: aragonite ratio and wt% $MgCO_3$ in calcite were calculated using peak locations and heights (after Smith et al., 2006). The remaining whole samples were examined using scanning electron microscopy.

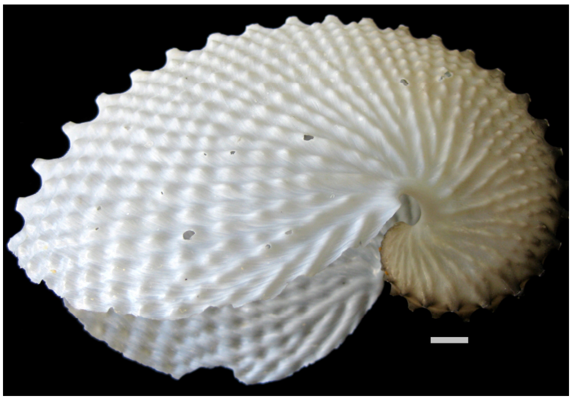

Figure 1: Calcitic egg case of *Argonauta nodosa* from Bateman's Bay, NSW, Australia. Scale bar = 1 cm.

Results

Weight loss over the two-week immersion period varied from none in the control to almost 20% in the lowest pH treatment (Table 1). Skeletal carbonate mineralogy of fragments did not vary in any significant way with pH exposure ($F_{6.25} = 0.59$, $p = 0.732$), though the lowest $MgCO_3$ content was also at the lowest pH level. Shell fragments with substantial weight loss (pH $\leq$ 7.4) showed dissolution and etching on both inner and outer surfaces.

pH	Mean % wt loss after 14 days ± SD (N = 12)	Mean wt% $MgCO_3$ in calcite ± SD (N = 3)
8.1	0.0 ± 0.6	5.0 ± 0.4
7.8	0.6 ± 1.0	5.7 ± 1.7
7.6	2.0 ± 1.5	4.7 ± 0.6
7.4	5.0 ± 3.5	5.1 ± 0.4
7.1	8.7 ± 2.8	4.9 ± 0.4
6.7	19.2 ± 7.4	4.6 ± 0.5

Table 1: Effects of immersion in controlled pH sea water on weight and mineralogy of fragments of *Argonauta nodosa* egg cases from Bateman's Bay, Australia.

Discussion

The thin egg cases produced by female argonauts are unique among the Mollusca. *Argonauta nodosa* secretes calcite using the female's dorsal arms rather than the mantle as in most molluscs. Crystals are precipitated on both the inner and outer shell surface in radiating fan-like units from a biogenic core (Mitchell *et al.*, 1994). Skeletal carbonate mineralogy, too, is unusual among cephalopods in being 100% calcite. The degree of substitution by $MgCO_3$ (normally about 5 wt% but ranging from 3.9 to 7.4 wt% $MgCO_3$) is higher than that reported for most other molluscs (Taylor & Reid, 1990; Mitchell *et al.*, 1994; Saul & Stadum, 2005). The addition of Mg to the calcite lattice reduces its stability and increases its solubility but even 7 wt% $MgCO_3$ is still less soluble than aragonite (Bertram et al., 1991).

This is the first study to examine the effects of CO_2-driven ocean acidification on an argonaut species. Shell dissolution was observed in all low-pH treatments, including those projected for the coming decades (pH 7.8-7.6 by 2100; IPCC, 2007). For isolated shells of a variety of other molluscs, significant dissolution and deterioration were noted after five weeks incubation in pH 7.4 (McClintock et al., 2009). Observable dissolution of *A. nodosa* egg cases occurred in only two weeks of immersion, suggesting deleterious consequences for this shell in a near-future ocean. In contrast to argonauts, the internal aragonitic skeletons produced by other cephalopods (e.g. in cuttlefish and squid) are protected from decreasing environmental pH while the animal is alive. Development of internal skeletons may not be impaired by ocean acidification, as shown for the cuttlefish *S. officinalis*, which is capable of unaffected calcification at sea water pH as low as 7.1 (Gutowska et al., 2008) . As a thin structure lacking a protective cover, the *A. nodosa* shell is directly bathed in seawater and is therefore particularly vulnerable to changing seawater chemistry. The fragile *A. nodosa* shell may be the most vulnerable shell to ocean acidification produced by an adult mollusc.

No significant trend in minerological change was observed in *A. nodosa* shells incubated in different acidification treatments. It may be that the low number of replicates per treatment (n=3) failed to detect any change, or, more likely, the egg cases do not change mineralogy as they dissolve. It might, however, be possible for the female argonaut to respond to lowered pH in sea water and secrete a different, more stable mineral. Or it might be too difficult for this octopus to adjust if, as has been suggested, argonauts have little control over their shell structure compared to most other molluscs (Mitchell *et al*., 1994).

Even if a female *A. nodosa* were able to modify her biomineralisation strategy, the metabolic demands of constantly maintaining the shell could reduce energy available for other processes, because biomineralisation is energetically demanding (Wood et al., 2011). Metabolic and energetic costs could limit calcification rates and the capacity of the female to control mineralisation.

The argonaut shell serves as the brood chamber where the female incubates its young in the pelagic realm (Finn & Norman, 2010). Etching and weight loss as observed here would compromise shell integrity, biomechanics and the ability to protect embryos, thereby reducing the reproductive capacity of argonauts.

The integrity of the non-living argonaut shell aside, the effects of ocean change on other life history processes such as fertilisation and development in these molluscs are also of concern. It is possible that shell-less argonauts (juveniles and minute males) may also be at risk in a changing ocean. Shell-less argonauts travel deeper into the pelagic zone, to depths of 200 m (Nixon & Young, 2003).

Argonauta nodosa has a wide geographic range throughout the Southern Ocean (Norman, 2003), where waters are among the most vulnerable to changes in pH/$p\mathrm{CO}_2$ and the undersaturation of carbonate (Orr et al., 2005; Fabry et al., 2008). Further, surface waters have been projected to change rapidly over the next century, since this is the direct site of thermal and gas exchange with the atmosphere (IPCC, 2007; Brierley and Kingsford, 2009). Since female *A. nodosa* are generally epipelagic, living between 0-50 m (Finn & Norman, 2010), they will be directly exposed to changing surface ocean conditions, and dissolution may occur in nature as recorded here (pH $\leq$7.8). On the other hand, since it has been suggested that broadly distributed species may have different "races," each adapted to cope with local stressors, some populations could show plasticity with respect to environmental change (Byrne *et al*., 2011). The wide geographic range of *A. nodosa* might include the phenotypic (acclimatisation) and genetic (adaptive) capacity for this species to persist in some regions in a changing ocean.

It is becoming increasingly important to consider the synergistic effects of stressors in ocean change research (e.g. ocean warming, acidification and hypercapnia; Byrne, 2011). Examining the effects of ocean change stressors on living *A. nodosa* in multifactor experiments would provide greater insight into how this organism will fare in a future ocean. The inverse relationship between the solubility of CaCO_3 and temperature is well known (Harper, 2000), and increased temperatures can even induce higher magnesium concentrations in some invertebrate species (Hermans et al., 2010). The effects of ocean acidification in conjunction with other ocean change stressors on the shell and various life stages of *A. nodosa* are therefore particularly important to determine.

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