

Will krill fare well under Southern Ocean acidification?

So Kawaguchi^{1,2,*}, Haruko Kurihara³, Robert King¹, Lillian Hale⁴, Thomas Berli⁴, James P. Robinson⁴, Akio Ishida⁵, Masahide Wakita^{5,6}, Patti Virtue⁴, Stephen Nicol^{1,2} and Atsushi Ishimatsu⁷

¹Australian Antarctic Division, Kingston, Tasmania 7050, Australia

²Antarctic Climate and Ecosystems Cooperative Research Centre, Sandy Bay, Hobart, Tasmania 7001, Australia

³University of the Ryukyus, Okinawa 903-0213, Japan

⁴University of Tasmania, Sandy Bay, Tasmania 7005, Australia

⁵Research Institute for Global Change, JAMSTEC, Yokosuka 237-0061, Japan

⁶Mutsu Institute for Oceanography, JAMSTEC, Mutsu 035-0022, Japan

⁷Nagasaki University, Nagasaki 851-2213, Japan

*Author for correspondence (so.kawaguchi@aad.gov.au).

Antarctic krill embryos and larvae were experimentally exposed to 380 (control), 1000 and 2000 μatm $p\text{CO}_2$ in order to assess the possible impact of ocean acidification on early development of krill. No significant effects were detected on embryonic development or larval behaviour at 1000 μatm $p\text{CO}_2$; however, at 2000 μatm $p\text{CO}_2$ development was disrupted before gastrulation in 90 per cent of embryos, and no larvae hatched successfully. Our model projections demonstrated that Southern Ocean sea water $p\text{CO}_2$ could rise up to 1400 μatm in krill's depth range under the IPCC IS92a scenario by the year 2100 (atmospheric $p\text{CO}_2$ 788 μatm). These results point out the urgent need for understanding the $p\text{CO}_2$ -response relationship for krill developmental and later stages, in order to predict the possible fate of this key species in the Southern Ocean.

Keywords: Antarctic krill; ocean acidification; early development; habitat $p\text{CO}_2$; Southern Ocean

1. INTRODUCTION

The ecosystems of the Southern Ocean are expected to be most severely affected by ocean acidification (OA) because of the higher solubilities of CO_2 and CaCO_3 in cold waters and because of regional upwelling of hypercapnic deep sea water [1,2]. Moreover, a future rise in surface water $p\text{CO}_2$ may be augmented at great depths [3], where sea water $p\text{CO}_2$ is already much higher than at the surface ([4]; figure 1). Hence, vertically migrating animals in the Southern Ocean will probably experience the most drastic changes in carbonate chemistry in future oceans. However, OA research has mainly dealt with tropical and temperate shallow-water calcifying organisms [1], and little attention has been paid to polar species [5].

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2010.0777> or via <http://rsbl.royalsocietypublishing.org>.

Received 23 August 2010

Accepted 21 September 2010

Antarctic krill (*Euphausia superba*, hereafter krill) is the key species of the Southern Ocean ecosystem, and is found in a range of water depths. Krill spawn eggs at the surface which sink to 700–1000 m before larvae hatch to swim back to the surface [6]. The post-larval vertical distribution ranges from the surface to at least 3500 m ([7]; figure 1). Thus, krill are already exposed to high CO_2 conditions at depth, which will probably become far more hypercapnic than surface waters (electronic supplementary material, S1).

The purpose of this study is to examine how elevated CO_2 conditions affect krill. We focused on early developmental stages, since larvae and juveniles are generally more vulnerable to environmental perturbations, and their survival will largely determine population abundance, distribution and community structure [8].

2. MATERIAL AND METHODS

The stock population of krill was collected from the Indian Ocean sector of the Southern Ocean between January and March in the 2005–2006 field season [9]. The krill were maintained in the Australian Antarctic Division's marine research aquarium, where they matured and spawned naturally [10].

(a) Experimental set-up

Experimental sea water was supplied from a 70 l header tank and equilibrated with air (control) or CO_2 -enriched air before being delivered to experimental jars (250 ml clear polycarbonate) containing krill eggs (see electronic supplementary material, S2). The CO_2 -enriched air was prepared with a mass flow controller (Horiba STEC SEC-E-40) and by an air valve, to regulate flow rates of pure CO_2 and atmospheric air, respectively. The $p\text{CO}_2$ levels of the CO_2 -enriched air and sea water were monitored by a CO_2 monitor (Telaire 7001) and indirectly from pH measurement (Radiometer PHM 210 pH metre), respectively. Experimental temperature was set at 0.5°C. Effluent from each jar was drained into a 70 l sump, and recirculated through a degassing unit before returning back to the header tank via a filtration and cooling system. For details, see Kawaguchi *et al.* [10]. Total alkalinity was measured through a two-stage, potentiometric, open-cell titration. The carbonate chemistry of the experimental sea water is summarized in the electronic supplementary material, S3.

(b) Hatching experiment

Fertilized eggs were obtained in January 2008 and 2009. In the 2008 experiments, three batches of eggs originating from three different females were used. Each batch was randomly distributed into experimental jars, with approximately 20–30 eggs per jar. The embryos were incubated at one of the three target CO_2 levels: control (380 μatm), medium (1000 μatm) or high (2000 μatm). In the 2009 experiments, four batches of eggs were incubated as in 2008. Hatch rates were determined for each jar after 7–10 days of spawning. The number of jars in each treatment is summarized in table 1. Embryonic stages were classified at the end of each 2009 experiment after George [11].

(c) Observation of larval swimming activity

Three of the four batches in the hatching experiments in 2009 (S2, W2 and Y2) were used for this observation. R2 was not used because of the limited capacity of the set-up. CO_2 exposure started within 1 day of spawning and continued throughout the experimental period. Larval behaviour was observed on an average of 3 days after hatching, when they were in the nauplius stage. Since almost no eggs hatched in 2000 μatm (table 1), observations were made only for the 380 and 1000 μatm groups.

(d) Statistical tests

Statistical tests were performed using SPPLUS v. 8 software.

3. RESULTS

The hatch rates of control eggs used in our experiment were highly variable (16.7–74.7%, table 1) but the range is in fact comparable to the rates in field experiments (0–89% [12,13]). There were significant

Table 1. Summary of hatch rates from all experiments conducted in this study.

| batch ID | year | CO ₂ level | <i>p</i> CO ₂ (μatm) | hatch rate (%) | <i>n</i> ^a |
|----------|------|-----------------------|---------------------------------|----------------|-----------------------|
| | | | | mean ± s.d. | |
| A1 | 2008 | control | 380 | 27.9 ± 14.5 | 12 |
| | | medium | 1000 | 32.0 ± 12.6 | 12 |
| | | high | 2000 | 0.6 ± 0.0 | 12 |
| B1 | 2008 | control | 380 | 21.0 ± 14.9 | 15 |
| | | medium | 1000 | 29.8 ± 11.4 | 9 |
| | | high | 2000 | 0.0 ± 0.0 | 9 |
| C1 | 2008 | control | 380 | 22.4 ± 12.2 | 9 |
| | | medium | 1000 | 18.6 ± 12.2 | 9 |
| | | high | 2000 | 0.0 ± 0.0 | 9 |
| R2 | 2009 | control | 380 | 39.2 ± 9.8 | 3 |
| | | medium | 1000 | 45.0 ± 10.2 | 3 |
| | | high | 2000 | 0.0 ± 0.0 | 3 |
| S2 | 2009 | control | 380 | 16.7 ± 14.4 | 3 |
| | | medium | 1000 | 23.7 ± 5.5 | 3 |
| | | high | 2000 | 0.0 ± 0.0 | 3 |
| W2 | 2009 | control | 380 | 56.1 ± 4.4 | 3 |
| | | medium | 1000 | 50.0 ± 12.5 | 3 |
| | | high | 2000 | 0.0 ± 0.0 | 3 |
| Y2 | 2009 | control | 380 | 74.7 ± 22.5 | 3 |
| | | medium | 1000 | 66.5 ± 8.6 | 3 |
| | | high | 2000 | 0.0 ± 0.0 | 3 |

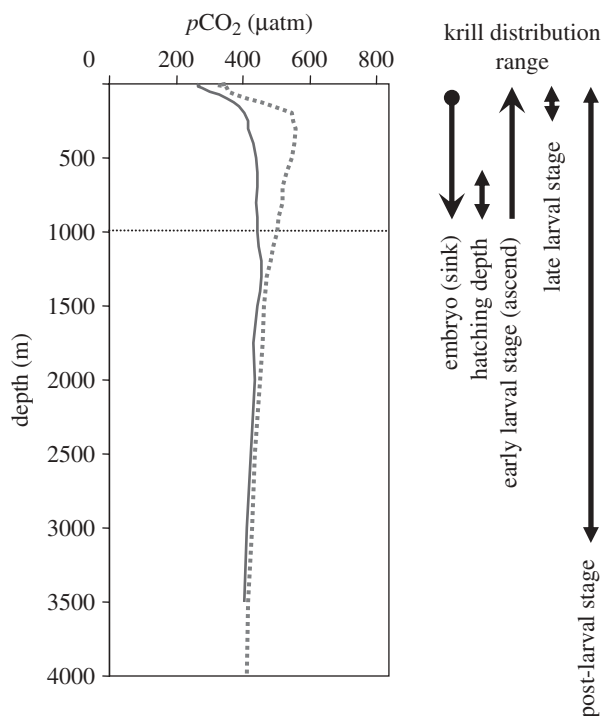
^aNumber of replicates.

Figure 1. Vertical distribution range of krill and *p*CO₂ vertical profile at Scotia Sea (59–30° S, 47–30° W; thick line) and Weddell Sea (64–30° S, 34–30° W; dotted line), the known main krill habitats around the Antarctic. The values of *p*CO₂ were calculated from DIC and TA (GLODAP: [20]), and *in situ* temperature and salinity [21] using the CO2SYS program [22] with silicate and phosphate contents set to zero. Data on krill depths from Quetin & Ross [6] (embryo); [23] (larvae); and [7] (post-larvae).

negative effects on hatch rates at 2000 μatm *p*CO₂ but not at 1000 μatm (table 2). At 2000 μatm *p*CO₂, development was disrupted by gastrulation stage in 90 per cent of embryos and no embryos survived to

hatch except one (batch A1, 0.6%) (figure 2 and table 2). Egg batch, CO₂ level and their interactions all significantly affected egg hatch rates when results from the three CO₂ levels were compared. When the data from control and 1000 μatm groups were compared, CO₂ level was not a significant factor, but the egg batch was (table 2). Neither egg batch, CO₂ level nor their interactions had a significant effect on nauplius swimming at 380 and 1000 μatm (table 2).

4. DISCUSSION

Our results demonstrated that krill embryos develop normally under a *p*CO₂ range of up to 1000 μatm but their development is almost totally inhibited at 2000 μatm (figure 2 and table 1). An important question is then whether the Southern Ocean *p*CO₂ will reach the levels detrimental to krill or not. According to model projections with a model of the Ocean Carbon Model Intercomparison Project or OCMIP-2, forced by IPCC IS92a scenario (atmospheric *p*CO₂ 788 μatm by the year 2010 [14]), the sea water *p*CO₂ is unlikely to reach 2000 μatm within this century even at depths, but may rise up to 1400 μatm (see electronic supplementary material, S1 for details). Currently, it is unclear whether CO₂ exerts its impacts on krill in a *p*CO₂-dependent manner or some threshold exists above which harmful CO₂ effects will manifest suddenly. Krill apparently have evolved a certain level of resistance to increased *p*CO₂, probably through their natural exposure from surface (380 μatm) to deep-sea *p*CO₂ levels (figure 1), as a result of evolutionary adaptation, but they might be highly vulnerable to higher *p*CO₂ levels.

The present study is merely the first step towards scientific understanding of krill's future in the era of OA and warming. The following topics are of primary

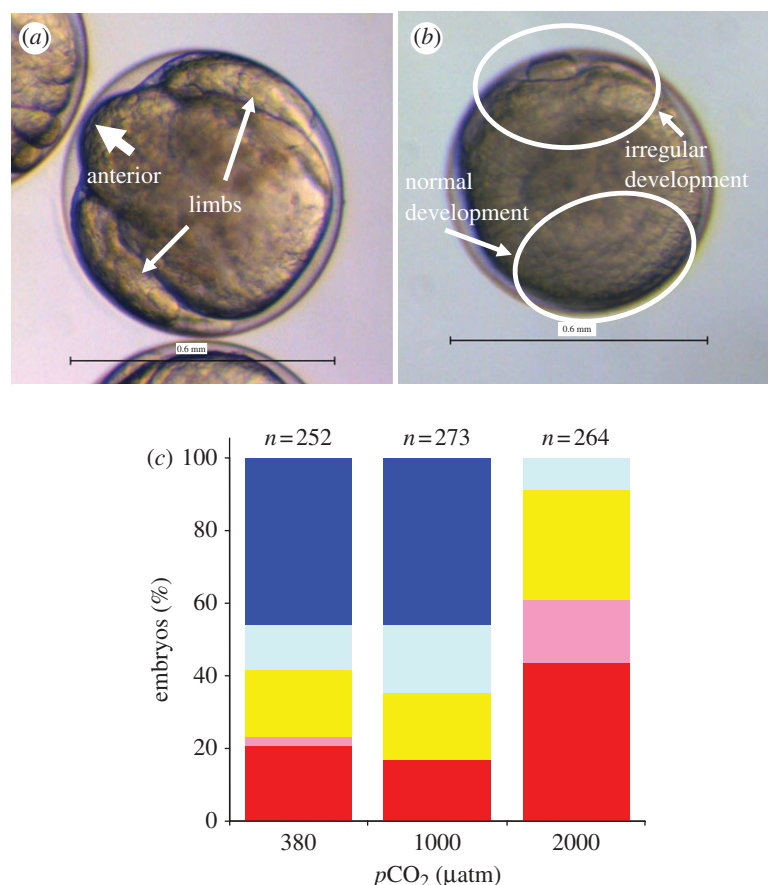


Figure 2. Effects of CO₂ on krill development. (a) An embryo reared under current surface pCO₂ developed into the limb bud stage; (b) an embryo reared at 2000 μatm pCO₂. Embryonic development was disrupted during gastrulation with a larger portion of the ectoderm appearing irregular. (c) Comparison of embryonic development under 380, 1000 and 2000 μatm pCO₂ conditions determined 7–10 days of spawning during 2009 experiments. Dark blue, hatched; light blue, limb formation; yellow, blastula–gastrula; pink, cleavage; red, no development. Scale bar, (a,b) 0.6 mm.

Table 2. Results of statistical tests (analysis of variance) assessing effects of egg batch, CO₂ level, and their interaction on hatch rates and larval behaviour.

| factors | d.f. | statistics | <i>p</i> |
|---|------|------------|----------|
| hatch rates (3CO ₂ levels: control, 1000 and 2000 μatm) | | | |
| egg batch | 6 | 14.2 | <0.00001 |
| CO ₂ level | 2 | 106.3 | <0.00001 |
| egg batch × CO ₂ level | 12 | 4.0 | <0.00001 |
| hatch rates (2CO ₂ levels: control and 1000 μatm) | | | |
| egg batch | 6 | 16.5 | <0.00001 |
| CO ₂ level | 1 | 1.9 | >0.1 |
| egg batch × CO ₂ level | 6 | 0.5 | >0.5 |
| swimming behaviour of nauplius (2CO ₂ levels: control and 1000 μatm) | | | |
| egg batch | 2 | 0.8 | >0.1 |
| CO ₂ level | 1 | 0.4 | >0.5 |
| egg batch × CO ₂ level | 2 | 0.8 | >0.1 |

importance for future studies. First, it is essential to develop a krill husbandry technique that makes larger number of krill available for laboratory investigations at different life-cycle stages. Even using the most advanced krill husbandry technique [10], the delicate nature of krill still poses a significant challenge to laboratory experiments. This has resulted in the limited number (3), and the coarse resolution

on krill's CO₂ sensitivity obtained in this study, both of which reduce our ability to predict krill's future. Second, we need to establish the finer CO₂-and-effect relationship for the pCO₂ range between 1000 and 2000 μatm, covering all developmental stages (particularly maturation stage because of its decisive influence on offspring population size). Third, the combined effects of OA and other environmental changes, such as warming, must be considered. Polar aquatic organisms are believed to live at near-stressful temperatures and could be vulnerable to warming [15]. The combined effects can be purely physiological but may also include various changes in krill habitats; e.g. reductions in sea-ice area, which have been regarded as a cause for recent declines in krill density in the South Atlantic [16], recent regional recovery of predators [17] and expanding fisheries [18] that have exerted increasing pressures on krill populations. Finally, mechanistic understanding of OA effects is also needed. Our experimental sea water at 2000 μatm pCO₂ was undersaturated with regard to calcite (see electronic supplementary material, S3), the CaCO₃ formed by crustaceans [19] and therefore the observed detrimental impacts could be brought about through undersaturation of sea water CaCO₃. Further detailed investigation should be conducted using physiological, biochemical and molecular approaches.

Another important message of the present study relates to the importance of incorporating possible temporal and spatial ranges of ocean conditions into experimental protocols of OA research. This is particularly relevant to the prediction on animals that migrate vertically in their life cycle, such as krill.

We thank Hans-Otto Pörtner, Robin Ross and Langdon Quetin and the two anonymous reviewers for their useful comments. This research was supported by the Australian Antarctic Division and by the Australian Government Co-operative Research Centres Program through the Antarctic Climate & Ecosystems Co-operative Research Centre (ACE CRC).

- 1 Doney, S. C., Fabry, V. J., Richard, A., Feely, R. A. & Kleypas, J. A. 2009 Ocean acidification: the other CO₂ problem. *Ann. Rev. Mar. Sci.* **1**, 169–192. (doi:10.1146/annurev.marine.010908.163834)
- 2 Sabine, C. L. *et al.* 2004 The oceanic sink for anthropogenic CO₂. *Science* **305**, 367–371. (doi:10.1126/science.1097403)
- 3 Brewer, P. G. & Peltzer, E. T. 2009 Limits to marine life. *Science* **324**, 347–348. (doi:10.1126/science.1170756)
- 4 Feely, R. A., Doney, S. C. & Cooley, S. R. 2009 Present conditions and future changes in a high-CO₂ world. *Oceanography* **22**, 36–47.
- 5 Fabry, V. J., McClintock, J. B., Mathis, J. T. & Grebmeier, J. M. 2009 Ocean acidification at high latitudes: the bellwether. *Oceanography* **22**, 160–171.
- 6 Quetin, L. B. & Ross, R. M. 1984 Depth distribution of developing *Euphausia superba* embryos, predicted from sinking rates. *Mar. Biol.* **79**, 47–53. (doi:10.1007/BF00404984)
- 7 Clarke, A. & Tyler, P. 2008 Adult Antarctic krill feeding at abyssal depths. *Curr. Biol.* **18**, 282–285. (doi:10.1016/j.cub.2008.01.059)
- 8 Kurihara, H. 2008 Effects of CO₂-driven ocean acidification on the early developmental stages of invertebrates. *Mar. Ecol. Prog. Ser.* **373**, 175–284. (doi:10.3354/meps07802)
- 9 Nicol, S., Meiners, K. & Raymond, B. 2010 BROKE-West, a large ecosystem survey of the South West Indian Ocean sector of the Southern Ocean 30–80° E (CCAMLR Division 58.4.2). *Deep-Sea Res. II* **57**, 693–700. (doi:10.1016/j.dsr2.2009.11.002)
- 10 Kawaguchi, S., King, R., Meijers, R., Osborn, J. E., Swadling, K. M., Ritz, D. A. & Nicol, S. 2010 An experimental aquarium for observing the schooling behaviour of Antarctic krill (*Euphausia superba*). *Deep-Sea Res. II* **57**, 683–692. (doi:10.1016/j.dsr2.2009.10.017)
- 11 George, R. Y. 1984 Ontogenetic adaptation in growth and respiration of *Euphausia superba* in relation to temperature and pressure. *J. Crustacean Biol.* **4**, 252–262.
- 12 Harrington, S. A. & Ikeda, T. 1986 Laboratory observations on spawning, brood size and egg hatchability of the Antarctic krill *Euphausia superba* from Prydz Bay, Antarctica. *Mar. Biol.* **92**, 231–235.
- 13 Kikuno, T. 1981 Spawning behaviour and early development of the Antarctic krill, *Euphausia superba* Dana, observed on board *R.V. Kaiyo Maru* in 1979/80. *Nankyoku Shiryo (Antarctic Record)* **73**, 93–102.
- 14 Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. & Miller, H. L. (eds) 2007 *Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.
- 15 Tewksbury, J. J., Huey, R. B. & Deutsch, C. A. 2008 Putting the heat on tropical animals. *Science* **320**, 1296–1297. (doi:10.1126/science.1159328)
- 16 Atkinson, A., Siegel, V., Pakhomov, E. & Rothery, P. 2004 Long-term decline in krill stock and increase in salps within the Southern Ocean. *Nature* **432**, 100–103. (doi:10.1038/nature02996)
- 17 Trathan, P. N. & Reid, K. 2009 Exploitation of the marine ecosystem in the sub-Antarctic: historical impacts and current consequences. *Papers Proc. R. Soc. Tasmania* **143**, 9–14.
- 18 Kawaguchi, S., Nicol, S. & Press, A. J. 2009 Direct effects of climate change on the Antarctic krill fishery. *Fish. Manag. Ecol.* **16**, 424–427. (doi:10.1111/j.1365-2400.2009.00686.x)
- 19 Wilt, F. H., Killian, C. E. & Livingston, B. T. 2003 Development of calcareous skeletal elements in invertebrates. *Differentiation* **71**, 237–250.
- 20 Key, R. M. *et al.* 2004 A global ocean carbon climatology: Results from GLODAP. *Global Biogeochem. Cycles* **18**, GB4031. (doi:10.1029/2004GB002247)
- 21 Conkright, M. E., Locarnini, R. A., Garcia, H. E., O'Brien, T. D., Boyer, T. P., Stephens, C. & Antonov, J. I. 2002 *World Ocean Atlas 2001: Objective Analyses, Data Statistics, and Figures, CD-ROM Documentation*. Silver Spring, MD: National Oceanographic Data Center.
- 22 Pierrot, D., Lewis, E. & Wallace, D. W. R. 2006 *MS Excel program developed for CO₂ system calculations*. ORNL/CDIAC-105. Oak Ridge, TN: US Department of Energy.
- 23 Daly, K. L. 2004 Overwintering growth and development of larval *Euphausia superba*: an interannual comparison under varying environmental conditions west of the Antarctic Peninsula. *Deep-Sea Res. II* **51**, 2139–2168. (doi:10.1016/j.dsr2.2004.07.010)