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CO_2-induced acidification affects hatching success in *Calanus finmarchicus*

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ABSTRACT: Bottle incubations were conducted to examine how exposure to seawater containing 8000 ppm carbon dioxide (CO_2; pH 6.95) influenced the growth and reproduction of the keystone copepod *Calanus finmarchicus*. The chosen concentration of CO_2 is expected to occur over 100s of cubic kilometres of seawater as a result of marine CO_2 storage/disposal, and is also representative of the predicted 'worst-case' atmospheric CO_2 scenario in the year 2300. Growth (egg production and biomass loss) in adult female copepods was not affected by the simulated ocean acidification. In contrast, a maximum of only 4% of the eggs successfully yielded nauplii after 72 h in the experimental treatment. Our results demonstrate that environmental risk assessments for marine CO_2 storage/disposal must look beyond adult mortality as an endpoint. Furthermore, if CO_2 is to be disposed of in the deep sea, the location and timing of such activities must take into consideration the overwintering populations of *C. finmarchicus*.

KEY WORDS: Carbon dioxide · Ocean acidification · *Calanus finmarchicus* · Egg production · Hatching success · Carbon capture and storage

INTRODUCTION

The atmospheric concentration of carbon dioxide (CO_2) has increased from 280 to 380 ppm over the past 2 centuries, and human dependence on fossil fuels makes further increases inevitable (Raven et al. 2005). It is now widely accepted that increasing atmospheric concentrations of CO_2 are causing a global increase in mean sea surface acidity (Raven et al. 2005). Worst-case scenario predictions suggest that CO_2 concentrations could potentially exceed 8000 ppm by the year 2300 (Caldeira & Wickett 2005), although the global economic implications of climate change have highlighted the necessity to stabilise concentrations at far lower levels (Stern 2007). Carbon capture and storage (CCS) initiatives are practicable options to help mitigate the effects of continued fossil fuel-derived energy production (Metz et al. 2005). New international rules to allow storage of CO_2 under the seabed entered into force on 10 February 2007, and millions of tonnes of CO_2 have already been stored beneath the seabed in geological reservoirs (e.g. Torp & Gale 2004). Injecting compressed CO_2 into the ocean is also a possible option. At depths >500 m there is sufficient pressure from the overlying water to ensure that the CO_2 will exist as a liquid. Between approximately 500 and 2500 m, liquid CO_2 will remain less dense than seawater. Plans to inject liquefied CO_2 at depths above and below 2500 m will therefore produce rising and sinking plumes of acidified seawater, respectively (Metz et al. 2005). To date, little is known about the long-term stability of marine CO_2 sequestration (Holloway 2005). Direct injection, or the mass release of CO_2 into seawater that would follow cap rock failure at geological reservoirs, will inevitably produce 100s of cubic kilometres of acidified (pH < 7) seawater (Herzog et al. 1996, Caldeira & Wickett 2005). It is important to understand the chronic effects of CO_2-induced acidifi-

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cation on keystone marine organisms in order to achieve a complete and balanced assessment of the risks associated with CO_2 storage/disposal. To date, only Kurihara et al. (2004) have studied the sub-lethal effects of elevated CO_2 on important non-calcifying marine organisms.

The copepod *Calanus finmarchicus* seasonally dominates the zooplankton biomass in the surface waters of the northern North Sea and the North Atlantic (Planque & Batten 2000). Their population size influences that of commercially important juvenile fish (Beaugrand et al. 2003), and their feeding activities contribute significantly to biogenic carbon export and nutrient recycling. The ecological success of these high-latitude copepods is partially attributable to their ability to avoid the seasonal scarcity of food by overwintering at depths between 500 and 1500 m, where temperatures range between approximately 0 and 8°C (e.g. Heath et al. 2004, Edvardsen et al. 2006). There are potentially 2 routes through which *C. finmarchicus* may be exposed to CO_2-acidified seawater during their annual life cycle: (1) via atmospheric deposition/geological reservoir release of CO_2 in surface waters and (2) the passage of rising CO_2 plumes when in deeper waters. We investigated how an atmospheric/seawater CO_2 concentration of 8000 ppm (pH = 6.95 ± 0.04 SD) affected the growth and reproduction of adult female *C. finmarchicus* under controlled laboratory conditions. The experiments determined treatment effects on egg production, hatching success and biomass loss over a 5 d period. The chosen CO_2 concentration is expected to occur over 100s of cubic kilometres of seawater as a result of marine CO_2 disposal (Herzog et al. 1996, Caldeira & Wickett 2005). It is also representative of the predicted 'worst-case' atmospheric CO_2 scenario in the year 2300 (Caldeira & Wickett 2005).

MATERIALS AND METHODS

Experimental work. Adult female *Calanus finmarchicus* and fresh surface seawater were collected off Stonehaven, NE Scotland, in June, and returned to a temperature-controlled laboratory within 2 h. Experimental copepods were fed *ad libitum* with exponential growth phase dinoflagellates *Prorocentum micans* (>500 µg C l^{-1}) and acclimated to laboratory conditions for 5 d prior to incubation. Healthy individual adult female *C. finmarchicus* were incubated for 5 consecutive 24 h periods in 500 ml purpose-built egg production chambers. These were filled to excess with fresh control or experimental seawater (n = 12 in both cases) that contained a natural, screened (95 µm) microplankton assemblage. After the addition of a female, each chamber was sealed with an air-tight lid to ensure that CO_2 out-gassing did not occur during incubation. Concentrations of chlorophyll *a* in the incubation water at the start of each 24 h period ranged between 0.53 and 0.80 µg l^{-1}. The experimental seawater was acidified prior to experimentation by bubbling compressed air containing 8000 ppm CO_2 through it until the pH stabilised (6.95 ± 0.04 SD). The control seawater pH was 8.23 (±0.04 SD). All pH measurements were made using a Hanna bench top meter (H1859), calibrated daily using standard solutions (Sigma) at pH 6, 7 and 9.2. Egg cannibalism is known to occur in *Calanus*, and was minimised by separating the females from their settling eggs via a 350 µm mesh. The eggs produced each day were counted and gently transferred by micropipette into air-tight 75 ml culture flasks containing fresh seawater at the same pH as the maternal incubation. These egg batches were subsequently incubated for 72 h, after which the numbers of nauplii and unhatched eggs were recorded. Hatching success (%) of the eggs produced each day was calculated as the number of nauplii present after 72 h / the number of eggs added at the start of the incubation × 100, because eggs without properly formed membranes may disintegrate quickly (Runge & Roff 2000). All incubations were conducted in a constant temperature room at the *in situ* temperature of 8.8°C. Samples of individual female *C. finmarchicus* were taken immediately before (n = 11) and after the 5 d incubation period to calculate losses of carbon and nitrogen in the control (n = 11) and experimental (n = 8) treatments. All individuals for elemental analysis were placed into separate tin cups and stored frozen (–20°C). The organic carbon and nitrogen content of freeze-dried samples was determined with a Fisons NA 1500 elemental analyser using sulphanillic acid as a standard.

Statistics. The quantities of carbon and nitrogen in the females before (initial, n = 11) and after the 5 d incubation period in the control (ctrl, n = 11) and experimental (+8000, n = 8) treatments were compared using 1-way ANOVAs. Pairwise comparisons were made using Tukey's test. The different number of post-incubation replicates of control and experimental females reflects the different mortalities in these treatments (1 and 4, respectively). Statistical comparison of the mortality data, and testing the elemental data for normality and homogeneity of variance were inappropriate because of the small sample sizes.

All of the egg-related data can be described as longitudinal, because they represent repeated measures over time (5 sequential days). Egg production rate (EPR) data were fitted to a Gaussian regression (Zuur et al. 2007). The other egg-related data were binomial, e.g. eggs either hatched or did not hatch, and therefore their analysis required a form of logistic regression

(Zuur et al. 2007). The analysis of all these data was complicated by the fact that the measured response variables (EPR, proportions of eggs; hatching, failing to hatch and disintegrating) for each individual female were possibly correlated over time, i.e. the proportions of eggs hatching on Day *i* could have been influenced by the proportions of eggs hatching on Day *h*, etc. All these data were analysed using generalised estimation equations (GEE), allowing for a structure of dependence for sequential observations on the same female (Liang & Zeger 1986, Hardin & Hilbe 2003, Fitzmaurice et al. 2004). Correlations between the 5 longitudinal observations, i.e. between measurements on Days 1 and 2, Days 1 and 3, etc., were taken into account using a compound symmetrical correlation matrix. Other correlation structures exist, but this structure is the most appropriate for short time series (Diggle et al. 2002, Fitzmaurice et al. 2004). The GEE model was specified to allow for a treatment (CO_2) effect, a time (day) effect and an interaction between these variables. Some epidemiological studies have revealed quadratic functions of time (West et al. 2006). However, we did not consider such functions for several reasons: (1) the significant loss of female biomass over the duration of the experiment (Fig. 1) indicates that the experimental animals were food limited; therefore, we expected EPR to decline linearly over time (e.g. Niehoff 2004), (2) there are only 5 sequential measurements, and (3) polynomial functions have a tendency to behave rather oddly towards the edges of the (time) gradient. The analysis of all egg-related data (EPR, proportions of eggs; hatching, failing to hatch and disintegrating) was conducted using the GEE library from the R software package. The GEE takes into account the different numbers of egg incubations by using the binomial distribution of eggs incubated in each daily replicate (Table 1). Robust *Z*-statistics were used to separately assess the significance of interaction and main terms.

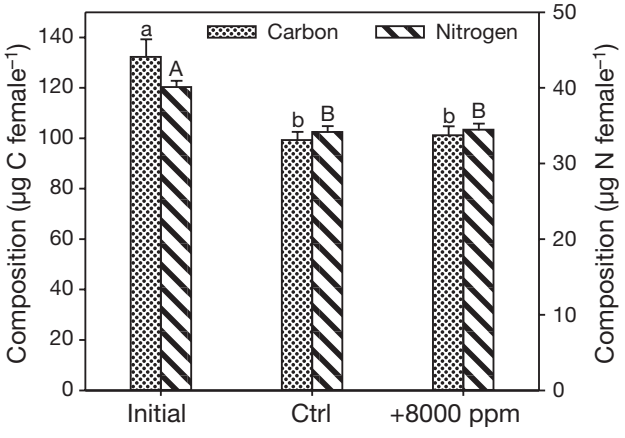


Fig. 1. *Calanus finmarchicus*. Elemental composition of adult females before and after 5 d exposure to 8000 ppm CO_2 seawater. Mean female carbon and nitrogen content before (Initial, n = 11) and after the 5 consecutive 24 h incubation periods in the control (Ctrl, n = 11) and experimental (+8000 ppm CO_2, n = 8) treatments. Error bars = ±SEM. Identical letters (lower case: carbon comparison; upper case: nitrogen comparison) indicate no significant difference (Tukey's test, $p > 0.05$)

Table 1. *Calanus finmarchicus* egg production. Total number of eggs produced/incubated each day. Values in parentheses represent the number of females that produced eggs

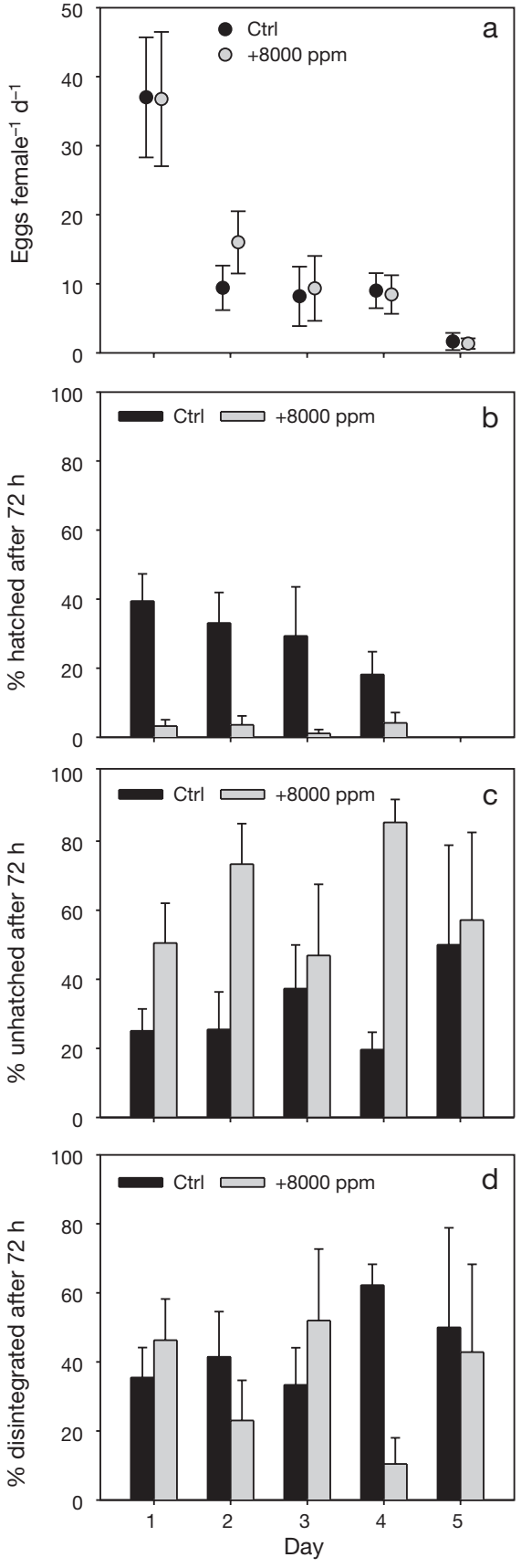
	Day				
	1	2	3	4	5
Control	444 (10)	113 (9)	90 (5)	99 (7)	18 (4)
+8000 ppm CO_2	441 (12)	160 (8)	84 (5)	76 (5)	12 (4)

RESULTS

The carbon and nitrogen content of the pre- and post-incubation females differed significantly (Fig. 1; 1-way ANOVA, $F = 12.289$, $p < 0.001$ and $F = 14.537$, $p < 0.001$, respectively). However, pairwise multiple comparisons (Tukey's test) revealed that treatment effects were not apparent (Table 2). Significant reductions in egg production rates were also observed over the duration of the experiments (Fig. 2a), but again, the experimental treatment had no discernable effect (Table 3a). In contrast, our results and analysis clearly demonstrate that the viability of eggs produced and incubated in the CO_2-acidified seawater was significantly impaired (Fig. 2b, Table 3b), with a maximum of only 4% of the eggs successfully hatching as nauplii after 72 h. Hatching success in the control incubations also remained <50% throughout the incubations,

Table 2. *Calanus finmarchicus*. Results of Tukey's tests for differences in (a) carbon (C) and (b) nitrogen (N) content of adult females before (Ini) and after 5 consecutive 24 h periods of exposure to control (Ctrl) and CO_2-acidified (8000 ppm) seawater. *q*: studentized range statistic

Comparison	Difference of means	*q*	p
(a) Ini C vs. Ctrl C	33.033	6.379	<0.001
Ini C vs. 8000 ppm C	31.099	5.511	0.002
8000 ppm C vs. Ctrl C	1.934	0.343	0.968
(b) Ini N vs. Ctrl N	5.943	6.910	<0.001
Ini N vs. 8000 ppm N	5.657	6.036	<0.001
8000 ppm N vs. Ctrl N	0.286	0.305	0.975



which is not atypical for *Calanus finmarchicus* (e.g. Jónasdóttir et al. 2002). We explored the mechanism behind the observed reproductive failure by examining treatment effects on the proportions of eggs failing to hatch and disintegrating. Exposure to CO_2-acidified seawater doubled the frequency of eggs that remained unhatched (average of 63% compared to 32% in the controls; Fig. 2c, Table 3c). Our initial analysis of how the CO_2-acidified seawater affected the production of eggs that subsequently disintegrated (Fig. 2d) suggested that eggs incubated in acidified seawater were less likely to disintegrate than those in the controls (Table $3d_i$). Furthermore, it indicated that there was a weakly significant ($p = 0.034$) interaction between the effects of treatment and time. We failed to attribute any mechanism of biological significance that could explain this result. Furthermore, closer examination of the data suggested that this trend was predominantly driven by the result observed on Day 4 (Fig. 2d). Reanalysis of the data excluding those from Day 4 yielded a GEE model that did not have a significant interaction (Table $3d_{ii}$), confirming that data from this day were particularly influential in the outcome of our analysis. The resulting model demonstrated that the proportions of eggs produced on Days 1, 2, 3 and 5 that disintegrated during the incubations were not affected by treatment or day ($p > 0.2$ in both cases; Table $3d_{ii}$). We are unsure of the exact reason for the observed result on Day 4, but cannot rule out that it occurred as a result of observer error or simply by chance. Nonetheless, considering that 4 of the 5 days yielded a non-significant effect of day and treatment, we believe it correct to exclude Day 4 from our analysis.

DISCUSSION

EPR and hatching success in both the control and experimental treatments were low, which is typical for female *Calanus finmarchicus* reproducing at the Stonehaven sampling site in June (2003 to 2007 averages ± SEM: 22.9 ± 5.0 eggs female^{-1} d^{-1} and 46.5 ± 10.6%, respectively; K. Cook unpubl. data). The significant reductions in female biomass and individual EPR in both experimental and control treatments indicate that the females were food-limited during the incuba-

Fig. 2. *Calanus finmarchicus.* Effect of an atmospheric CO_2 concentration of 8000 ppm on reproduction in adult females. For the control (Ctrl) and experimental (+8000 ppm) treatments: (a) individual daily egg production rates, and the percentage of eggs that (b) successfully produced nauplii after 72 h, (c) remained unhatched after 72 h and (d) had disintegrated after 72 h. All data presented are mean values ± SEM

Table 3. *Calanus finmarchicus*. Model coefficients of optimal generalised estimation equations investigating how time (Day) and 8000 ppm [CO_2] (Treatment) influence: (a) egg production rate, and the proportion of eggs that (b) yielded nauplii (hatching success), (c) remained unhatched, (d_i) disintegrate (all data included) and (d_{ii}) disintegrate (data from Day 4 excluded) (see 'Results'). *Z*: estimated value of a regression parameter divided by its SE. Naïve and robust estimates of this value ignore and include the temporal dependence structure imposed by the GEE methodology

		Estimate	Naïve SE	Naïve *Z*	Robust SE	Robust *Z*	p
(a)	(Intercept)	41.55	4.64	8.97	4.81	8.64	0.000
	Day	−7.62	1.57	−4.84	1.27	−6.01	0.000
	Treatment	−0.35	4.19	−0.09	4.90	−0.07	0.944
(b)	(Intercept)	−0.33	0.13	−2.49	0.11	−2.93	0.003
	Day	−2.20	0.20	−10.81	0.38	−5.83	0.000
	Treatment	−0.31	0.10	−4.09	0.05	−5.80	0.000
(c)	(Intercept)	−1.46	0.11	−13.89	0.16	−9.40	0.000
	Treatment	1.14	0.13	9.01	0.16	6.94	0.000
(d_i)	(Intercept)	−1.49	0.19	−7.86	0.22	−6.73	0.000
	Day	0.16	0.31	0.51	0.30	0.52	0.601
	Treatment	0.21	0.08	2.64	0.08	2.75	0.006
	Treatment:Day	−0.34	0.15	−2.19	0.16	−2.12	0.034
(d_{ii})	(Intercept)	−1.27	0.19	−6.79	0.20	−6.43	0.000
	Day	−0.25	0.19	−1.32	0.20	−1.27	0.203
	Treatment	0.03	0.10	0.28	0.12	0.23	0.816

tions (e.g. Niehoff 2004), which agrees well with the low concentrations of chlorophyll *a* at the start of each daily incubation. Nonetheless, food-quality effects cannot be discounted (e.g. Jónasdóttir et al. 2002). *C. finmarchicus* is known to reproduce during periods of food scarcity by catabolising internal reserves (Niehoff 2004, Mayor et al. 2006); therefore, a loss of biomass is not unusual. However, reduced pH has previously been shown to depress rates of growth and metabolic activity in marine invertebrates (Pörtner et al. 2004, Michaelidis et al. 2005); thus, we had anticipated that both egg production rates and biomass losses would be significantly lower in females exposed to the CO_2-acidified seawater. Metabolic depression in adult *C. finmarchicus* resulting from the CO_2-acidified seawater was not discernable in our results, and may therefore be small in relation to the effects of other stressors, e.g. low food concentrations that the adults typically encounter in the natural environment. It is possible that the number of females incubated in each experimental treatment was too low to detect subtle differences in EPR and biomass changes between treatments, although the relatively low variability in both EPR and female elemental content suggest that this was not the case.

The dramatic reduction in hatching success as a result of CO_2-acidified seawater demonstrates that reproduction in *Calanus finmarchicus*, and possibly other calanoid copepods, is pH sensitive (Kurihara et al. 2004). Indeed, previous work has shown that reproductive processes in *Acartia* spp. are also affected by elevated CO_2 (Kurihara et al. 2004). Kurihara et al. (2004) also demonstrated that hatching success in *A. erythraea* was significantly affected at 10 000 ppm CO_2, but not at 5000 ppm. The present study reports a significant reduction in hatching success at 8000 ppm CO_2, suggesting that a threshold concentration somewhere between 5000 and 8000 ppm CO_2 may exist, above which hatching success in calanoid copepods is significantly impaired. The data presented here do not allow the effects of decreased pH and elevated CO_2 concentration to be distinguished, although a recent study comparing the effects of CO_2- and inorganic-acid-induced acidification on reproductive processes in sea urchins demonstrated that both have similar, negative effects (Kurihara & Shirayama 2004). This suggests that the reduced hatching success observed here was primarily mediated via an increase in the concentration of H^+, although elevated CO_2 concentrations may themselves have inhibitory effects (Kurihara & Shirayama 2004, Pörtner et al. 2004). Other work examining the effects of hydrochloric acid (HCl)-induced pH reductions on invertebrate reproduction provides several potential mechanisms to explain this result, none of which are mutually exclusive. Elevated CO_2 and the associated decrease in pH could have: (1) facilitated polyspermy by interfering with membrane depolarisation during fertilisation (e.g. Tyler & Sheer 1937); (2) inhibited the acrosomal reaction and thus fertilisation (Gregg & Metz 1976); (3) depressed gametic and embryonic rates of respiration and protein synthesis (Grainger et al. 1979, Christen et al. 1983); and/or (4) induced mitotic, hence genetic, abnormalities in the embryo (Pagano et al. 1985). Much is known about the reproductive biology of *C. finmarchicus* (reviewed by Hirche 1996), although there is a lack of detailed information on the fertilisation process. It is not possible to deduce the mechanism(s) driving the hatching failure in this study, and it is clear that more detailed physiological experiments are required in order to achieve this goal.

Irrespectively, egg mortality plays an important role in the population dynamics of *Calanus finmarchicus* (Ohman & Hirche 2001). It is therefore of concern to find that the elevated CO_2 concentration investigated here had such a dramatic effect on egg mortality. Whilst it is unlikely that anthropogenic CO_2 emissions

will acidify the surface ocean to the extent investigated here (8000 ppm [CO_2]), the catastrophic failure of large CO_2 reservoirs will inevitably acidify (pH < 7) 100s of cubic kilometres of seawater (Herzog et al. 1996, Caldeira & Wickett 2005). This could have a significant effect on the reproductive efforts of *C. finmarchicus* and possibly other species of copepods inhabiting the overlying waters (e.g. Kurihara et al. 2004). The large, slowly rising plumes of acidified seawater that will result from the direct injection of compressed CO_2 into the ocean at depths <2500 m (Metz et al. 2005) may therefore also affect these copepods. The development of reproductive organs in *C. finmarchicus* begins in the late juvenile Copepodite Stages IV and V (Hirche 1996), both of which are found in the overwintering populations. The process of mating in *C. finmarchicus* has yet to be observed (Hirche 1996), but since mature females are often observed to spawn soon after their arrival in surface waters, well in advance of the spring bloom (e.g. Niehoff et al. 1999), it is probable that this also occurs in the deep sea as the animals exit diapause. Slow-rising plumes of CO_2-acidified seawater that coincide with *C. finmarchicus* populations as they ascend from diapause could therefore pose a potential threat to the reproductive efforts of these copepods. We acknowledge that the poikilothermic nature of *C. finmarchicus* may cause the effects of CO_2-induced acidification in deeper, colder waters to differ from those presented here. However, it would be prudent to take deep-water populations of overwintering copepods into consideration when choosing locations for any marine CO_2 sequestration initiative. This is of particular importance for the 'epicentre' populations of *C. finmarchicus*, e.g. those in the Gulf of Maine and the Norwegian Sea (Speirs et al. 2006 and references therein), where animals overwinter at depths between 500 and 1200 m (Miller et al. 1991, Edvardsen et al. 2006).

The present study demonstrates that future risk/environmental impact assessments of marine CO_2 disposal/storage options must look beyond adult mortality as an endpoint (e.g. Herzog et al. 1996). Nonetheless, industrial CO_2 emissions will continue to drive a global decrease in the pH of the surface ocean (Caldeira & Wickett 2005). If these continue unabated, the acidification of surface waters could ultimately lead to widespread effects on invertebrate reproductive processes (references above). The ecological risks of marine CO_2 sequestration must therefore be weighed against those associated with surface-water acidification. More research urgently needs to be focussed towards understanding the ontogenetic changes in susceptibility to the effects of elevated CO_2 in a range of calcifying and non-calcifying keystone marine organisms.

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LITERATURE CITED

Beaugrand G, Brander KM, Lindley SS, Reid PC (2003) Plankton effect on cod recruitment in the North Sea. Nature 426: 661–664

Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J Geophys Res Oceans 110: CO9S04, doi:101029/2004JC002671

Christen R, Schackmann RW, Shapiro BM (1983) Metabolism of sea urchin sperm. J Biol Chem 258:5392–5399

Diggle PJ, Heagerty P, Liang KY, Zeger SL (2002) Analysis of longitudinal data, 2nd edn. Oxford University Press, New York

Edvardsen A, Pedersen JM, Slagstad D, Semenova T, Timonin A (2006) Distribution of overwintering *Calanus* in the north Norwegian Sea. Ocean Sci Discuss 2:87–96

Fitzmaurice GM, Laird NM, Ware JH (2004) Applied longitudinal analysis. John Wiley & Sons, Hoboken

Grainger JL, Winkler MM, Shen SS, Steinhardt RA (1979) Intracellular pH controls protein synthesis rate in the sea urchin egg and early embryo. Dev Biol 68:396–406

Gregg KW, Metz CB (1976) Physiological parameters of the sea urchin acrosome reaction. Biol Reprod 14:405–411

Hardin JW, Hilbe JM (2003) Generalized estimating equations. Chapman & Hall/CRC, Boca Raton, FL

Heath MR, Boyle PR, Gislason A, Gurney WSC and others (2004) Comparative ecology of over-wintering *Calanus finmarchicus* in the northern North Atlantic, and implications for life-cycle patterns. ICES J Mar Sci 61:698–708

Herzog HJ, Adams EE, Auerbach D, Caulfield J (1996) Environmental impacts of ocean disposal of CO_2. Energ Conserv Manag 37:999–1005

Hirche HJ (1996) The reproductive biology of the marine copepod, *Calanus finmarchicus* — a review. Ophelia 44: 111–128

Holloway S (2005) Underground sequestration of carbon dioxide — a viable greenhouse gas mitigation option. Energy 30:2318–2333

Jónasdóttir SH, Gudfinnsson HG, Gislason A, Astthorsson OS (2002) Diet composition and quality for *Calanus finmarchicus* egg production and hatching success off southwest Iceland. Mar Biol 140:1195–1206

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) Effects of raised CO_2 concentration on the egg production rates and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). Mar Pollut Bull 49:721–727

Liang KY, Zeger SL (1986) Longitudinal data analysis using generalized linear models. Biometrika 73:13–22

Mayor DJ, Anderson TR, Irigoien X, Harris R (2006) Feeding and reproduction of *Calanus finmarchicus* during non-bloom conditions in the Irminger Sea. J Plankton Res 28: 1167–1179

Metz B, Davidson O, de Coninck H, Loos M, Meyer L (2005) Carbon dioxide capture and storage. Working Group III of the IPCC, Cambridge University Press, Cambridge

Michaelidis B, Ouzounis C, Paleras A, Pörtner HO (2005) Effect of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis.* Mar Ecol Prog Ser 293:109–118

Miller CB, Cowles TJ, Wiebe PH, Copley NJ, Grigg H (1991) Phenology in *Calanus finmarchicus*; hypotheses about control mechanisms. Mar Ecol Prog Ser 72:79–91

Niehoff B (2004) The effect of food limitation on gonad development and egg production of the planktonic copepod *Calanus finmarchicus.* J Exp Mar Biol Ecol 307: 237–259

Niehoff B, Klenke U, Hirche HJ, Irigoien X, Head R, Harris R (1999) A high frequency time series at Weathership M, Norwegian Sea, during the 1997 spring bloom: the reproductive biology of *Calanus finmarchicus.* Mar Ecol Prog Ser 176:81–92

Ohman MD, Hirche HJ (2001) Density-dependent mortality in an oceanic copepod population. Nature 412:638–641

Pagano G, Cipollaro M, Corsale G, Esposito A, Ragucci E, Giordano GG (1985) pH-induced changes in mitotic and developmental patterns in sea urchin embryogenesis. I. Exposure of embryos. Teratog Carcinog Mutagen 5:101–112

Planque B, Batten SD (2000) *Calanus finmarchicus* in the North Atlantic: the year of *Calanus* in the context of interdecadal change. ICES J Mar Sci 57:1528–1535

Pörtner HO, Langenbuch M, Reipschläger A (2004) Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. J Oceanogr 60: 705–718

Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O and others (2005) Ocean acidification due to increasing atmospheric carbon dioxide. The Royal Society policy document 12/05, Royal Society, London. Available to download from:http://wwwroyalsocacuk/displaypagedocasp?id=13539

Runge JA, Roff JC (2000) The measurement of growth and reproductive rates. In: Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley M (eds) Zooplankton methodology manual. Academic Press, San Diego, CA, p 401–454

Speirs DC, Gurney WSC, Heath MR, Horbelt W, Wood SN, de Cuevas BA (2006) Ocean-scale modelling of the distribution, abundance, and seasonal dynamics of the copepod *Calanus finmarchicus.* Mar Ecol Prog Ser 313:173–192

Stern N (2007) The economics of climate change. Cambridge University Press, Cambridge

Torp TA, Gale J (2004) Demonstrating storage of CO_2 in geological reservoirs: the Sleipner and SACS projects. Energy 29:1361–1369

Tyler A, Scheer BT (1937) Inhibition of fertilization in eggs of marine animals by means of acid. J Exp Zool 75:179–195

West B, Welch KB, Galecki A T (2006) Linear mixed models: a practical guide using statistical software. Chapman & Hall/CRC, Boca Raton; FL

Zuur AF, Ieno EN, Smith GM (2007) Analysing ecological data. Springer, New York

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