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SHORT NOTE

Behavioural responses of Antarctic krill (*Euphausia superba*) to CO_2-induced ocean acidification: would krill really notice?

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Abstract

The Southern Ocean is expected to be significantly affected by future ocean acidification. Antarctic krill (*Euphausia superba*) is the key species of the Southern Ocean ecosystem. Understanding their behavioural responses to acidification is critical for assessing the impacts of ocean acidification on the ecosystem. Adult Antarctic krill reared in different holding tanks with various CO_2 levels for 6 months prior to the experiments were tested for their behavioural responses to different carbon dioxide partial pressures (pCO_2) (400, 1000, 1500, 2000, and 4000 µatm pCO_2) in a two-channel flume. The time krill occupied either of the flume channels (with high or ambient CO_2 levels) was highly variable in all tests. In most cases no significant preference to either side of the flume was found. The krill did not display any systematic discrimination to the sea water with different CO_2 levels regardless of the CO_2 levels that krill were acclimated for in the 6 months prior to the experiment. Poor ability to discriminate high CO_2 waters may have an important implication to their life history in the future as ocean acidification rapidly progresses in parts of Southern Ocean.

Keywords Antarctic krill · Ocean acidification · Behavioural response · Southern Ocean

Introduction

Antarctic krill (*Euphausia superba*) is a dominant species of the Southern Ocean ecosystem (Atkinson et al. 2004). They perform a key trophic link in Antarctic food webs and transfer carbon and energy from phytoplankton to higher trophic level species (Perissinotto et al. 1997; Atkinson et al. 2004). Antarctic krill is also suggested to play an important role in the recycling of nutrients including iron in the Southern Ocean through its diel vertical migration (Tovar-Sanchez et al. 2007; Nicol et al. 2010; Schmidt et al. 2011; Whitehouse et al. 2011; Ratnarajah et al. 2016).

Ocean acidification is expected to have severe impacts on the ecosystems in the Southern Ocean due to the higher solubility of CO_2 in cold waters and the degree to which ocean acidification would be augmented in deep waters (Sabine et al. 2004; Brewer and Peltzer 2009; Kawaguchi et al. 2011). Previous research found that the embryonic development of krill can be significantly affected at high CO_2 levels (Kawaguchi et al. 2011; 2013). Physiology and metabolism of adult krill were also demonstrated to be negatively impacted under high CO_2 treatment (Saba et al. 2012).

Our understanding of the effects of ocean acidification on Antarctic krill is still limited (Kawaguchi et al. 2011, 2013; Saba et al. 2012) and nothing is known about the impacts of increased levels of CO_2 on their behaviour. Species would respond firstly to the changing environment through behavioural modification (Tuomainen and Candolin 2011; Nagelkerken and Munday 2016). Krill have been observed to feed rapidly in aquaria by swimming fast in tight circles,

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presumably after sensing chemical cues in the food (Hamner et al. 1983). It was also demonstrated that krill show aversion responses to ammonia and urine and re-aggregation responses to varying light conditions (Strand and Hamner 1990; Kawaguchi et al. 2010). Antarctic krill is an obligate schooler and therefore any change in their behaviour is expected to have implications for all aspects of the biology of krill (Hamner et al. 1983). Further, this behavioural characteristic, i.e. to form swarms at various scales from tens of meters to the orders of tens or hundreds of kilometres, is the prime reason why this species can function as suitable prey for a wide range of higher predators in the region. Therefore, understanding the effects of ocean acidification on behavioural responses in Antarctic krill is fundamental for evaluating the ecosystem effects of ocean acidification that can be expected in the future.

We conducted a series of experiments to test the behavioural response of adult Antarctic krill to five different pCO_2 levels: 400 µatm (present-day level), 1000 µatm (projected CO_2 level in 2100, van Vuuren et al. 2011), 1500 µatm (projected CO_2 level between 2100 and 2200, Meinshausen et al. 2011), 2000 µatm (projected CO_2 level in 2300, van Vuuren et al. 2011) and 4000 µatm (one extreme level). This research is a first step to examine if krill exhibit any selectivity or preference to different CO_2 levels. This knowledge could help us better evaluate the impact of ocean acidification on Antarctic krill and any flow on effects for the entire Southern Ocean ecosystem.

Materials and methods

Krill rearing

Live Antarctic krill were collected on 22–23 February 2015 (66–03°S, 59–25°E and 66–33°S, 59–35°E) from the RSV *Aurora Australis* and were transported back to the research aquarium at the Australian Antarctic Division in Kingston, and maintained at 0.5 °C until the start of this study. At the commencement of this study, adult krill were randomly distributed and reared in five 200-L tanks. Seawater was supplied through the seawater recirculating system (Kawaguchi et al. 2010). The CO_2 levels in the five rearing tanks were 400, 1000, 1500, 2000 and 4000 µatm pCO_2, respectively. The temperature was maintained at 0.5 °C. Krill were reared in each holding tank for 6 months before the behaviour experiment was conducted.

Experimental set-up

Response of adult krill was tested in a 2-channel choice flume (Fig. 1) using pCO_2 levels of 400, 1000, 1500, 2000, and 4000 µatm. The temperature for the flume trials was set to 0.5 °C, identical to the krill rearing tanks. Channels 1 and 2 of the flume were supplied with seawater through lines A and B respectively. A constant gravity-driven flow of 300 mL min^{-1} per channel was maintained throughout all trials. For each trial, one single adult krill from a holding tank was randomly collected by gently using a hand net and the krill was released at the downstream end of the flume where it could move towards either side of the chamber. Each trial took 30 min, which included an acclimation period (5 min), the first observation period (5 min), water-switching period (15 min) and the second observation period (5 min). After the acclimation period and the first observation period, the two supply lines were switched (channels 1 and 2 were supplied through lines B and A, respectively) in order to provide a control for potential side preferences that were not related to seawater source (Munday et al. 2009). According to the flow rate and volume of the flume (about 7.4 L), it would take about 13 min for all the water in the flume to be switched between the two channels. This was tested and was proven by monitoring the pH value in regions 1, 2, 6 and 7 of the flume during the process of water switching between channel 1 (400 µatm pCO_2) and channel 2 (1000 µatm pCO_2) (Online Resource 1). Based on this monitoring result, the water-switching period was of 15 min duration to make sure the seawater in the two channels of the flume had been switched entirely. A digital video recorder mounted above the flume was used to record the position of the krill in the flume for the 30-min period. Each krill was tested once only.

Behaviour experiment

A total of 13 experiments, with identical or different CO_2 levels in the two channels of the flume, were conducted (Table 1). Most experiments consisted of 12 trials except for experiment 11 and experiment 13 due to the limited number of krill available in the holding tank with CO_2 level of 4000 µatm (Table 1).

Experiments 1, 2, 5, 8, 11 were designed as blank tests. During these tests, the flume was supplied with seawater at ambient CO_2 level (400 µatm pCO_2) through two separate seawater supply lines A and B (Table 1). Experiments 3, 4, 6, 7, 9, 10, 12 and 13 were designed as response tests. During these tests, the flume was supplied with seawater of ambient pCO_2 level (400 µatm) from seawater supply line A and seawater of high pCO_2 level (1000 µatm, 1500 µatm, 2000 µatm and 4000 µatm) from seawater supply line B (Table 1). Krill tested in these 13 experiments were collected from different tanks with various pCO_2 levels (Table 1).

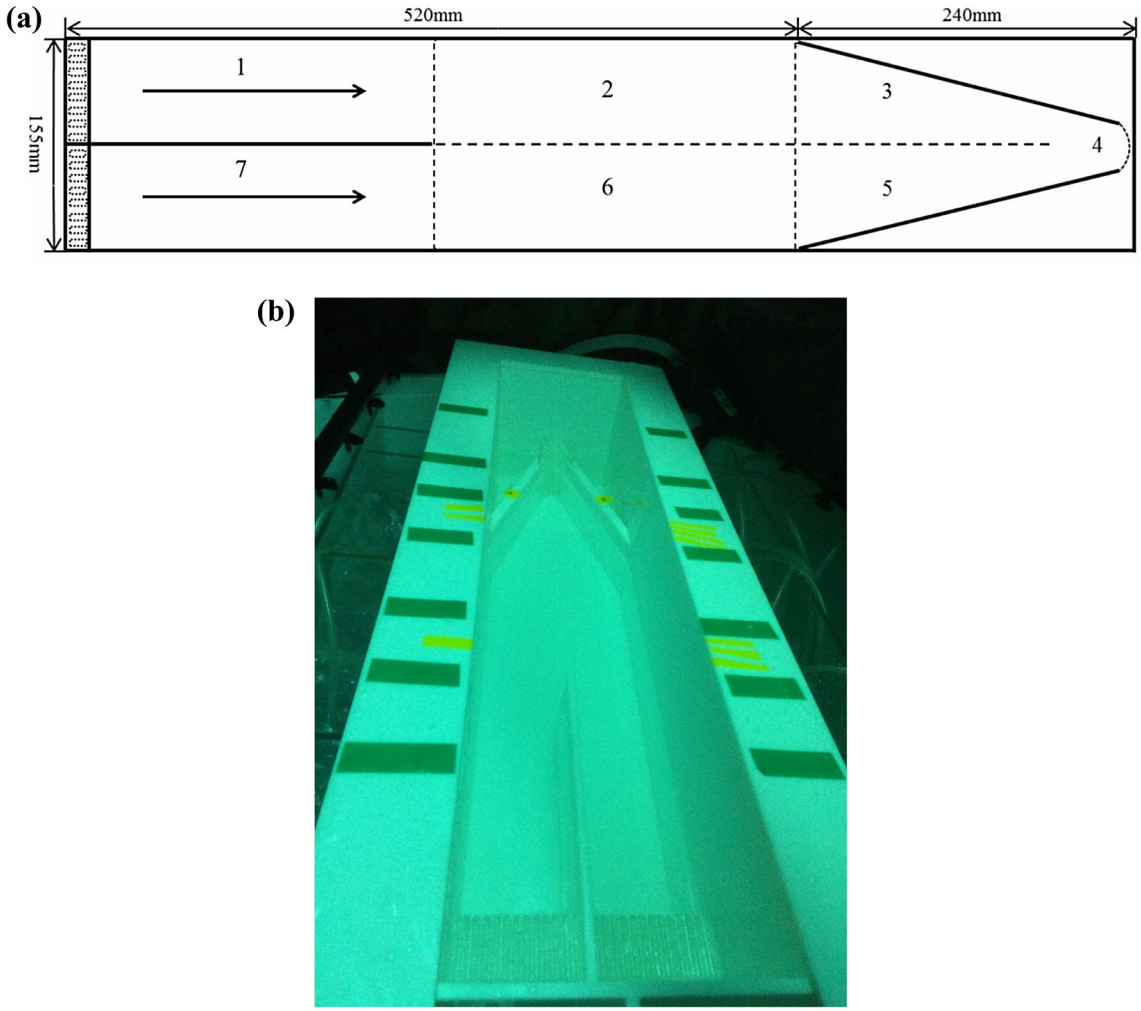

Fig. 1 Description of the flume. **a** A sketch map showing seven regions, named from 1 to 7, which were divided arbitrarily. Positions 1 and 2 were considered as Channel 1, positions 3, 4 and 5 were considered as downstream, positions 6 and 7 were considered as Channel 2 in this study. **b** A photograph of the flume taken from the upstream looking towards downstream

Data analysis

The positions of krill at 5-second intervals were recorded during the first observation period (prior to water switch) and during the second observation period (after water switch) and were used for the statistical analysis.

Paired samples *T* test was used to: (1) in blank tests (experiment 1, 2, 5, 8 and 11) to compare the proportion of time that each krill spent in channel 1 and channel 2 prior to the water switch (the first observation period of each trial) and after the water switch (the second observation period of each trial). This enabled the determination of whether or not there was an effect of the switching manipulation on krill behaviour. (2) In response tests (experiment 3, 4, 6, 7, 9, 10, 12 and 13) to compare the proportion of time that each krill spent in the high CO_2 channel and the ambient CO_2 channel prior to and after the water switch (the first and second observation periods respectively of each trial).

One-way ANOVA was used to test the amount of time krill stayed in channel 1 and channel 2 of the flume for blank test experiments and also used to test the amount of time krill stayed in the high CO_2 channel and the ambient CO_2 channel of the flume in response test experiments.

Results

In most cases, the krill swam around the flume and moved between the two channels and downstream during the 30-min trial. Sometimes the krill remained in the downstream region for extended periods, in some cases the entire 30-min of the test.

Table 1 Initial target CO_2 level of channel 1 and channel 2 of the flume and the tank where the krill was collected from for the experiment. Water in channel 1 and channel 2 was switched after 10 min

Experiment number and replicates (*n*)	CO_2 level (µatm pCO_2) of the krill holding tank	Channel 1 (µatm pCO_2)	Channel 2 (µatm pCO_2)
Exp.1 ($n = 12$)	400	400	400
Exp.2 ($n = 12$)	1000	400	400
Exp.3 ($n = 12$)	400	1000	400
Exp.4 ($n = 12$)	1000	1000	400
Exp.5 ($n = 12$)	1500	400	400
Exp.6 ($n = 12$)	400	1500	400
Exp.7 ($n = 12$)	1500	1500	400
Exp.8 ($n = 12$)	2000	400	400
Exp.9 ($n = 12$)	400	2000	400
Exp.10 ($n = 12$)	2000	2000	400
Exp.11 ($n = 8$)	4000	400	400
Exp.12 ($n = 12$)	400	4000	400
Exp.13 ($n = 9$)	5000	4000	400

In the blank tests (pCO_2 level of both channels was 400 µatm), krill from various holding tanks with different pCO_2 levels showed no significant channel preference of the flume in most cases (Fig. 2a, b). The only exceptions were krill from the 400 µatm tank spending more time in channel 2 before the water switch, and krill from the 2000 µatm tank spending more time in channel 2 after the water switch (Online Resource 2). One-way ANOVA showed that there were no significant differences in the amount of time spent in either channel of the flume by krill from different tanks (Online Resource 3).

In the response tests with high CO_2 levels in one channel and ambient CO_2 in the other, krill showed no preference for either channel of the flume before the water switch (Online Resource 4), while krill reared in the1500 µatm pCO_2 tank prior to the experiment spent more time in the high CO_2 channel compared with the ambient CO_2 channel after water switch (Online Resource 4). In contrast, krill reared in the 4000 µatm pCO_2 tank prior to the experiment exhibited a strong preference for the ambient CO_2 channel after water switch (Online Resource 4). Krill from different tanks spent similar time in either channel of the flume before water switch, except for krill reared in the 1500 µatm pCO_2 tank prior to the experiment which spent more time in the channel with high CO_2 levels and less time in the channel with ambient CO_2 levels after water switch compared with krill reared in the 4000 µatm pCO_2 tank, determined by one-way ANOVA (Online Resource 5).

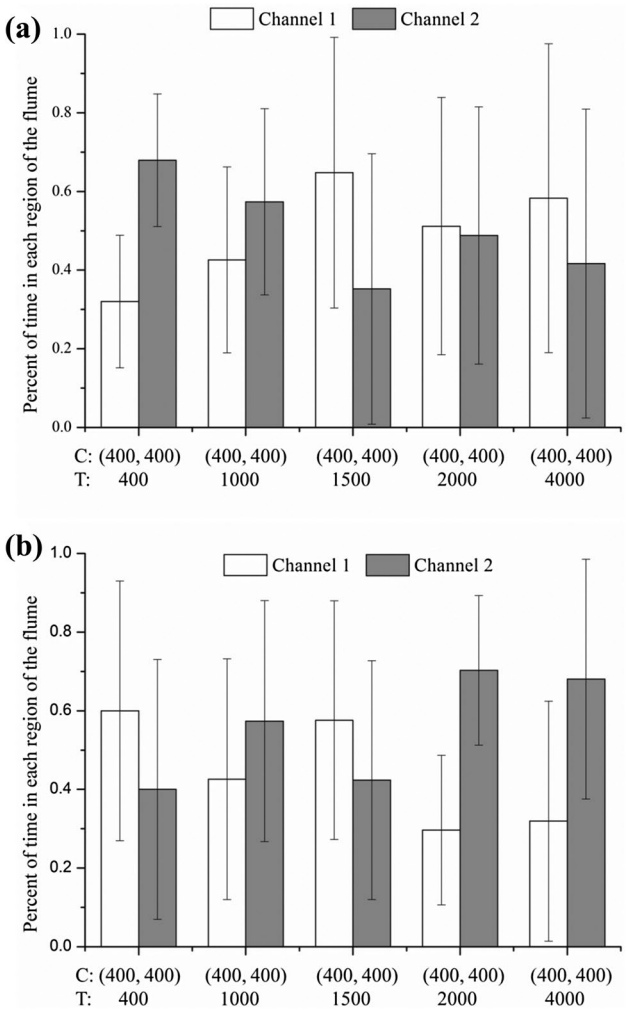

Fig. 2 Percent of time that krill stay in channel 1 and channel 2 of the flume in the blank tests. **a** Before water switching. **b** After water switching (**b**). CO_2 level (µatm pCO_2) in the two channels of the flume (C) and that in the krill holding tank (T) of each test are shown in the abscissa. Error bars indicate standard deviation

Discussion

This research is the first report on the behavioural responses of krill to elevated levels of CO_2. Much of the research about the effects of ocean acidification on the behaviour of marine organisms has been conducted on fish (Munday et al. 2009, 2010; Simpson et al. 2011) and the results have found that CO_2-induced ocean acidification impairs olfactory discrimination and homing behaviour of the coastal clownfish (Munday et al. 2009). Our results suggest that neither krill reared in ambient seawater, nor krill acclimated in high CO_2 seawater for 6 months prior to the response experiment could effectively avoid the seawater with high CO_2 level (Fig. 3). The only exceptions were for krill reared in the 1500 µatm pCO_2 tank that spent more time in the high CO_2 channel after water switch in experiment 7 (Fig. 2).

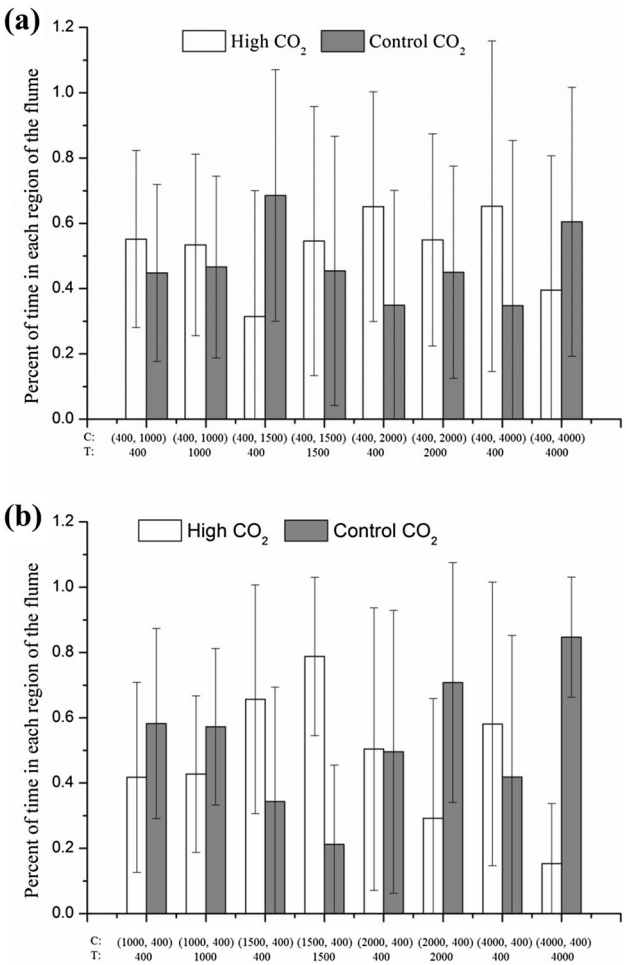

Fig. 3 Percent of time that krill stay in each region of the flume (high CO_2 channel, ambient CO_2 channel) in the response tests. **a** Before water switching. **b** After water switching. CO_2 level (µatm pCO_2) in the two channels of the flume (C) and that in the krill holding tank (T) of each experiment are shown in the abscissa. Error bars indicate standard deviation

In fact, in most cases krill just moved from one channel to the other or remained in the downstream location. Only two out of twelve trials showed that krill stayed in the high CO_2 channel for the entire period after water switch. Therefore we are not able to draw a conclusion that the krill reared in the 1500 µatm pCO_2 tank preferred the high CO_2 environment. Moreover, the krill reared in the high CO_2 water for 6 months did not select the control CO_2 channel or reject the high CO_2 channel effectively when they were transferred into the flume. One possible explanation could be a change in the sensory threshold where krill reared in the high CO_2 water become insensitive to the chemical cues from the acidified environment and therefore they would not respond to it during short term exposure. Another possible explanation could be made in relation to a change in their overall fitness affecting their behavioural response. It has been suggested that elevated CO_2 levels could compromise acid–base balance in krill, and the cost is that krill then have to increase their feeding rate, nutrient release rate and metabolic activity (Saba et al. 2012). Based on visual observation, a larger proportion of krill were less active and swimming closer to the bottom of the tank in the 4000 µatm pCO_2 tank compared to krill reared in other CO_2 levels. Mortality rate of krill reared in the 4000 µatm pCO_2 tank was also higher (31%) compared to those reared in 400–2000 µatm pCO_2 tanks (6.5–12.5%) (Ericson personal communication) which indicates that krill in the higher pCO_2 tank were likely to be in a stressed condition compared to krill exposed to lower CO_2 levels. This could also be manifested by the higher frequencies of krill reared in the 4000 µatm pCO_2 tank staying in the downstream of the flume during the whole test in experiments 11 and 13. On occasions with low food availability (i.e., winter time), krill may not be able to obtain sufficient energy not only to maintain growth and reproduction but also to compensate the acid–base equilibration at high CO_2 (Saba et al. 2012; Wittmann and Portner 2013).

Zooplankton species with natural exposures to various pCO_2 levels have been shown to cope better to manipulated ocean acidification compared to species that are experiencing narrower natural pCO_2 ranges (Lewis et al. 2013). Krill may be evolutionally adapted to the pCO_2 levels that they experience within their natural habitat range especially through their seasonal and ontogenetic vertical migrations from the surface (ambient CO_2 level) to the deep sea where higher CO_2 levels are encountered (Kawaguchi et al. 2011). However, the krill embryo has been reported to sink passively and hatch at a depth of 700–1000 m after fertilized eggs were laid at surface (Quetin and Ross 1984). The pCO_2 levels in the upper 1000 m will be higher than 1000 µatm pCO_2 in parts of the Southern Ocean in the next 100 years (Meinshausen et al. 2011). The embryonic development of krill has been demonstrated to be negatively impacted at these pCO_2 levels (Kawaguchi et al. 2013). On the other hand, adult organisms are generally considered to be more resilient compared with the early life stages (Kurihara 2008; Portner and Ferrel 2008; Cripps et al. 2014). The implication of this is that, if adults are not able to discriminate the threat from high CO_2 habitat, it could be detrimental to the krill because they may continue to reproduce in the spawning ground that has become unfavorable for their embryos due to ocean acidification. Indeed, krill were observed staying in the channel with the high CO_2 water for a long time in some cases (nearly half an hour). This process would in turn have a negative effect on the krill population and therefore a further effect on the Southern Ocean ecosystem. Since group behaviour is an important characteristic of krill, future studies may also need to look into aspects of the effects of increased levels of CO_2 on their group behaviour.

In this study, we demonstrated Antarctic krill's inability to discriminate high CO_2 waters in experimental conditions. Our study clearly underscores the need for detailed studies on both behavioural and physiological responses of krill to ocean acidification and the interactions with rising ocean temperature (Ferrari et al. 2015) to better understand the link between krill behaviour and population responses to environmental stressors in the changing Southern Ocean.

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References

Atkinson A, Siegel V, Pakhomov E, Rothry P (2004) Long-term decline in krill stock and increase in salps within the Southern Ocean. Nature 432:100–103

Brewer PG, Peltzer ET (2009) Limits to marine life. Science 324:347–348

Cripps G, Lindeque P, Flynn KJ (2014) Have we been underestimating the effects of ocean acidification in zooplankton? Glob Change Biol 20:3377–3385

Ferrari MCO, Munday PL, Rummer JL, McCormick MI, Corkill K, Watson S, Allan BJM, Meekan MG, Chivers DP (2015) Interactive effects of ocean acidification and rising sea temperatures alter predation rat and predator selectivity in reef fish communities. Glob Change Biol 21:1848–1855

Hamner WG, Hamner PP, Strand SW, Gilmer RW (1983) Behavior of Antarctic krill, *Euphausia superba*: chemoreception, feeding, schooling, and molting. Science 220:433–435

Kawaguchi S, King R, Mijers R, Osborn JE, Swadling KM, Ritz DA, Nicol S (2010) An experimental aquarium for observing the schooling behaviour of Antarctic krill (*Euphausia superb*). Deep-Sea Res II 57:683–692

Kawaguchi S, Kurihara H, King R, Hale L, Berli T, Robinson JP, Ishida A, Wakita M, Virtue P, Nicol S, Ishimatsu A (2011) Will krill fare well under Southern Ocean acidification? Biol Lett 7:288–291

Kawaguchi S, Ishida A, King R, Raymond B, Waller N, Constable A, Nicol S, Wakita M, Ishimatsu A (2013) Risk maps for Antarctic krill under projected Southern Ocean acidification. Nat Clim Change 3:843–847

Kurihara H (2008) Effects of CO_2-driven ocean acidification on early developmental stages of invertebrates. Mar Ecol Prog Ser 373:275–284

Lewis CN, Brown KA, Edwards LA, Cooper G, Findlay HS (2013) Sensitivity to ocean acidification parallels natural pCO_2 gradients experienced by Arctic copepods under winter sea ice. Proc Natl Acad Sci USA 110(51):4960–4967

Meinshausen M, Smith SJ, Calvin K, Daniel JS, Kainuma MLT, Lamarque JF, Matsumoto K, Montzka SA, Raper SCB, Riahi K, Thomson A, Velders JM, Vuuren DP (2011) The RCP greenhouse gas concentrations and their extensions from 1765 to 2300. Clim Change 109:213–241

Munday PL, Dixson DL, Donelson JM, Joes GP, Pratchett MS, Devitsina GV, Doving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. Proc Natl Acad Sci USA 106(6):1848–1852

Munday PL, Dixson DL, McCormick MI, Meekab M, Ferrari MCO, Chivers DP (2010) Replenishment of fish populations is threatened by ocean acidification. Proc Natl Acad Sci USA 107:12930–12934

Nagelkerken I, Munday PL (2016) Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level resonses. Glob Change Biol 22:974–989

Nicol S, Bowie A, Jarman SN, Lannuzel D, Meiners KM, Van Der Merwe P (2010) Southern Ocean iron fertilization by baleen whales and Antarctic krill. Fish Fish 11:203–209

Perissinotto R, Pakhomov EA, McQuaid CD, Froneman PW (1997) In situ grazing rates and daily ration of Antarctic krill *Euphausia superba* feeding on phytoplankton at the Antarctic Polar Front and the Marginal Ice Zone. Mar Ecol Prog Ser 160:77–91

Portner HO, Farrell AP (2008) Physiology and climate change. Science 322:690–692

Quetin L, Ross RM (1984) Depth distribution of developing *Euphausia superba* embryos, predicted from sinking rates. Mar Biol 79:47–53

Ratnarajah L, Nicol S, Kawaguchi S, Townsend AT, Lannuzel D, Meiners KM, Bowie AR (2016) Understanding the variability in the iron concentration of Antarctic krill. Limnol Oceanogr 61(5):1651–1660

Saba GK, Schofield O, Torres JJ, Ombres EH, Steinberg DK (2012) Increased feeding and nutrient excretion of adult Antarctic krill, *Euphausia sperba*, exposed to enhanced carbon dioxide (CO_2). PLoS ONE 7:1–12

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng TH, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science 305:367–371

Schmidt K, Atkinson A, Steigenberger S, Fielding S, Lindsay MCM, Pond DW, Tarling GA, Klevjer TA, Allen CS, Nicol S, Achterberg EP (2011) Seabed foraging by Antarctic krill: impactions for stock assessment, bentho-pelagic coupling, and the vertical transfer of iron. Limnol Oceanogr 56:1411–1428

Simpson SD, Munday PL, Wittenrich ML, Manassa R, Dixson DL, Gagliano M, Yan HY (2011) Ocean acidification erodes crucial auditory behaviour in a marine fish. Biol Lett 7:917–920

Strand SW, Hamner WM (1990) Schooling behavior of Antarctic krill (*Euphausia superba*) in laboratory aquaria: reactions to chemical and visual stimuli. Mar Biol 106:355–359

Tovar-Sanchez A, Duarte CM, Hernandez-Leon S, Sanudo-Wilhelmy SA (2007) Krill as a central node for iron cycling in the Southern Ocean. Geophys Res Lett 34:L11601

Tuomainen U, Candolin U (2011) Behavioural responses to human-induced environmental change. Biol Rev 86:640–657

van Vuuren DP, Edmonds J, Kainuma M, Riahi K, Thomson A, Hibbard K, Hurtt GC, Kram T, Krey V, Lamarque JF, Masui T, Meinshausen M, Nakicenovic N, Smith SJ, Rose SK (2011) The representative concentration pathways: an overview. Clim Change 109:5–31

Whitehouse MJ, Atkinson A, Rees AP (2011) Close coupling between ammonium uptake by phytoplankton and excretion by Antarctic krill, *Euphausia superba*. Deep-Sea Res I 58:725–732

Wittmann AC, Portner HO (2013) Sensitivities of extant animal taxa to ocean acidification. Nat Clim Change 3:995–1001