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Potential ecotoxicological effects of elevated bicarbonate ion concentrations on marine organisms

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ABSTRACT

Recently, a novel method for carbon capture and storage has been proposed, which converts gaseous CO_2 into aqueous bicarbonate ions (HCO_3^-), allowing it to be deposited into the ocean. This alkalinization method could be used to dispose large amounts of CO_2 without acidifying seawater pH, but there is no information on the potential adverse effects of consequently elevated HCO_3^- concentrations on marine organisms. In this study, we evaluated the ecotoxicological effects of elevated concentrations of dissolved inorganic carbon (DIC) (max 193 mM) on 10 marine organisms. We found species-specific ecotoxicological effects of elevated DIC on marine organisms, with EC50-DIC (causing 50% inhibition) of 11–85 mM. The tentative criteria for protecting 80% of individuals of marine organisms are suggested to be pH 7.8 and 11 mM DIC, based on acidification data previously documented and alkalinization data newly obtained from this study. Overall, the results of this study are useful for providing baseline information on ecotoxicological effects of elevated DIC on marine organisms. More complementary studies are needed on the alkalinization method to determine DIC effects on seawater chemistry and marine organisms.

1. Introduction

Anthropogenic increases in atmospheric carbon dioxide (CO_2) are causing the Earth's ocean to acidify, which is then driving a change in carbonate chemistry by reducing the concentration of carbonate ions (CO_3^{2-}) and increasing concentrations of bicarbonate ions (HCO_3^-) (Orr et al., 2005). Ocean acidification inhibits the growth of living organisms composed of $CaCO_3$, such as coral and coccolithophorids (Orr et al., 2005; Hoegh-Guldberg et al., 2007; Beaufort et al., 2011). Other organisms, such as sea urchins and fish larvae, might be inhibited by declines in pH as slight as 0.1–0.2 pH (Munday et al., 2009; Crim et al., 2011; Watson et al., 2012; Sung et al., 2014). Although carbon capture and storage (CCS) techniques could reduce atmospheric CO_2, direct injection of CO_2 into the oceans may provide potential risks to marine ecosystems (Noble et al., 2012). An alternative technology for reducing atmospheric CO_2 has been proposed wherein CO_2 is converted and concentrated into HCO_3^- by the accelerated weathering of limestone (AWL) technique (Lee et al., 2017; Renforth and Henderson, 2017). Bicarbonate ions are the dominant form of dissolved inorganic carbon (DIC) in seawater, which can then be discharged directly into the ocean. This alkalinization approach would be less prone than direct CO_2 injections to reduce seawater pH, and would thus reduce the ecological risk of oceanic CCS to marine ecosystems.

Recently, KEPRI (Korea Electric Power Research Institute) has proposed a method for alkalinizing CO_2 (producing highly concentrated HCO_3^-) using the following reactions (Lee et al., 2017) (Eqs (1) and (2)):

$$CaO(s) + \text{seawater}\ [H_2O(l) + Mg^{2+}(aq) + \ldots] \rightarrow Ca^{2+}(aq) + Mg(OH)_2(s) \downarrow \quad (1)$$

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$$Mg(OH)_2(s) + 2CO_2(g) + H_2O(l) \rightleftarrows Mg^{2+} + 2HCO_3^-(aq) + MgCO_3(s) + H_2O(l) \quad (2)$$

With this method, DIC could be produced at a concentration of more than 100 times greater than ocean background concentrations (i.e., ~15 kg of CO_2 per ton of seawater). However, high concentrations of HCO_3^- could have negative effects on various marine organisms. Previous studies have shown that elevated concentrations of DIC can affect freshwater organisms adversely (Hoke et al., 1992; Harper et al., 2014; Vera et al., 2014; Ciparis et al., 2015). However, toxic effects of elevated DIC concentrations on marine life have not yet been studied.

In this study, we evaluated the ecotoxicological effects of elevated DIC concentrations on various types of marine organisms. We also compared ecotoxicological effects caused by changes in pH and DIC related to acidification and alkalinization methods. Lastly, we summarize the advantages and disadvantages of using the alkalinization method for CCS and propose future research directions.

2. Materials and methods

2.1. Experimental setting: production of HCO_3^- by alkalinization

Concentrations of DIC in seawater increased by converting gaseous CO_2 into aqueous HCO_3^- with magnesium hydroxide (Eq. (2)). To accomplish this, 1 L of artificial seawater and 13.0 g of magnesium hydroxide ($Mg(OH)_2$, Sigma-Aldrich, St. Louis, MO) were mixed with a magnetic stirrer for 30 min in a 2-L glass beaker. Fifteen percent CO_2 gas were injected at a rate of 500 mL min^{-1} (using a mass flow controller) while we observed changes in pH values (SevenCompact, Mettler Toledo, Greifensee, Switzerland). When the reactant reached a pH of 7.8, we stopped the flow of CO_2 gas and recorded the resultant change in pH. When the pH did not change further during a subsequent 1-h period, the resulting product was filtered through a 0.45 μm cellulose acetate filter, creating a stock solution of elevated DIC concentration (filtrate) that was then poured into 40-mL vials (without voids) and sealed with a Teflon-coated screw cap. To analyze the amount of gaseous CO_2 converted into aqueous HCO_3^-, the sample aliquot was diluted five times with artificial seawater and then the DIC concentration was measured using a total organic carbon analyzer (Shimadzu TOC-L) with a non-dispersive infrared detector (Shimadzu, Kyoto, Japan). The concentration of magnesium ions was measured with an ion-selective electrode (C-CIT Sensors, Wädenswil, Switzerland).

2.2. Exposure tests on various marine organisms

We assessed the potential ecotoxicological effects of elevated DIC concentrations on 10 marine species, including a marine bacterium (*Vibrio fischeri*), three microalgae (*Dunaliella salina*, *Isochrysis galbana*, and *Nannochloropsis oculata*), a copepod (*Tigriopus japonicus*), a rotifer (*Brachionus plicatilis*), an amphipod (*Monocorophium acherusicum*), an echinoderm (*Strongylocentrotus nudus*), a bivalve (*Crassostrea gigas*), and a fish (*Cyprinodon variegatus*). The prepared stock solution was diluted with sterile natural seawater to several different concentrations of DIC, which were then used to conduct exposure tests on marine organisms. Detailed experimental conditions and testing methods are provided in Table 1. Exposed concentrations of DIC ranged from 3.0 mM to 190 mM, while pH values ranged from 7.61 to 8.45. Each exposure test was conducted in triplicate or quadruplicate with 7–13 DIC concentrations (Table 1).

After 30 min of exposure to elevated DIC, we measured bacterial bioluminescence inhibition using a luminescent bacteria toxicity measurement apparatus (N-TOX, NeoEnBiz Inc., Korea) by the standard method (ISO 11348-2008) (ISO, 2007). Ecotoxicity tests using three microalgae species were performed following the standard ASTM E1218-97 protocol (ASTM, 1997). Inhibition of growth rate on microalgae to elevated DIC concentrations was measured after 72 h of exposure. Lethal toxic effects of elevated DIC concentrations were evaluated (after exposures for 48 h, 24 h, and 96 h) for a copepod, a rotifer, and an amphipod, according to the standard protocols, ASTM E2317-04, ASTM E1440-91, and ASTM E1367-03, respectively (ASTM, 2012a, 2012b, 2014). A sea urchin fertilization test was conducted according to guidelines established by the US EPA method 1008.0 (US EPA, 2002). Finally, mortalities of test organisms, such as bivalve and fish, were measured after exposure to elevated DIC concentrations for 96 h, according to previously developed protocols (US EPA, 2002; Kurihara et al., 2007).

2.3. Data collection: enrichment of pCO_2 (acidification)

To compare ecotoxicological effects between alkalinity and acidity, data from the literature on ecotoxicity to elevated pCO_2 were collected and reviewed for different marine organisms (Table S1 of the Supplementary Materials (S)). Most previous studies assessing lethal and sub-lethal effects of oceanic acidification on marine organisms and on CCS have been performed after exposure to lowered pH in seawater (using injected CO_2 gas). We collected 102 data points on the ecotoxicological effects of pCO_2, pH, and DIC on 19 marine organisms (Kikkawa et al., 2004; Wu et al., 2010; Sung et al., 2010; Crim et al., 2011; Basallote et al., 2012; Dickinson et al., 2012; Watson et al., 2012; Bignami et al., 2013; Moon et al., 2013a, 2013b; Tatters et al., 2013; Van de Waal et al., 2013; Sung et al., 2014; Eberlein et al., 2016). Exposure concentrations of pCO_2 were from 196 to 97,705 ppmv, and the corresponding pH values ranged from 5.50 to 8.30. DIC concentrations were calculated by a CO2SYS program (http://cdiac.ornl.gov/ftp/co2sys) based on pH, pCO_2, salinity, and temperature (Lewis and Wallace, 1998).

2.4. Analysis of toxicity data

Dose-response curves were plotted using DIC concentrations and ecotoxicological effects data for marine organisms and fitted with a sigmoid relationship using SigmaPlot for Windows (Version 10.0, SPSS, Chicago, IL). We calculated effective concentrations (EC) causing 20% (EC20), 50% (EC50), and 80% (EC 80) inhibitions. These inhibitions were based on the results of our ecotoxicological tests of marine organisms subjected to our DIC concentration gradient in seawater samples, using ToxCalc™ software (Ver. 5.0, Tidepool Scientific Software, McKinleyville, CA).

3. Results and discussion

3.1. Changes in water chemistry

The pH, Mg, and DIC concentrations in the highly concentrated bicarbonate seawater produced via the alkalinization method are provided in Fig. S1. pH values declined drastically after injection of gaseous CO_2 and reached 7.8 about 360 min after the start of the reaction (Fig. S1a). During the same period, concentrations of Mg ions increased 4.8 times more than that of controls (without $Mg(OH)_2$), while DIC increased 42 times more than that of controls (DIC concentrations after 360 min was 293 mM; Mg concentration was 4550 mg L^{-1}) (Fig. S1b-c). Magnesium hydroxide changed into Mg ions (producing HCO_3^-) and the concentration increased as reaction time increased. DIC concentrations were correlated well

Table 1
Experimental conditions and testing organisms for evaluation of ecotoxicological effects of elevated dissolved inorganic carbon (DIC) concentrations used in this study.

Taxon & Species	DIC concentrations	Bicarbonate concentrations	pH	Replicates	Exposure duration	Endpoints	Test methods & References
	(mM)	(mM)		(n)	(h)		
Bacterium							
Vibrio fischeri	3.0−190 [a] (8 levels)	2.8−180	7.72−8.04	3	0.5	Luminescence inhibition	ISO 11348-2008 (ISO, 2007)
Microalgae							
Dunaliella salina	4.0−180 (10 levels)	3.8−170	7.61−8.01	4	72	Growth rate inhibition	ASTM E1218-97 (ASTM, 1997)
Isocrysis galbana	3.0−180 (8 levels)	2.8−170	7.87−8.05	4	72	Growth rate inhibition	
Nannochloropsis oculata	4.0−140 (10 levels)	3.9−140	8.03−8.37	4	72	Growth rate inhibition	
Copepod							
Tigriopus japonicus	3.0−170 (8 levels)	2.9−160	8.00−8.23	4	48	Mortality	ASTM E2317-04 (ASTM, 2012b)
Rotifer							
Brachions plicatilis	4.0−140 (7 levels)	3.8−140	7.89−8.09	4	24	Mortality	ASTM E1440-91 (ASTM, 2012a)
Amphipod							
Monocorophium acherusicum	3.0−170 (8 levels)	2.9−160	8.00−8.23	4	96	Mortality	ASTM E1367-03 (ASTM, 2014)
Echinoderm							
Strongylocentrotus nudus	3.0−140 (13 levels)	3.0−130	7.83−8.00	3	0.66	Fertility inhibition	US EPA 1008.0 (US EPA, 2002)
Bivalve							
Crassostrea gigas	5.0−180 (7 levels)	4.9−170	8.06−8.29	4	96	Mortality	Kurihara et al., 2007
Fish							
Cyprinodon variegatus	4.0−190 (12 levels)	3.1−190	7.75−8.45	4	96	Mortality	US EPA 1004.0 (US EPA, 2002)

[a] Test solutions for DIC exposure were prepared using the serial dilution method.

with the Mg^{2+} concentrations (Fig. S1d). Thus, when magnesium hydroxide was added to seawater and injected CO_2 reacted with it, HCO_3^-, CO_3^{2-}, and OH^- ions were formed, and subsequent reactions between these ions reduced the pH of seawater. The concentration of HCO_3^- increases when CO_2 and OH^- reacted with HCO_3^- to form CO_3^{2-}, which in turn decreases the concentration of HCO_3^-. Carbonate ions are formed by OH^- ions, which increase in the concentration soon after injecting CO_2, but then they react with calcium ions in seawater to form calcium carbonate (Zhao et al., 2013, 2017).

Acidification data showed that pH in seawater declined with increased pCO_2 (Fig. 1a). Acidified seawater (using injected gaseous CO_2) declined to a minimum of pH 5.5 for exposure tests on marine organisms (Table S1). Calculated DIC concentrations increased only slightly to a maximum of 7.7 mM, indicating that the acidification method slightly increased DIC concentrations. In contrast, HCO_3^- production by the alkalinization method (as applied in our study) increased DIC concentrations tremendously. The maximum DIC concentration obtained using the alkalinization method was 193 mM, which was 25 times greater than the concentration obtained using the acidification method (Lee et al., 2017). Thus, the alkalinization method can concentrate a large amount of gaseous CO_2 into aqueous HCO_3^- without lowering the pH of seawater.

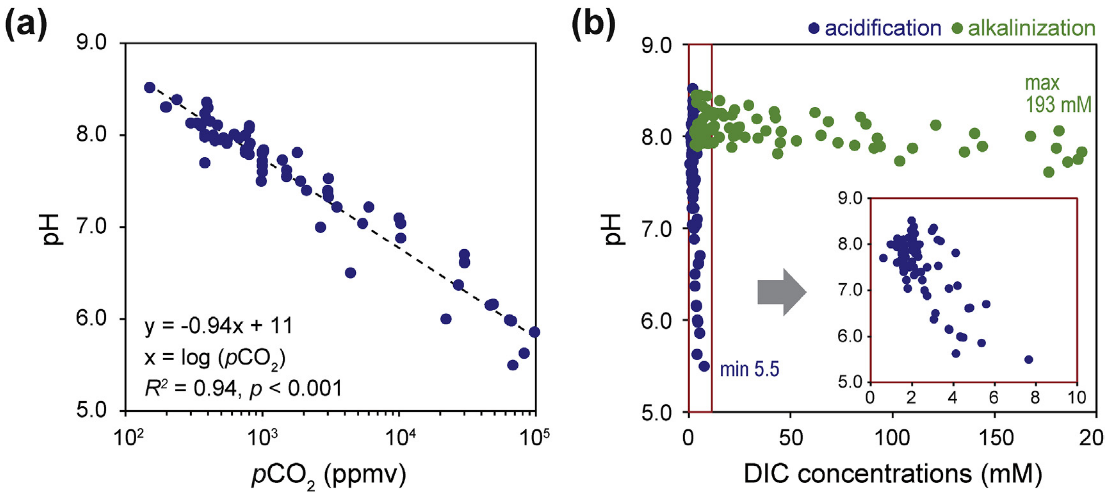

Fig. 1. Scatter plots generated using acidification and alkalinization methods: (a) between pCO_2 and pH and (b) between DIC concentrations and pH in water. Data on pCO_2, pH values, and DIC concentrations obtained from previous acidification studies given in Table S1. DIC concentrations by alkalinization method were obtained from the present study.

3.2. Ecotoxicological effects of elevated DIC

Concentrations of Mg ions were elevated by alkalinization (Fig. S1), but our exposure tests on marine organisms were conducted with diluted stock solutions, at concentrations that should not cause Mg toxicity (Table S2). Rather, it is likely that any ecotoxicological effects on organisms in this study can be attributed to elevated HCO_3^- concentration. Our results suggested a dose-response relationship between DIC concentration and degree of toxic effects (Fig. 2 and Table S3). Ecotoxicological effects in response to elevated DIC concentrations were species-specific and effective concentrations (i.e., EC20, EC50, and EC80) varied among species (Fig. S2) (e.g., EC50 values of DIC among species ranged from 11 mM to 85 mM).

The mortality rate for the copepod *T. japonicus* was the most sensitive endpoint at the lowest exposure concentration of DIC, followed by those of the amphipod *M. acherusicum* and the rotifer *B. plicatilis*. Bioluminescence inhibition of the bacterium *V. fischeri* was not observed at any of the exposure DIC concentrations tested (range: 3.0–190 mM, Table S3). Growth inhibition effects on microalgae varied tremendously among species (Fig. 2 and Table S3). For example, *D. salina* was more sensitive than the other two microalgae species (*I. galbana* and *N. oculata*), suggesting that microalgae differ in their toxicological responses to DIC concentrations.

The mode of HCO_3^- toxicity may result from a disruption in ionic regulation (Ciparis et al., 2015; Harper et al., 2014). A previous study suggested that ionoregulation in freshwater organisms (via ionocytes) is a critical mechanism regulating homeostasis (Harper et al., 2014). In particular, ion exchanges through the Na^+/K^+ pump (Na^+/K^+ ATPase) and HCO_3^-/Cl^- co-transporter (i.e., ionocytes excrete HCO_3^- in exchange for Cl^-) may be inhibited by elevated HCO_3^- concentrations (Hoke et al., 1992; Harper et al., 2014; Vera et al., 2014; Ciparis et al., 2015). Therefore, freshwater organisms cannot maintain intracellular and hemolymph acid-base balance if inhibited by elevated HCO_3^- (Vera et al., 2014). Elevated concentrations of some dissolved ions were toxic to freshwater and marine organisms, which is a physiological response to ion imbalance (SETAC, 2004). Potentially-toxic ions include K^+, HCO_3^-, and Mg^{2+} for freshwater organisms, whereas K^+ and HCO_3^- ions are

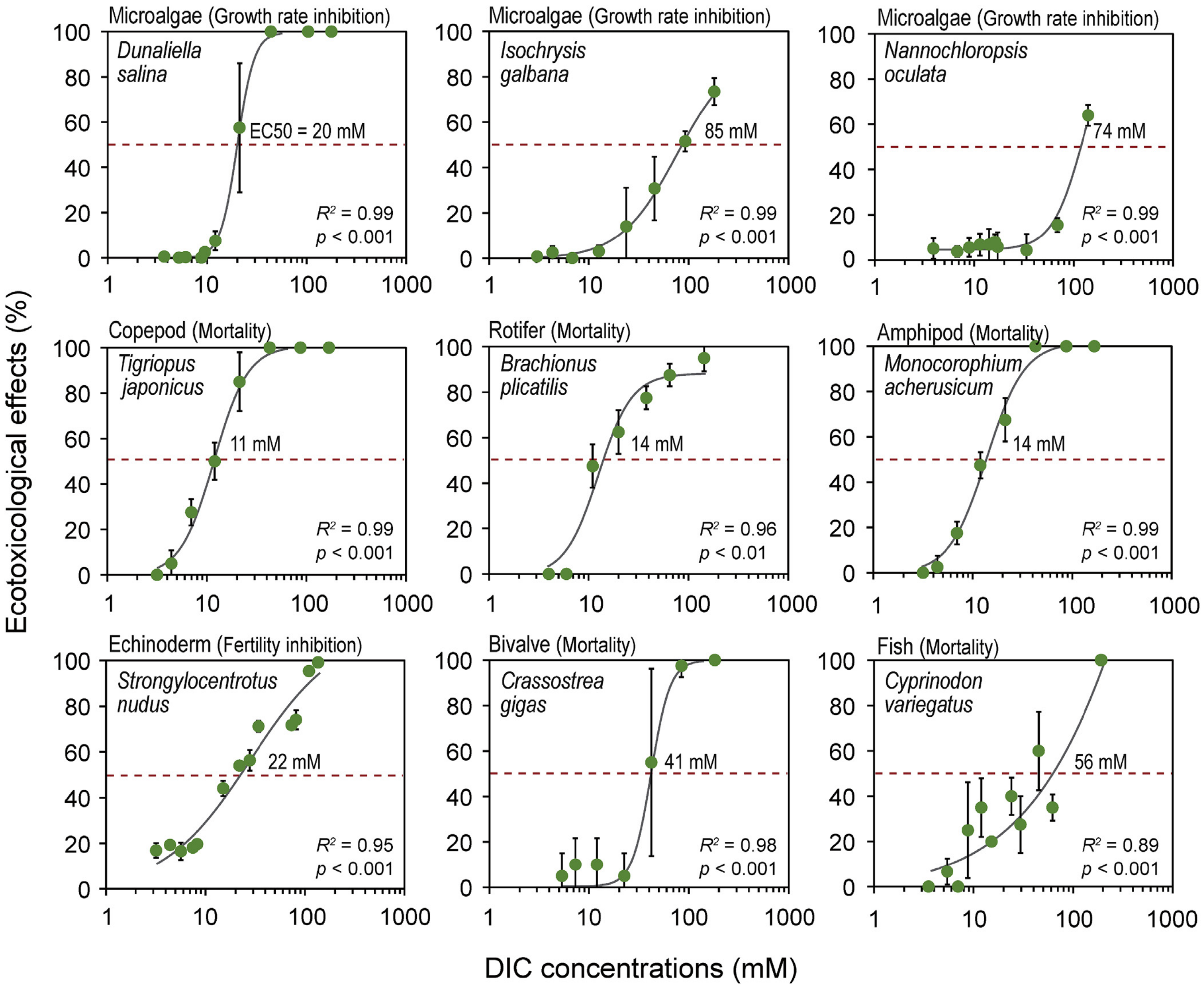

Fig. 2. Dose-response relationship between DIC concentrations and ecotoxicity of various marine organisms. The ecotoxicology data were fitted to sigmoid curves. Data on bacterium *Vibrio fischeri* was not included in this figure because no inhibitions of bioluminescence were observed (Table S1). The toxicological endpoints and effective concentrations are presented in Table 1 and Fig. S2.

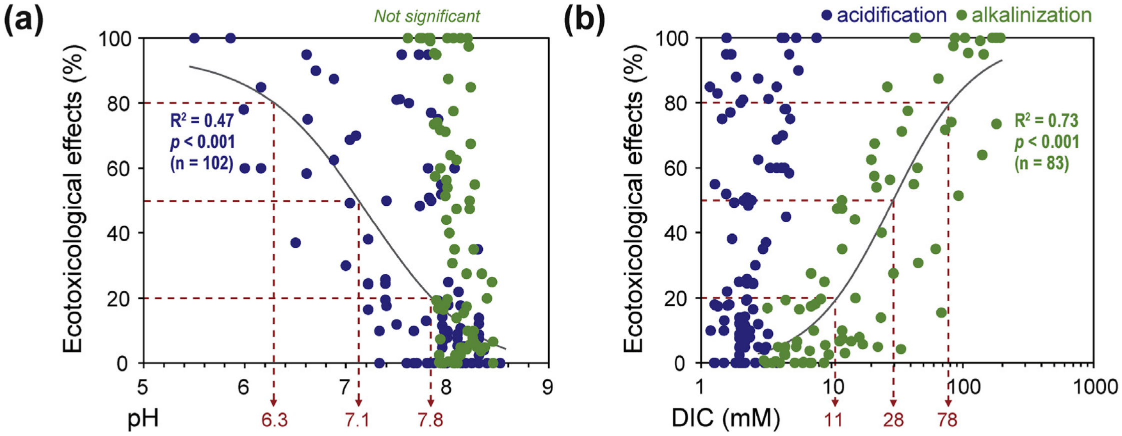

Fig. 3. Scatter plots of ecotoxicological effects on various marine organisms tested under the conditions of acidification (reviewed data, provided in Table S1) and alkalinization (the present data, provided in Table S3): (a) between pH in water and its inhibitory effects and (b) between DIC concentration and its inhibitory effects.

generally considered to be potentially toxic to marine organisms (SETAC, 2004). Until recently, there have been few studies examining the toxicity of the HCO_3^- to freshwater organisms and no studies on toxicity to marine organisms. The ion imbalance toxicity on marine organisms may be the ultimate mode of toxic action, but this supposition requires further investigation.

3.3. *Effective pH values and DIC concentrations*

In our literature review of seawater acidification studies, we found a significant dose-response relationship ($R^2 = 0.47$; $p < 0.001$) between pH and its ecotoxicological effects on 19 marine organisms (Fig. 3a). Similarly, the alkalinization method (applied in our study) showed a significant dose-response relationship between the ecotoxicological effects on marine organisms and DIC concentrations ($R^2 = 0.73$; $p < 0.001$) (Fig. 3b). That is, ecotoxicological impacts to marine organisms depend strongly on pH values in the acidification method and on DIC concentrations in the alkalinization method. The pH value of marine waters is usually stable (about 8.0), because buffering is sufficient to maintain pH values in fairly consistent manner (CCME, 1999). The ecotoxicological effects on marine organisms associated with reductions in pH were also species-specific relative to DIC concentration. Some species of echinoids (e.g., *Strongylocentrotus nudus*), bivalves (e.g., *Tridacna squamosa*), and gastropods (e.g., *Haliotis kamtschatkana*) are very sensitive to small reductions in pH ($\Delta pH = 0.1–0.2$) (Crim et al., 2011; Watson et al., 2012; Sung et al., 2014).

Effective pH values causing toxic effects in marine organisms were calculated to be 7.8 (20% inhibition), and 7.1 (50% inhibition), and 6.3 (80% inhibition). Comparable effective concentrations for DIC were 11 mM (EC20), 28 mM (EC50), and 78 mM (EC80), respectively. Thus, we suggest tentatively that pH 7.8 and 11 mM DIC are reasonable criteria for protecting 80% of individuals of marine organisms. Marine organisms have generally similar ecotoxicological sensitivities on elevated DIC to freshwater organisms. A previous study showed that the LC50 (median lethal concentration) of DIC (HCO_3^-) on freshwater zooplankton *Ceriodaphnia dubia*, freshwater mussel *Lampsilis siliquoidea*, pallid sturgeon *Scaphirhynchus albus*, and shovelnose sturgeon *Scaphirhynchus platorynchus* were 11 mM, 14 mM, 14 mM, and 17 mM HCO_3^-, respectively (Harper et al., 2014). Other previous studies reported similar results, LC50 values of HCO_3^- on freshwater zooplanktons *C. dubia* and *Daphnia magna* were 12–16 mM and 14–26 mM, respectively (Hoke et al., 1992; Vera et al., 2014). In this study, we could not elucidate the specific mechanism causing DIC toxicity on marine organisms, but we found that potential ecotoxicological effects seem to occur only at great concentrations.

3.4. *Implication for carbon dioxide capture and ocean storage*

Applying the acidification method (injection of CO_2 directly into the ocean) would cause a decline in pH and could lead to a variety of toxic effects on marine organisms (Fabry et al., 2008; Guinotte and Fabry, 2008; Crim et al., 2011; Campbell et al., 2014; Lewis et al., 2016). In contrast, the alkalinization should be considered as an alternative CCS method because it can be used to dispose of a large amount of atmospheric CO_2 in the ocean (in the form of DIC) without reducing the pH of the seawater (Renforth and Henderson, 2017). The density of highly-concentrated DIC seawater is about 0.1% greater than that of normal seawater. Thus, when highly-concentrated DIC seawater is disposed into the ocean, it is likely that it would sink to deeper layers (Rau, 2011; Lee et al., 2017). This sinking would prevent CO_2 from getting re-emitted into the atmosphere and thus minimize its impact on marine ecosystems.

However, marine organisms could be affected by elevated concentrations of DIC, as shown in this study. In addition, the production of highly-concentrated DIC is based on the assumption that Mg ions present in natural seawater are used in the production of DIC (Eq. (1)), which could lead to a deficiency of Mg in seawater, at least locally. Because Mg is an essential element in phytoplankton photosynthesis, a limitation of Mg could lead eventually to a decline in primary production and a decline in the movement of organic carbon (as phytoplankton and/or particulate organic carbon) to the deep ocean (viz., biological carbon pump). In addition, after a long period of time due to the deep seawater circulation, high-concentrated DIC water masses in bottom layer would rise eventually to the surface (Libes, 2009). Overall, CCS based on the alkalinization method has several advantages, but it also has risks and so needs further consideration. In particular, further research is needed, specifically addressing: (1) changes of *in situ* seawater chemistry in response to alkalinization, (2) mechanisms responsible for bicarbonate toxicity on estuarine and marine organisms, and (3) integrated ecological risk assessments for elevated DIC.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.05.057.

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<Supplementary Materials>

Potential ecotoxicological effects of elevated bicarbonate ion concentrations on marine organisms

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Supplementary Tables

Table S1. Summary of previously reported ecotoxicological effects of elevated CO_2 on marine organisms.

Taxon & species		Endpoints	pCO_2 (ppmv)	pH	Calculated DIC (mM)	Inhibition (%)	References
Bacteriim	*Vibrio fischeri*	Luminescence inhibition	380	7.70	0.61	0.0	Sung et al., 2010
			1000	7.60	1.28	0.0	
			3000	7.40	2.43	50.0	
			10000	7.10	4.20	70.0	
			30000	6.70	5.58	90.0	
Microalgae	*Alexandrium fundyense*	Growth rate inhibition	237	8.39	2.08	0.0	Eberlein et al., 2016
			813	7.91	2.22	19.3	
			1018	7.82	2.25	50.9	
	Chaetoceros	Growth rate inhibition	196	8.31	1.96	2.4	Tatters et al., 2013
			199	8.31	1.96	0.0	
			333	8.13	2.02	0.0	
			355	8.10	2.03	0.0	
			519	7.97	2.15	7.5	
			553	7.95	2.16	6.5	
	Coscinodiscus	Growth rate inhibition	196	8.31	1.96	0.0	Tatters et al., 2013
			199	8.31	1.96	14.0	
			333	8.13	2.02	0.0	
			355	8.10	2.03	0.0	
			519	7.97	2.15	10.0	
			553	7.95	2.16	0.0	
	Cylindrotheca	Growth rate inhibition	196	8.31	1.96	4.8	Tatters et al., 2013
			199	8.31	1.96	0.0	
			333	8.13	2.02	10.8	
			355	8.10	2.03	0.0	
			519	7.97	2.15	0.0	
			553	7.95	2.16	0.0	
	Navicula	Growth rate inhibition	196	8.31	1.96	0.0	Tatters et al., 2013
			199	8.31	1.96	12.2	
			333	8.13	2.02	5.2	
			355	8.10	2.03	7.7	
			519	7.97	2.15	0.0	
			553	7.95	2.16	4.8	
	Phaeodactylum tricornutum	Growth rate inhibition	388	8.18	1.98	5.2	Wu et al., 2010
			388	8.16	2.00	5.2	

Table S1. (Continued).

Taxon & species		Endpoints	pCO$_2$ (ppmv)	pH	Calculated DIC (mM)	Inhibition (%)	References
			1000	7.82	2.14	0.0	
			1000	7.80	2.15	0.0	
	Pseudo-nitzxchia	Growth rate inhibition	196	8.31	1.96	8.9	Tatters et al., 2013
			199	8.31	1.96	0.0	
			333	8.13	2.02	0.0	
			355	8.10	2.03	0.0	
			519	7.97	2.15	0.0	
			553	7.95	2.16	11.6	
	Thalassiosira	Growth rate inhibition	196	8.31	1.96	7.7	Tatters et al., 2013
			199	8.31	1.96	0.0	
			333	8.13	2.02	5.0	
			355	8.10	2.03	5.4	
			519	7.97	2.15	0.0	
			553	7.95	2.16	14.3	
	Thoracosphaera heimii	Growth rate inhibition	150	8.52	1.98	0.0	Van de Waal et al., 2013
			380	8.24	2.13	0.0	
			750	8.00	2.23	9.7	
			1400	7.73	2.29	48.4	
Copepod	*Tisbe* sp.	Mortality	395	7.99	1.26	0.0	Moon et al., 2013a
			998	7.67	1.49	10.0	
			3030	7.33	2.10	10.0	
			10300	6.88	2.73	87.5	
			30100	6.61	4.73	95.0	
Amphipod	*Monocorophium acherusicum*	Mortality	395	7.99	1.26	0.0	Moon et al., 2013a
			998	7.67	1.49	0.0	
			3030	7.33	2.10	0.0	
			10300	6.88	2.73	62.5	
			30100	6.61	4.73	58.3	
Polychaete	*Perinereis aibuhitensis*	Cellular energy allocation inhibition	390	8.18	1.95	0.0	Moon et al., 2013b
			3030	7.53	3.27	81.3	
			10300	7.04	3.78	68.8	
			30100	6.62	4.80	75.0	
Echinoderm	*Strongylocentrotus nudus*	Fertility inhibition	380	7.98	1.18	10.0	Sung et al., 2014
			380	8.01	1.27	18.0	
			450	7.94	1.27	55.0	
			550	7.91	1.45	75.0	

Table S1. (Continued).

Taxon & species		Endpoints	pCO$_2$ (ppmv)	pH	Calculated DIC (mM)	Inhibition (%)	References
			550	7.94	1.56	52.0	
			750	7.84	1.67	77.0	
			750	7.81	1.56	95.0	
			1000	7.72	1.68	95.0	
			1500	7.62	2.00	80.0	
			1500	7.55	1.71	95.0	
Bivalve	*Crassostrea virginica*	Mortality	392	8.36	3.05	4.0	Dickinson et al., 2012
			470	8.11	1.58	22.0	
			676	7.97	1.64	18.0	
			802	8.10	3.24	9.0	
	Tridacna squamosa	Mortality	416	8.15	1.84	0.0	Watson et al., 2012
			622	8.01	1.95	25.0	
			1019	7.84	2.11	50.0	
Gastropod	*Haliotis kamtschatkana*	Growth rate inhibition	400	8.30	2.97	35.0	Crim et al., 2011
			800	8.07	3.38	60.0	
			1800	7.81	4.11	60.0	
Fish	*Sparus aurata*	Mortality	436	8.00	2.36	10.0	Basallote et al., 2011
			981	7.50	2.73	12.0	
			2659	7.00	2.60	30.0	
			4406	6.50	3.13	37.0	
			22089	6.00	4.33	60.0	
			67763	5.50	7.66	100.0	
	Pagrus major	Mortality	48853	6.16	3.77	85.0	Kikkawa et al., 2004
			48853	6.16	3.77	60.0	
			97705	5.86	5.36	100.0	
			97705	5.86	5.36	100.0	
	Rachycentron canadum	Mortality	300	8.13	1.29	17.8	Bignami et al., 2013
			500	7.95	1.35	17.5	
			800	7.79	1.50	13.1	
			2100	7.40	1.60	17.7	
			3500	7.22	1.72	38.1	
			5400	7.04	1.79	49.3	

Table S2. Sub-lethal and lethal effects of $MgCl_2$ on marine fish (*Cyprinodon variegatus*).

Treatment	Concentration (mg L^{-1})	Replicates	Mobility inhibition (%, 96 h)		Mortality (%, 96 h)	
			Mean	SD	Mean	SD
Control	-	4	0	0	0	0
$MgCl_2$	625	4	0	0	0	0
	1250	4	0	0	0	0
	2500	4	0	0	0	0
	5000	4	2.5	5.0	2.5	5.0
	10000	4	15	13	15	13
	20000	4	100	0	100	0

Table S3. Summary of ecotoxicological effects of elevated DIC on marine organisms observed in this study.

Taxon & species		Endpoints	pH	Measured DIC (mM)	Inhibition (%)	
					Mean	SD
Bacterium	*Vibrio fischeri*	Luminescence inhibition	7.72	186	0.0	0.0
			7.89	94	0.0	0.0
			7.95	53	0.0	0.0
			8.10	25	0.0	0.0
			8.07	12	0.0	0.0
			8.05	8	0.0	0.0
			8.05	5	0.0	0.0
			8.04	3	0.0	0.0
Microalgae	*Dunaliella salina*	Growth rate inhibition	7.61	177	100.0	0.0
			7.73	104	100.0	0.0
			7.81	44	100.0	0.0
			7.88	21	57.5	28.5
			7.93	12	7.5	4.2
			7.92	9	2.5	1.9
			7.93	9	0.0	0.0
			7.92	6	0.3	0.5
			7.96	5	0.0	0.0
			8.01	4	0.5	1.0
	Isochrysis galbana	Growth rate inhibition	7.87	180	73.5	6.0
			7.98	93	51.5	4.4
			8.05	46	30.8	14.0
			8.09	24	14.0	17.1
			8.11	13	3.0	2.6
			8.10	7	0.0	0.0
			8.08	4	2.5	2.9
			8.05	3	0.8	1.0
	Nannochloropsis oculata	Growth rate inhibition	8.03	140	64.0	4.7
			8.16	69	15.5	3.1
			8.19	34	4.3	7.2
			8.22	17	5.8	6.5
			8.25	16	8.0	3.4
			8.25	14	6.8	7.0
			8.26	11	6.8	4.9
			8.29	9	5.5	4.1
			8.33	7	3.8	2.4
			8.37	4	5.0	4.5

Table S3. (Continued).

Taxon & species		Endpoints	pH	Measured DIC (mM)	Inhibition (%) Mean	SD
Copepod	*Tigriopus japonicus*	Mortality	8.00	168	100.0	0.0
			8.13	87	100.0	0.0
			8.20	43	100.0	0.0
			8.23	21	85.0	12.9
			8.22	12	50.0	8.2
			8.19	7	27.5	5.8
			8.13	4	5.0	5.8
			8.07	3	0.0	0.0
Rotifer	*Brachionus plicatilis*	Mortality	7.89	144	95.0	5.8
			8.01	65	87.5	5.0
			8.06	38	77.5	5.0
			8.09	20	62.5	9.6
			8.09	11	47.5	9.6
			8.08	6	0.0	0.0
			8.06	4	0.0	0.0
Amphipod	*Monocorophium acherusicum*	Mortality	8.00	168	100.0	0.0
			8.13	87	100.0	0.0
			8.20	43	100.0	0.0
			8.23	21	67.5	9.6
			8.22	12	47.5	5.8
			8.19	7	17.5	5.0
			8.13	4	2.5	5.0
			8.07	3	0.0	0.0
Echinoderm	*Strongylocentrotus nudus*	Fertility inhibition	7.83	135	99.2	0.8
			7.87	110	95.4	0.5
			7.90	81	74.1	4.2
			7.93	73	71.7	1.3
			7.98	34	71.2	2.4
			7.99	28	56.3	4.5
			8.00	22	54.0	1.7
			7.99	15	44.1	3.3
			8.00	8	19.7	1.8
			7.98	7	18.2	1.9
			7.96	6	16.5	3.8
			7.89	4	19.4	1.8
			7.91	3	16.9	3.2

Table S3. (Continued).

Taxon & species		Endpoints	pH	Measured DIC (mM)	Inhibition (%)	
					Mean	SD
Bivalve	*Crassostrea gigas*	Mortality	8.06	182	100.0	0.0
			8.21	84	97.5	5.0
			8.27	42	55.0	41.2
			8.29	22	5.0	10.0
			8.26	12	10.0	11.5
			8.20	7	10.0	11.5
			8.14	5	5.0	10.0
Fish	*Cyprinodon variegatus*	Mortality	7.83	193	100.0	0.0
			8.26	62	35.0	5.8
			8.34	30	27.5	12.6
			8.39	15	20.0	0.0
			8.44	9	25.0	21.2
			8.45	5	6.7	5.8
			8.45	4	0.0	0.0
			7.75	191	100.0	0.0
			7.93	45	60.0	17.3
			8.02	24	40.0	8.2
			8.07	12	35.0	12.9
			8.06	7	0.0	0.0

Supplementary Figures

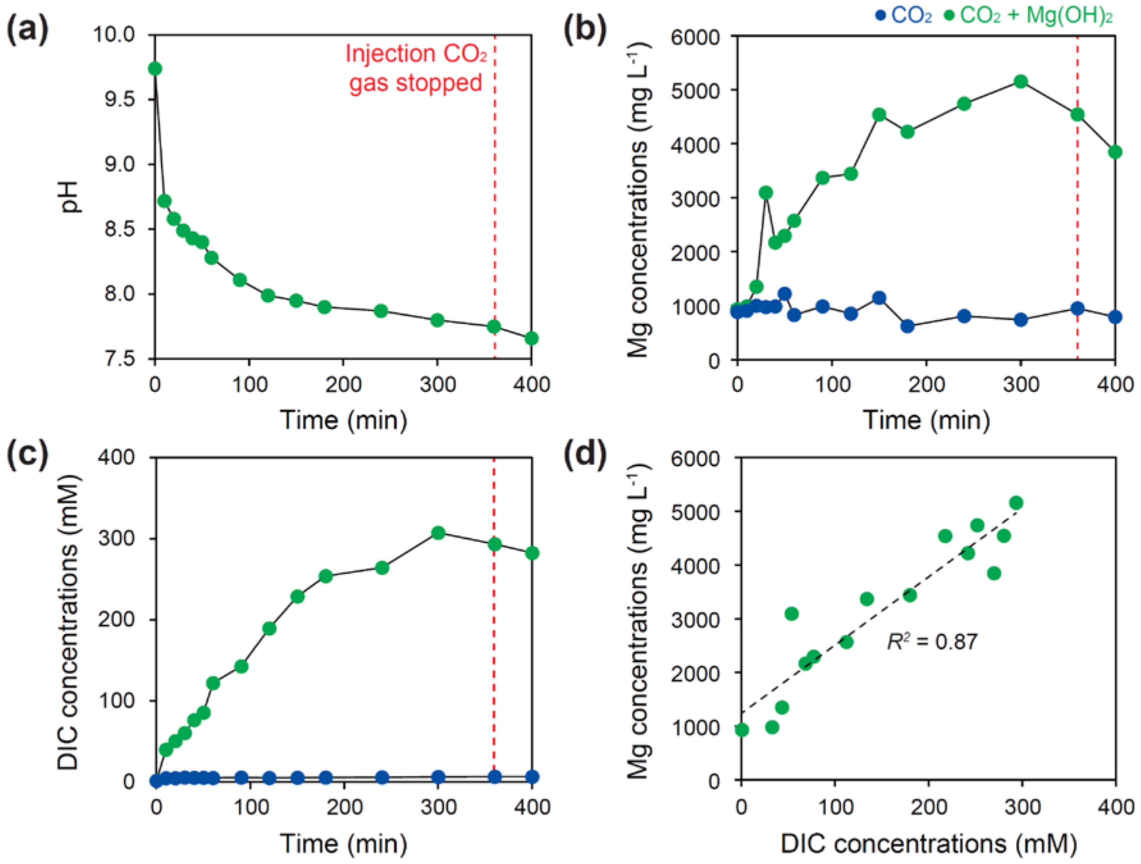

Fig. S1. (a) Changes of pH values, (b) Mg concentrations, and (c) DIC concentrations with reaction time after injection of CO_2 gas into the magnesium hydroxide solution. (d) Scatter plot between DIC concentrations and Mg concentrations in the solution. Red dotted lines indicate gaseous CO_2 injection stop times (~360 min).

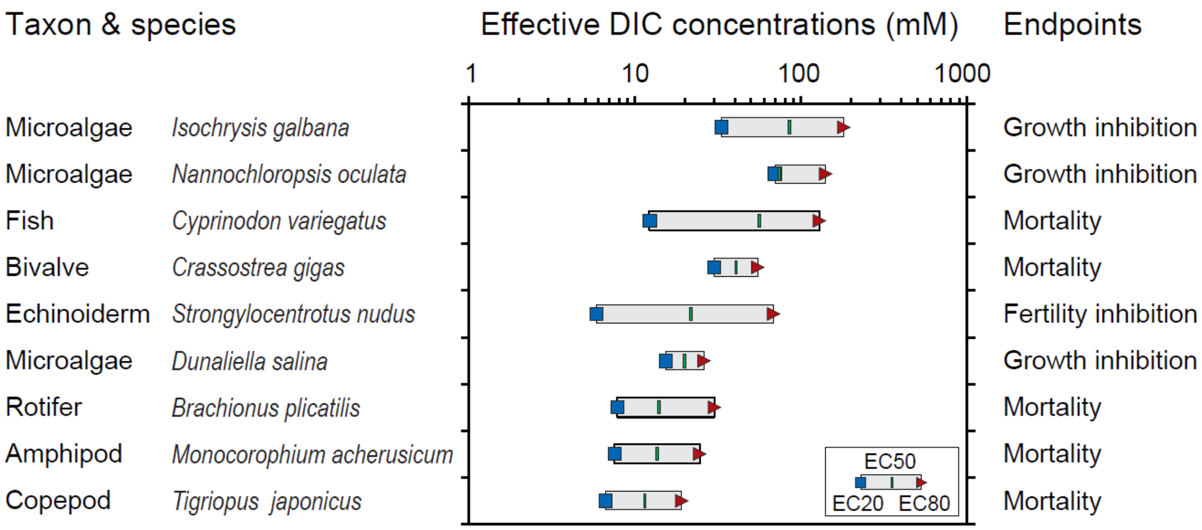

Fig. S2. Effective DIC concentrations (EC20, EC50, and EC80) of various marine organisms obtained in this study.

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