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Role of seaweeds in neutralizing the impact of seawater acidification- A laboratory study with beached shells of certain bivalves and spines of a sea urchin

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Original Article

Abstract

Ocean acidification is one of the major impacts of climate change in sea which is manifested by the decrease in hydrogen ion concentration (pH) of seawater mainly due to increased uptake of CO_2 and reduction in carbonate ions. This is a report on the dissolution rate of dead shells of four marine bivalves and spines of a sea urchin when treated with different levels of CO_2 dissolved in seawater for 48 hours which was measured gravimetrically. Dissolution of dead shells expressed as reduction in shell weight was directly proportional to the concentration of dissolved CO_2. Live thallus of green seaweed *Chaetomorpha antennina* did reduce the magnitude of dissolution rates ($P<0.05$) of all the shells and spines considerably as well as the change in pH of ambient seawater due to the addition of CO_2. The remedial property of seaweeds was more effective at lower concentrations of dissolved CO_2. The induced change in pH was restored by green seaweed only at concentrations above 250 ppm. Although we noticed strong impact of dissolved CO_2 on the dead shells of *Mactrinula plicataria* even at 100 ppm level, the remedial action by the green seaweed was maximum in *Siliqua radiata* followed by *Perna viridis*. Results of this laboratory study shows the positive role of seaweeds in neutralizing the acidification impacts.

Keywords: *Seawater acidification, carbon sequestration, seaweeds, marine bivalves, CO_2, pH, shell dissolution*

Introduction

Global anthropogenic emission of CO_2 is on the rise in atmosphere which will have significant effects on the marine systems. The average concentration of CO_2 has increased from 315 ppm in 1960 to 414 ppm in April 2019 and the anthropogenic greenhouse gas emission in the globe during the year 2000 - 2010 were the highest in history (IPCC, 2019; CO_2.earth, 2019). It is estimated that nearly one third of all CO_2 emissions for the past 200 years has been absorbed by the oceans (Sabine *et al.*, 2004). Ocean acidification refers to the gradual drop in pH of seawater going on currently due to mixing of CO_2 from atmosphere emitted through anthropogenic interventions. It is predicted that the CO_2 levels would exceed 1000 ppm and the average pH of the oceans could fall by 0.5 units (equivalent to a threefold increase in the concentration of hydrogen ions) by the year 2100 if global emissions of CO_2 continue to rise on current trends (Royal Society, 2005). Present levels of absorption of atmospheric

CO_2 by the oceans is measured as 22 million tonnes per day (Smithsonian Ocean, 2019).

A number of studies suggest that the current trend of pH reduction will have negative impacts on number of marine organisms (Jokiel, 2008; Marubini *et al*., 2008; Cohen *et al*., 2009; Albright *et al*., 2010). Reviews and technical reports available on the ocean acidification (Royal Society, 2005; Waldbusser and Salisbury, 2014; Smithsonian Ocean, 2019) have identified the gap in ocean acidification research which is still in infancy stage and they reiterate the need to launch global monitoring, experimental mesocosm and field studies rapidly so as to predict acidification impacts and to reduce the cost and challenges of climate change mitigation. Nienhuis *et al.* (2010) worked on a marine snail *Nucella lamellosa* and found that higher levels of dissolved CO_2 affects the shell dissolution rate but not the calcification rate.

Marine primary producers such as planktonic algae and seaweeds are known to relegate excess CO_2 from seawater (Kaladharan *et al*., 2009) although they are not considered as true carbon sequesters as the carbon fixed is recycled soon upon decay. Seagrasses and seaweeds are capable of growing more rapidly under elevated CO_2 levels (Zimmerman *et al*., 1997; Unsworth *et al*., 2012; Manzello *et al*., 2012). However, coralline algae do not grow so well under acidified conditions (Kufner *et al*., 2008). Large scale seaweed mariculture has been recognized as one of the climate resilient aquaculture techniques to mitigate ocean acidification (Kaladharan, 2013; Zacharia *et al*., 2015; Duarte *et al*., 2017). Several bivalve species show strong habitat association with seagrass and seaweed beds (Peterson and Heck, 2001; Marinelli and Waldbusser, 2005). In this communication we attempt to gather quantitative evidence on the impact of acidic seawater pH (in various levels of dissolved CO_2) on dead bivalve shells and on the spines of sea urchin, in the presence and absence of seaweeds under laboratory conditions.

Material and methods

Shells and seaweed samples

Small, thin and freshly beached intact shells of different marine bivalves such as *Siliqua radiata, Perna viridis, Mactrinula plicataria* and *Sunetta scripta* and the spines of seaurchin *Echinometra mathaei* were collected from the high tide line of Thirumullavaram coast (08° 54′427″ N; 76° 38′ 213″ E) along the Kerala coast. In the laboratory, these shells were thoroughly washed and cleaned with brush and freshwater to remove any attached organisms, residual organic matters, sand debris and other plankters attached to the mineralized skeletal structures of marine organisms. The moisture adhering to the shells was removed using tissue paper and dried in an oven at 60 ± 2°C for 12 hours. Shells and spine were weighed the next day using the digital balance (Sartorius CP. 225D).

Samples of seaweed, *Chaetomorpha antennina* (Bory) Kutzing were freshly collected from the Thirumullavaram coast during the low tide hours by carefully detaching them from granite stones habitat with a scalpel. Live seaweed samples were brought to the laboratory and acclimatized overnight in a 500 L tank with running seawater (32 PSU), provided with proper aeration and photoperiod. The fresh seaweed samples were cleaned with excess seawater to remove sand, debris and attached phytoplankton or other fauna if any. The moisture adhered to the fronds was removed using a tissue paper and weighed on the digital top-pan balance.

Seawater

Seawater was collected from the surface of 15 m deep station off Cochin (9°57′.681″N; 76°10′.069″E) on board FRV *Silver Pompano* and transported to the CMFRI Laboratory in plastic bins. Just before the experiment, seawater was filtered through a 0.45μ filter paper (47mm Whatmann, to remove major phytoplankton), salinity was checked and maintained at 32 PSU and the initial pH was noted using a digital pH Meter (WTW 303, Germany).

Carbonating seawater for incubation experiments

Desired levels of CO_2 (0, 100, 200, 250 and 300 ppm) was dispensed to the seawater over the ambient levels from a soda maker (Mr. Butler, Protech Appliances(P)Ltd., India) and corrected by further addition of CO_2 or seawater as the case may be and kept airtight (Kaladharan *et al*., 2009). Determined the CO_2 concentration before and after the incubation using the titration method as described by Dye (1958).

Rate of shell dissolution due to CO_2

To determine the impact of varying levels of dissolved CO_2 on the outer shells and spines of different marine organisms in the presence of seaweeds, 0.5g of each shell of different species were incubated together in 1.0 L seawater enriched with different concentrations of CO_2 such as 0, 100, 200, 250 and 300 ppm containing 5.0gm of fresh *Chaetomorpha antennina* seaweed samples in 1000 ml airtight glass bottles (Corning). Another set of five bottles with similar quantities of shells and spines and same levels of CO_2 but without 5.0g seaweed samples were maintained as control. These experimental and control sets of bottles labelled accordingly were incubated in sunlight under a running water trough for 48 hours in triplicates.

At the end of incubation period, respective water samples were transferred separately into 100 ml bottles for determining immediately the levels of CO_2 retained in it using titration method and into small beaker for determining the pH of the water using the digital pH meter. All the shells and spines were transferred carefully in to a large Petri plate, rinsed with filtered seawater and again with double distilled water. These shell samples were sorted by species using a brush and forceps with utmost care, dried in separate watch glasses at 60 ± 2°C for 12 hours before taking the final weight. Any change in their weight after the incubation with or without seaweed for 48 hours was used for calculating the rate of shell dissolution.

Results

Change in CO_2 and pH

In the presence of seaweed, *Chaetomorpha antennina* (5.0 g/l) there was significant reduction in the concentration of dissolved CO_2 and corresponding increment of pH was also observed with increase in the CO_2 concentration which was evident in each treatment with different levels of CO_2 (Table1). The recovery of pH due to the live seaweed treatment was uniform (0.02) up to 250 ppm of CO_2 and increased to 0.07 at 300 ppm of CO_2. However, the relegation of dissolved CO_2 due to the treatment of *Chaetomorpha* was maximum (26 ppm) at 200 ppm of CO_2 and beyond which gradual decrease was recorded (Table 1).

Table 1. Change in CO_2 and pH in carbonated seawater due to live seaweed samples

CO_2	Δ pH	Δ CO_2	Seaweed
(ppm)	(+)	(-)	(g)
0	0	0	0
	0	0.02	5.02
100	0.02	10	0
	0.04	27	5.034
200	0.03	13	0
	0.05	39	5.036
250	0.04	16	0
	0.06	28	5.02
300	0.08	19	0
	0.15	30	5.005

Reduction in shell weight due to exposure to dissolved CO_2

The shells of each species such as *Siliqua radiata, Perna viridis, Mactrinula plicataria, Sunetta scripta* and *Echinometra mathaei* registered considerable reduction in their shell weight when in different levels of CO_2 dissolved in seawater indicating the dissolution of carbonate materials from the skeletal structures of marine invertebrates. There was no change in the shell weight when treated with 0 ppm CO_2 (control). In the presence of seaweed *Chaetomorpha antennina* (5 g/l), the reduction in shell weight due to dissolved CO_2 was marginal compared to the weight reduction in shells when incubated with same levels of dissolved CO_2 but without the seaweed. Such reduction in shell weight was different in different species, matching with the thickness of the shell and the spines used for our study.

In the absence of seaweed, there was a marked reduction in the shell weight (mg) of *Siliqua radiata* in higher levels of dissolved CO_2 registering 12 ±0.2, 19.7 ±0.4, 27.6 ±0.65 and 36.3 ±0.38 mg in 100, 200, 250 and 300 ppm CO_2 respectively. However, in the presence of seaweed, the weight reduction in shells was only 3.7 ±0.19, 12.5 ±0.29, 21 ±0.29 and 32.1 ±0.28 mg in the order of increasing levels of dissolved CO_2 as shown in Fig. 1. With regard to the shells of green mussel *Perna viridis* there was 12.5 ±0.47, 14.2 ±0.62, 17.2 ±0.53 and 21.3 ±0.45 mg weight loss registered in 100, 200, 250 and 300 ppm CO_2 respectively. While in the presence of seaweed, the weight reduction due to the dissolved CO_2 was considerably lesser than that of its absence which registered only 6.1 ±0.78 in 100 ppm, 9.2 ±0.41 in 200 ppm, 12.5 ±0.54 in 250 ppm and 18.7 ±0.49 mg in 300 ppm of CO_2 (Fig. 2).

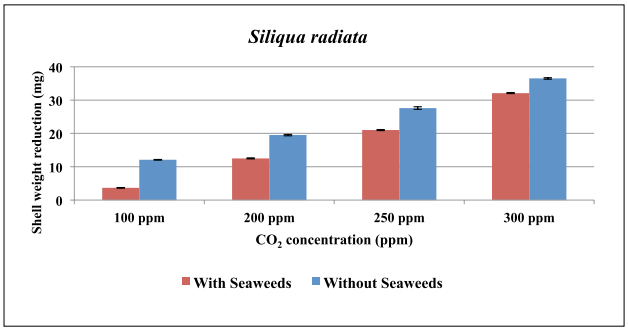

Fig. 1. Loss of shell weight (mg, mean ±SE) of *Siliqua radiata* due to exposure to dissolved CO_2.

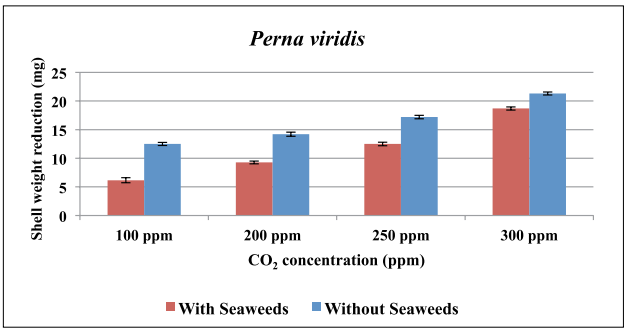

Fig. 2. Loss of shell weight (mg, mean±SE) of *Perna viridis* due to exposure to dissolved CO_2.

In the shells of *Mactrinula plicataria* there was 20.8 ±0.45, 24.1 ±1.4, 29.8 ±0.05 and 35.8 ±1.3 mg weight reduction registered in 100, 200, 250 and 300 ppm of CO_2 respectively. With the help of the seaweed the weight loss in the shells due to the dissolved CO_2 could be reduced to only 16.3 ±0.33, 20.2 ±0.45, 26.5 ±1.4 and 33.7 ±1.8 mg (Fig. 3) in 100, 200, 250, and 300 ppm of CO_2 respectively. The shells of *Sunetta scripta* collected were thick and hard and the registered weight reduction due to the contact with varying levels of CO_2 dissolved in seawater for 48 hours was 8.9 ±0.05, 9.5 ±0.24, 10.7 ±0.45 and 13.2 ±0.45 mg. With the presence of seaweed the shell weight loss was limited to only 6.2 ±0.33, 7.1 ±0.08, 8.6 ±0.52 and 12.2 ±0.37 mg in 100, 200, 250, and 300ppm of CO_2 respectively (Fig. 4).

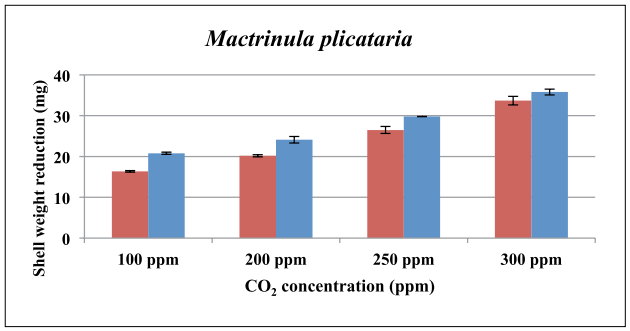

Fig. 3. Weight reduction (mg, mean ±SE) in the shells of *Mactrinula plicataria* due to exposure to dissolved CO_2

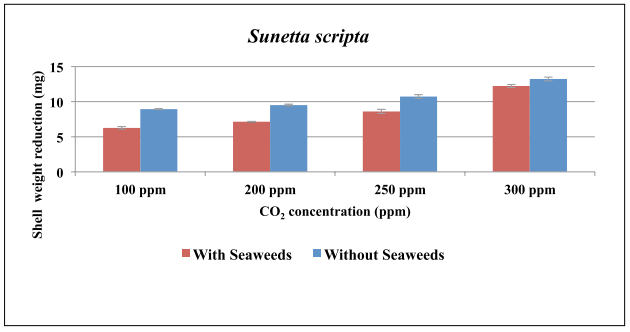

Fig. 4. Loss of shell weight (mg, mean ±SE) of *Sunetta scripta* due to exposure to dissolved CO_2

The outer spines of sea urchin, *Echinometra mathaei* also recorded notable reduction in their weight when exposed to dissolved CO_2 (Fig. 5). Although these spines were thicker than the bivalve shells they registered weight reduction of 9.3 ±0.65 mg in 100 ppm, 11.8 ±0.21mg in 200 ppm, 12.8 ±0.86 mg in 250 ppm and 16.9 ±0.57mg in 300 ppm CO_2 which could be limited to only 6.8 ±0.05, 9.8 ±0.45, 11.2 ±1.3 and 15.7 ±0.53 mg in the order of increasing levels dissolved CO_2. The results of statistical analysis of data are summarized in Table 2.

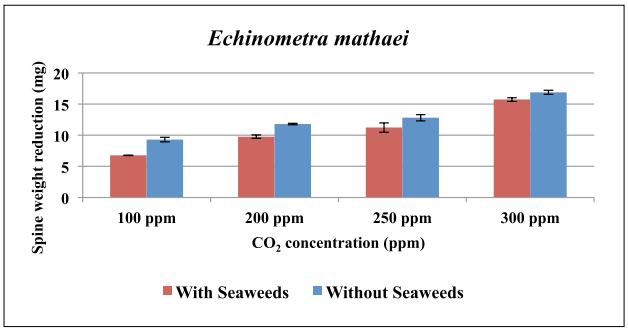

Fig. 5. Loss of shell weight (mg, mean ±SE) of *Echinometra mathaei* due to exposure to dissolved CO_2

Table 2. Result of T test showing the variation in dissolution of shells with seaweeds in different treatments with varying levels of dissolved CO_2

Species	Mean	t value	P value
Siliqua radiata	6.6	7.96	0.004
Perna viridis	4.82	5.87	0.009
Mactrinula plicataria	3.45	6.73	0.006
Sunetta scripta	2.03	5.53	0.011
Echinometra mathaei	1.82	6.56	0.007

Remedial role of live seaweed on the dissolution of shells and spines due to CO_2

In the presence of live seaweed thalli, the weight reduction in shells of four species of marine bivalves and spines of a sea urchin due to increasing levels of dissolved CO_2 was evidently lesser than that of treatments without seaweed. There was notable increase in the weight of shells saved by live seaweed which is shown in Fig. 6. The weight of the shells saved by seaweeds or would have lost if no live seaweed was different in different species closely matching with the thickness of shells. Mitigated effects of live *Chaetomorpha antennina* on the flat and thin shells of *Siliqua radiata* registered maximum (8.2 mg) and minimum in the thick spines of *Echinometra mathaei* (2.5 mg).

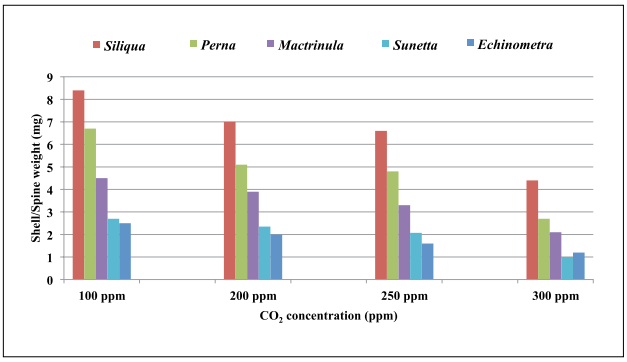

Fig. 6. Weight of shells/ spines (mg) saved by live seaweeds.

Discussion

Drop in pH indicates the increase in acidity and it interferes with the ability of ocean life to extract calcium from the water to build their shells and skeletons. As pH is logarithmic, a drop of 0.1 means roughly 30% increase in acidity (Rebecca, 2018). Being highly autotrophic, seaweed vegetation can utilize the carbon dioxide for photosynthesis which can remove the dissolved CO_2 from the seawater. Seaweed beds (Duarte and Cebrian, 1996) and seaweed farms (Chung *et al.*, 2013) are considered significant CO_2 sink and can play active role in mitigation and adaptation of climate change. It has been proved beyond doubt from a recent review (Jensen *et al.*, 2018) that seaweeds have been accepted as blue carbon sink par with other coastal and marine macrophytes.

It is largely evident from the results that the dissolution of shells of molluscs and spines of sea urchin as measured as weight reduction increased proportionately with increasing levels of CO_2 did take place even in the presence of live seaweed (Figs 1-5). However, its magnitude could be reduced due to the presence of seaweed thalli and this remedial action by seaweeds on shell dissolution was higher at lower levels of CO_2 in the ambient water (Fig. 6). This biological efficiency of green seaweed *Chaetomorpha antennina* can be attributed to sequestration of CO_2 during photosynthesis (Chung *et al.,* 2013) and subsequent release of dissolved organic carbon (DOC) by the seaweed (Barron and Duarte, 2015).

Waldbusser *et al.* (2011) have found that the shell dissolution rates in the shells of oyster (*Crassostrea virginica*) is influenced by pH and shell legacies. Although we have not considered the legacy of shells, we used only the freshly lodged shells at the intertidal beach. Ocean acidification due to increase in the levels of dissolved CO_2 is found detrimental to fertilization, settlement and post settlement growth of reef forming corals (Albright *et al.*, 2010). However, it is also unravelled that the higher levels of dissolved CO_2 affect only the shell dissolution rate but not the calcification rate in marine snail *Nucella lamellosa* (Nienhuis *et al.*, 2010). Although a strong impact of dissolved CO_2 was noticed on the shells of *Mactrinula plicataria*, even at 100 ppm level (Fig. 3), the remedial action by the seaweed was maximum in *Siliqua radiata* followed by *Perna viridis* (Fig. 6).

This valuable role played by seaweeds on shell dissolution as quantified as net reduction in shell/ spine weight was higher in lower levels of CO_2 which also suggest the need to optimise the quantity of live seaweed biomass to combat higher levels of dissolved CO_2. This trend warrants the need for more seaweed beds along the coast or extensive seaweed farms in sea to combat ocean acidification. In Korea, an innovative research approach of coastal CO_2 removal belt, CCRB (Chung *et al.*, 2013) had established huge farms of brown alga *Eklonia calva* that can draw 10 ton CO_2 per ha per year. Restoration of more habitats for seaweed growth along the coastline or large scale mariculture of seaweeds can more effectively mitigate the adverse effects of ocean acidification.

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