Marine Environmental Research 109 (2015) 9–20

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Colony-specific investigations reveal highly variable responses among individual corals to ocean acidification and warming

Javid Kavousi [a, *], James Davis Reimer [a, b], Yasuaki Tanaka [b, c], Takashi Nakamura [a, b]

[a] Graduate School of Engineering and Science, University of the Ryukyus, 1 Senbaru, Nishihara 903-0213, Okinawa, Japan
[b] Tropical Biosphere Research Center, University of the Ryukyus, Okinawa 905-0227, Japan
[c] Environment and Life Sciences, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE1410, Negara Brunei Darussalam

ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form
12 May 2015
Accepted 13 May 2015
Available online 14 May 2015

Keywords:
Scleractinian corals
Colony-specific responses
Ocean acidification
Global warming
Zooxanthellae
Bleaching

ABSTRACT

As anthropogenic climate change is an ongoing concern, scientific investigations on its impacts on coral reefs are increasing. Although impacts of combined ocean acidification (OA) and temperature stress (T) on reef-building scleractinian corals have been studied at the genus, species and population levels, there are little data available on how individual corals respond to combined OA and anomalous temperatures. In this study, we exposed individual colonies of *Acropora digitifera*, *Montipora digitata* and *Porites cylindrica* to four pCO_2-temperature treatments including 400 μatm-28 °C, 400 μatm-31 °C, 1000 μatm-28 °C and 1000 μatm-31 °C for 26 days. Physiological parameters including calcification, protein content, maximum photosynthetic efficiency, *Symbiodinium* density, and chlorophyll content along with *Symbiodinium* type of each colony were examined. Along with intercolonial responses, responses of individual colonies versus pooled data to the treatments were investigated. The main results were: 1) responses to either OA or T or their combination were different between individual colonies when considering physiological functions; 2) tolerance to either OA or T was not synonymous with tolerance to the other parameter; 3) tolerance to both OA and T did not necessarily lead to tolerance of OA and T combined (OAT) at the same time; 4) OAT had negative, positive or no impacts on physiological functions of coral colonies; and 5) pooled data were not representative of responses of all individual colonies. Indeed, the pooled data obscured actual responses of individual colonies or presented a response that was not observed in any individual. From the results of this study we recommend improving experimental designs of studies investigating physiological responses of corals to climate change by complementing them with colony-specific examinations.

1. Introduction

Unprecedented anthropogenic CO_2 emissions are considered a serious threat to global coral reefs due to resulting global warming and ocean acidification (Hoegh-Guldberg et al., 2007). Global sea surface temperatures increased approximately 0.6 °C in the last century and are predicted to increase another 2–3 °C by the end of this century (Meehl et al., 2007). Absorbance of approximately one third of the emitted CO_2 by oceans in the past century has resulted in ocean acidification (OA), which includes declines in pH and aragonite saturation state. Ocean pH has already declined by 0.1 units (Solomon, 2007) and it is predicted to drop another 0.3–0.4 units by 2100 (Orr et al., 2005). Aragonite, which has already decreased by 0.6 (from 4.6) units in tropical regions in the past hundred years, will drop to less than 2.8 by the end of this century (Kleypas et al., 1999).

Since reef-building scleractinian corals are calcifiers (producing $CaCO_3$) and aragonite is their precipitated calcium carbonate, the above-mentioned changes in ocean carbonate chemistry can result in less calcium carbonate precipitation and higher rates of dissolution of calcium carbonate structures of corals and reefs (Kleypas et al., 1999). Increases in temperatures also can have substantial impacts on reef-building corals. There have been several reports of declines in calcification rates of corals due to increases in temperature in the last decades (Tanzil et al., 2009; Cooper et al., 2008; Cantin et al., 2010). Increased temperatures can result in mass coral bleaching, loss of endosymbiotic *Symbiodinium* spp. and/or photosynthetic pigments, and ultimately

* Corresponding author.
E-mail address: javid.kavousi@gmail.com (J. Kavousi).

http://dx.doi.org/10.1016/j.marenvres.2015.05.004
0141-1136/

massive coral mortality (Goreau et al., 2000). Indeed, the expected changes in temperature and OA may cause several coral species to go extinct and also cause the decay of coral reefs worldwide (Hoegh-Guldberg et al., 2007; Carpenter et al., 2008). In spite of these predictions, unlike the effects of both thermal stress and ocean acidification, which have been generally thoroughly investigated, the impacts of combined OA and temperature on corals' physiology still requires more experiments to obtain a clearer understanding of the responses of corals.

Corals' responses to thermal stress and OA have been suggested to be genera- and species-specific (Goreau et al., 2000; Anthony et al., 2008; Ries et al., 2009; Edmunds et al., 2012). Responses of corals to high temperature stress at the colony-level, especially in natural conditions, are frequently being investigated (Berkelmans and Oliver, 1999; Ban et al., 2013; Depczynski et al., 2013). Responses of individual coral colonies under OA, however, have not been well studied with only a few studies having tested the effects of acidified waters on calcification of individual colonies (Marubini et al., 2003; Iguchi et al., 2012; Ohki et al., 2013). Furthermore, other physiological parameters including maximum photosynthetic efficiency, chlorophyll content of *Symbiodinium,* and *Symbiodinium* density of individual colonies have only been investigated for three colonies of *Porites australiensis* (Iguchi et al., 2012). Clearly, further investigations on how the physiological functions of coral colonies asides from calcification are affected by OA, and in coral species other than *P. australiensis*, are needed. Furthermore, how individual coral colonies and their symbiotic *Symbiodinium* respond to the combination of OA and high temperatures remains unexamined. Moreover, whether responses of individual coral colonies to the combined effects of OA and temperature can be predicted based on their responses to OA or temperature alone remains unknown.

Almost all physiological studies on calcifying corals have used pooled data from a few colonies of designated species to infer how most coral taxa will respond to either temperature or OA or their combination. Whether pooled data show different results compared to those from responses of individuals under OA, temperature stress, and their combination, and whether such differences can be observed when studying other physiological functions rather than calcification are other questions that need to be investigated. Answering these questions will provide opportunities to assess different methodological and experimental designs that will in turn affect experiments and predictions on future of corals and coral reefs.

In this study, we exposed three individual colonies each of *Acropora digitifera*, *Montipora digitata*, and *Porites cylindrica* to four pCO_2-temperature treatments including 400 μatm- 28 °C, 400 μatm-31 °C, 1000 μatm-28 °C and 1000 μatm-31 °C for 26 days. Physiological parameters including calcification, protein content, maximum photosynthetic efficiency, *Symbiodinium* density, and chlorophyll content along with *Symbiodinium* type of each colony were examined. The main questions that this study tried to answer were; 1) how each individual coral colony responds to OA, thermal stress, and OA + thermal stress; 2) if there are intercolonial differences in physiological responses of corals and their *Symbiodinium* under combined OA + thermal stress; and 3) whether pooled data are representative for responses of individual coral colonies.

2. Material and methods

2.1. Coral collection

Three healthy colonies each of *Acropora digitifera*, *Montipora digitata and Porites cylindrica* (nine colonies in total) were sampled from the shallow reef lagoon at Bise (26°42′34″N, 127°52′46″E), Okinawa, Japan in June 2014. Nubbins of similar sizes and shapes were attached to plastic screws using super-glue. The coral nubbins were kept in an outdoor tank with running ambient seawater for 5 weeks before starting the experiment. All coral nubbins were apparently healthy with no signs of disease or external algal growth prior to the experiment.

2.2. Experimental set-up

The experiment was conducted at the AICAL (Acidification Impact on CALcifiers) laboratory of the Tropical Biosphere Research Center Sesoko Station, University of the Ryukyus, Okinawa, Japan. A pH-stat system through CO_2 bubbling was used in our study (Tanaka et al., 2014). Briefly, the system was charged with running seawater filtered through 10 and 1 μm filters respectively. Pure CO_2 from compressed CO_2 tanks was bubbled into the seawater to provide the desired pCO_2 conditions. The computer program CO_2SYS was used to estimate the pH, HCO_3^-, CO_3^{2-} and aragonite saturation state (Ω_{arg}) from xCO_2, temperature, total alkalinity of 2267 μmol/kg, and salinity of 34.5 (Lewis and Wallace, 1998). The acidified waters were ejected to 4 L tanks with a flow rate for 150 ml min^{-1}. Four treatments including pCO_2-temperatures 400 μatm-28 °C, 400 μatm-31 °C, 1000 μatm-28 °C and 1000 μatm-31 °C (Table 1) with two tank replications were used. pCO_2 conditions of 400 μatm and 1000 μatm were chosen as present day and near-future scenarios (IPCC, 2013), respectively. 28 °C was chosen as ambient temperature, which was close to natural sea surface temperatures in Okinawa during the experiment, and 31 °C was chosen as the stress temperature. Similar 12:12 light: dark photoperiods (120–140 μmol m^{-2} s^{-1}) under metal-halide lamps (Funnel2 150W, Kamihata, Japan) were used for all tanks. Heaters and cooling systems were used to keep the temperature conditions in the aquaria stable.

2.3. Physiological analyses

Six nubbins of each colony were put in each pCO_2-temperature treatment (three in each tank replication) for 26 days. All nubbins were used to measure the following parameters. Buoyant weight was measured based on Davies (1989) as a representative of calcification rate. We represented calcification rate as percent change in final weight relative to the initial weight over 26 days. Soluble protein content was calculated using the Bradford Protein Assay (Bradford, 1976). Protein content then was converted to coral surface area and was presented as mg^{-1} cm^{-2} of coral surface. *Symbiodinium* density was counted for each nubbin using a Thoma hemocytometer (Erma, Japan). To convert the counted *Symbiodinium* cells and protein content per cm^{-2} of coral surface, the aluminum foil method (Marsh, 1970) was used to measure nubbin surface area. Maximum photosynthetic efficiency of the PSII of zooxanthellae, *Fv/Fm*, was measured by a pulse-amplitude modulated (Diving PAM; Walz, Effeltrich, Germany) fluorometer (Schreiber et al., 1986). Coral nubbins were kept in darkness for 30–40 min prior to measurements. Since the initial measured *Fv/Fm* of coral nubbins of colonies showed variations, we presented the data on *Fv/Fm* as the ration of final/initial measurements for each nubbin instead of using only the final measurements. This way of presenting *Fv/Fm* correctly shows changes in photosynthetic efficiency of *Symbiodinium* in each nubbin. Chlorophyll content (including chlorophyll $a + c_2$) was calculated based on Jeffrey and Humphrey (1975). Chlorophyll content and *Symbiodinium* density were then presented as pg^{-1} *Symbiodinium* $cell^{-1}$ and $cell^{-1}$ cm^{-2} of coral surface respectively.