学位論文

Spatial community shift from hard to soft corals in acidified water

(酸性化によるハードコーラルから

ソフトコーラルへの群集シフト)

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日本語要旨

人為的活動により大気中の二酸化炭素分圧(pCO_2)の上昇が報告されている．これに伴い，海水中のpCO_2が上昇し，pHが酸性に傾いて海洋酸性化が起こる．海洋酸性化は石灰化を抑制することから，造礁サンゴをはじめとする海洋生物に悪影響を及ぼすことが指摘されてきた．これまでの多くの水槽実験で個体・群体レベルでのCO_2の影響について調べられたが，水槽実験だけでは生態系としての応答を議論するのに不十分である．そのような中で，サンゴ礁における生態系のシフトに関する仮説として挙げられたのは，ハードコーラルから非石灰化藻類というシフトであった．

生態系としてのCO_2への応答に重要な知見を与え得るのが実際の野外CO_2シープにより酸性化している環境である．これまでサンゴ礁域におけるCO_2シープはパプアニューギニアの1例しかなかった．ここでは，サンゴ礁は発達せず，オープンな地形である．このサイトでは，酸性化が進むとともに非石灰化藻類の被度が高くなった．ただし，最大で17%，平均は3.3%と，優占種といえるほど高い被度でない．ハードコーラルは，酸性化が進んでも被度は下がらず，平均で30%と高い被度を持つ．ただし，酸性化が進んだ範囲では枝状のサンゴの被度が減り，塊状のサンゴのみが生息し多様性が下がった．これまでの飼育実験から，塊状のサンゴは，枝状のサンゴよりも酸性化によって石灰化が抑制される影響を受ける度合いが小さいことが報告されている．このような酸性化に対する影響を受ける度合いの相対的な差がパプアニューギニアでのCO_2シープ周辺でのサンゴ種の分布に影響を与えた可能性がある．

パプアニューギニアのCO_2シープで塊状サンゴが優占することは重要な情報であるが，塊状サンゴはどこでも生息するわけではなく，違った条件では，また他の生物が優占可能性もある．

発表者は沖縄県硫黄鳥島のサンゴ礁が発達した礁池内でのCO_2シープにおいて，酸性化が進んだ海においてソフトコーラルが密生することを発見した．ここから，ハードコーラルからソフトコーラルというあらたな群集シフトの可能性の仮説を立てた．

発表者が発見したCO_2シープはサンゴ礁礁嶺が発達した半閉鎖的な地形の中にある．CO_2シープによる酸性化の影響がない地点ではハードコーラルが生息し，ソフトコーラルは殆ど棲息しない．酸性化海域において、pCO_2の日中9時間の定点観測を行った．同地点でロガーを用いて約一ヶ月のpH変化を追ったところ，

潮位サイクルに合わせて酸性化が起こることが明らかになった．硫黄鳥島のサンゴ礁には礁嶺が発達していることから，干潮時には礁嶺部分が水面上まで上がり，礁池内が半閉鎖的になることで，酸性化した海水が外洋水とまざりにくくなったことによると考えられる．最干潮時のpCO_2と生物分布を見ると，medium pCO_2 サイト(平均Ω_a =2.82) ではソフトコーラル（ウミキノコ属）が密生していた．また，さらにpCO_2が高いHigh pCO_2 サイト(Ω_a =2.36)では，造礁サンゴもソフトコーラルも棲息していなかった．もっともpCO_2が低い地点ではソフトコーラルは生息せず，ハードコーラルのみの生息が確認された．

硫黄鳥島の調査から，ソフトコーラルが酸性化に対して長期的に群集スケールで生息する耐性を持つ可能性が示唆された．この整合性を確かめるため，酸性化に対する代謝の応答をハードコーラルとソフトコーラルについて調べその比較を行った．

飼育実験では，石灰化速度については，ソフトコーラルは，昼間は高pCO_2による影響を受けなかった．一方，ハードコーラルでは高pCO_2に伴う石灰化速度の減少がみられた．ソフトコーラルの石灰化部は軟体部に完全に覆われ，外の海水の影響を受けにくいことから，これは光合成を行う昼間には酸性化した外の海水の悪影響を受けにくい事を意味すると考えられる．

光合成については，ソフトコーラルの光合成はpCO_2増加により促進されることが明らかになった．一方，ハードコーラルでは，pCO_2増加による光合成速度の上昇はみられなかった．

以上から，CO_2に対する代謝応答の観察結果から，ソフトコーラルは造礁サンゴよりもCO_2増加に対して耐性があることが示唆される．これは，硫黄鳥島において CO_2が831μatmまで上がる地点では造礁サンゴに代わりソフトコーラルが密生していたことと整合的である．

また飼育実験における夜間の石灰化量をみると，ソフトコーラルにおいても，ハードコーラルにおいてもΩ_a <1の条件下では昼ではみられなかった溶解が生じた．これが，硫黄鳥島のHigh pCO_2サイトにおいてソフトコーラルでさえも不在であった原因であると考えられる．そこで，実際に硫黄鳥島のHigh pCO_2サイトにおいてΩ_a <1となる頻度を計算すると，4.26 h/dayであった．Medium pCO_2サイトにおいてはΩ_a <1は定期的には起こらないということが分かった．このことから, Medium pCO_2サイトにおいてはハードコーラルが完全に生息できないのではなく，ソフトコーラルとの空間競争において相対的排除されたと考えられる．

また，他の野外酸性化サイトにおけるハードコーラルの生息との比較を行った．これまでの他地域での報告から，酸性化サイトにおける生物相は，3つに分けられる．一つ目は，ハードコーラルの多様性が保たれる状態，二つ目は，リーフを形成する枝状のハードコーラルが減る，または生息せず，塊状サンゴやソフトコーラルなどの石灰化量が小さなサンゴが優占する状態，最後にハードコーラルもソフトコーラルも生息しない状態である．生物相がこれだけ変わる中，Ω_aの平均値は，ほぼ同じ範囲であることから，Ω_aのみから生物相の変化を議論することは出来ない．ハードコーラルの多様性が保たれる地点ではΩ_aの変動幅が小さいことと，実験結果で夜間において$\Omega_a <1$となるときのみ溶解が生じたことから，$\Omega_a <1$となる頻度が重要である可能性を提言した．

Abstract

Anthropogenic increases in the partial pressure of CO_2 (pCO_2) cause ocean acidification, declining calcium carbonate saturation states, reduced coral reef calcification and changes in the compositions of marine communities. This study shows that the spatial distributions of both hard and soft coral communities in volcanically acidified, semi-enclosed waters in the coral reef of Iwotorishima Island, Japan, are related to pCO_2 levels.

At Iwotorishima Island, few soft or hard corals live at the site with high pCO_2 (Average $\Omega_a = 2.36$), dense soft coral and little hard coral lives at the site with medium pCO_2 (Average $\Omega_a = 2.82$) and few soft corals and dominant hard corals inhabit the site with ambient pCO_2.

In the experimental part of this study, metabolic responses to elevated pCO_2 during daytime indicated advantages for soft coral in terms of living with elevated pCO_2. During daytime, the photosynthesis rate of soft coral was increased by increased pCO_2, whereas the calcification rate was not affected. The results indicate that soft coral is not harmed and soft coral photosynthesis is enhanced by ocean acidification. In contrast, photosynthesis and calcification rates of hard coral decreased with elevated pCO_2, indicating that hard coral is harmed by ocean acidification. The difference in the metabolic response of corals during daytime to elevated pCO_2 contributes to the dense community of soft coral at medium pCO_2.

The absence of the two corals in the high-pCO_2 area may be the result of dark decalcification. In the culture experiment, significant decalcification occurred for both soft and hard coral under the condition of $\Omega_a < 1$. The frequency of $\Omega_a < 1$ conditions was estimated as in the high-pCO_2 area, the average duration of $\Omega_a < 1$ per day should be 4.26 h. In contrast, in the medium-pCO_2 area, $\Omega_a < 1$ was estimated not to have occurred regularly. This suggests that hard coral would be absent in the medium-pCO_2 area because hard coral is at a disadvantage in competition with soft coral, as mentioned above, rather than because hard coral is absolutely unable to survive at low Ω_a.

The biota at these locally acidified sites are divided into three phases. The first phase is that hard coral diversity, cover, and calcification rates are maintained. The second phase is that branching-type hard corals, which are the main builders of reefs, decrease or are absent, and less-calcifying corals, including soft corals, are dominant. The third

phase, is with no corals, i.e., neither soft corals nor massive-type corals.

The average Ω_a was similar among these sites, with values in the range. It is suggested that discussing coral abundance with average Ω_a is not appropriate because biota change dramatically under similar Ω_a conditions. It is noteworthy that in the site with high diversity of hard corals, the lowest Ω_a was higher than the values at other sites. These points suggest that the frequency at which Ω_a falls below a certain threshold value, is more important for the survivability of corals rather than the average Ω_a value.

Contents

Chapter 1: Introduction

1-1. Ocean acidification

1-1-1. The influence of ocean acidification on coral reef organisms

Since the Industrial Revolution, carbon dioxide (CO_2) emission has increased drastically by human activities (Etheridge et al., 1996). Now partial pressure of CO_2 (pCO_2) is about 400 μatm, though pCO_2 had never exceeded about 280 μatm at any time during the last 650,000 years (IPCC, 2007). The increase in pCO_2 in atmosphere causes ocean acidification, declining pH and calcium carbonation states in oceans (Door et al., 2009).

In 2100, the average pCO_2 in atmosphere is projected up to 950 μatm in the worst scenario and the average surface pH in the ocean is calculated as decreasing to 7.8 in 2100 from 8.1 of today (Gattuso and Lavigne 2009).

When CO_2 dissolves in seawater, dissolved inorganic carbon comes to the gas-liquid equilibrium (1-1) and the chemical equilibria (1-2)~(1-4).

CO_2 (g) = CO_2 (aq)……………………….(1-1)
CO_2 (aq) + H_2O (l) = H_2CO_3…………….....(1-2)
H_2CO_3 (aq) = H^+ (aq) + HCO_3^- (aq)…….(1-3)
HCO_3^- (aq) = H^+ (aq) + CO_3^{2-}……….....…(1-4)

where (g), (aq) and (l) mean gas state, hydrated state and liquid state, respectively.

The concentration of H_2CO_3 (aq) [H_2CO_3 (aq)] is less than 0.3% of [CO_2 (aq)] and the sum of dissolved inorganic [CO_2 (aq)] and [H_2CO_3] is denoted as [CO_2^* (aq)] (Gattuso and Hansson, 2011). Therefore, the systems (1-1)~(1-4) can be translated as below.

CO_2(g) ⇔ CO_2^*(aq)……………….....….(1-1')
CO_2^*(aq) ⇔ H^+(aq) + HCO_3^-(aq)….…..(1-3')
HCO_3^-(aq) ⇔ H^+(aq) + CO_3^{2-}………….(1-4)

[CO_2^*(aq)] is proportional to the pCO_2 by Henry's law.

[CO_2^*(aq)] = $K_0 \times pCO_2$ …………………...(1-5)

Where K_0 is the solubility coefficient of CO_2 in seawater and reported by Weiss (1974).

The equilibria constants K_1 for systems (1-3') and K_2 for (1-4) are shown below.

K_1 = [H^+(aq)] [HCO_3^-(aq)] /[CO_2^*(aq)] ……………………(1-6)
K_2 = [H^+(aq)] [CO_3^{2-}(aq)]/ [HCO_3^-(aq)]……………………(1-7)

K_1 and K_2 are reported as functions of temperature and salinity under ordinary pressure by Mehrbach et al. (1973).

The sum of the dissolved carbonate species is denoted as total dissolved inorganic carbon (TIC):

TIC **= $[CO_2^*(aq)] + [HCO_3^-(aq)] + [CO_3^{2-}(aq)]$……………..(1-8)**

Therefore, the concentrations of each carbonate matters are written as below.

$[CO_2^*(aq)]$ = TIC **$\times\{1+ K_1/[H^+(aq)]+ K_1 K_2/[H^+(aq)]^2\}^{-1}$……..(1-9)**

$[HCO_3^-(aq)]$ = TIC **$\times\{[H^+(aq)]/ K_1 + 1 + K_2/ [H^+(aq)]\}^{-1}$……..(1-10)**

$[CO_3^{2-}(aq)]$ = TIC **$\times\{[H^+(aq)]^2/ K_1 K_2 + [H^+(aq)]/K_2 + 1\}^{-1}$……(1-11)**

Total alkalinity (**TA**) is a measure of the capacity of water to neutralize acids.

TA = $[HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] - [H^+]$ + minor components ($[HPO_4^{2-}] + 2[PO_4^{3-}] + [H_3SiO_4^{-1} + [NH_3] + [HS^-] - [HSO_4^-] - [HF] - [H_3PO_4]$)

In order to discuss the acidity index of seawater for a given mineral, saturation state is used. Ω is the saturation state of the calcium carbonate ($CaCO_3$), which are major minerals in shells and skeletons of marine organisms, and defined as follows,

$\Omega = [Ca^{2+}] \times [CO_3^{2-}]/K^*sp$ …………………………………(1-12)

where K*sp is the product of the equilibrium concentrations of Ca^{2+} and CO_3^{2-}, which differs among minerals of calcium carbonate such as aragonite, calcite and Mg-calcite.

Carbonate dissolution is described as follows:

$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2HCO_3^-$……………………. (1-13)

Based on the systems (1-9)~(1-11), dissolution of CO_2 in seawater does not effect on TA, but increases the concentration of bicarbonate ions ($[HCO_3^-]$) and the Total inorganic carbon (TIC), and lowers the pH and the concentration of carbonate ions ($[CO_3^{2-}]$). Therefore, ocean acidification inhibits calcification and has harmful effects on calcifying organisms.

1-1-2. Previous studies and remaining problems

Growing number of studies have investigated the effects of increasing pCO$_2$ on calcifiers (e.g., Kroeker, et al., 2013). Hard coral, which is the main builder of coral reefs, is one of the most focused animals as a loser acclimatized to elevated CO_2 concentrations (Kleypas et al., 2006). Many tank experiments have observed the decreased skeletal growth in hard corals (Pandolfi et al., 2013; Comeau et al., 2014).

However, it is difficult to apply experimental results to field conditions and estimate the response in community scale. The complex in situ oceanographic conditions cannot be recreated in laboratory experiments. For example, light, current and carbon chemistry, which effect calcification, may change significantly in situ. Also, experiment cannot take into account of community processes such as long term effect beyond generations, relations of organisms, and differences in sensitivity to high pCO$_2$ among species or individuals (Kleypas et al., 2006).

Although enough information is not available, community shift in coral reefs caused by ocean acidification is proposed. Most of projected community changes due to ocean acidification expect transitions from hard coral to non-calcifying algal communities (Bellwood et al., 2004; Hoegh-Guldberg et al., 2007; Norström et al., 2009). This is because experimental results report the increased growth of non-calcifying algae with elevated pCO_2 (Kroeker et al., 2013). In addition to it, non-calcifying algae is expected as a winner when coral reefs are stressed. Previously, many marine scientists have concluded that coral reefs are moving toward or are locked into a non-calcifying algae state by the stresses other than ocean acidification, such as a major hurricane (Woodley et al. 1981), coral predator and coral disease outbreaks (Knowlton et al. 1990). However, Bruno et al. (2007) note that the replacement of corals by non-calcifying algae as the dominant benthic functional group is less common and less geographically extensive than generally assumed. This is partly because other organisms than non-calcifying algae have received less attention, despite the biotic diversity of coral reef communities.

As a new approach to examine the effect of ocean acidification in community scale, observations of spatial community shifts under naturally high pCO_2 conditions can provide information on the relationship between community structure and pCO_2 levels. The first report on shift of marine organisms at CO_2 gas vents in the Mediterranean Sea, under reduced pH conditions showed decrease of calcifying organisms and increase of non-calcifying macroalgae and seagrasses (Hall-Spencer et al., 2008), as well as increase of recruitment of non-calcifying algae (Kroeker et al., 2009), suggesting a shift from a calcareous to non-calcareous algae dominant community. In case of coral reefs, there is only one example of field site that is acidified by CO_2 seep. The reported study assesses the effects of ocean acidification at a CO_2 seep off Papua New Guinea (PNG), where the site is acidified locally by pure CO_2 venting gas (Fabricius et al., 2011). The study showed that the abundance of non-calcifying algae increased with the calculated reduced pH conditions. The result is consistent with the experimental results in laboratory (Kroeker et al., 2013), which reported increased growth of non-calcifying algae with pCO_2 Increase. However the coverage of non-calcifying algae was 17% at most, which is not high enough to regard non-calcifying algae as dominant species. As to hard corals, pH did not affect their abundance, though their diversity was decreased as the abundance of structurally complex framework builders reduced and only massive type coral survive in lowered pH. This indicates that massive type hard coral acclimates to high pCO_2 in long term to overcome the harmful effect and survive. This is explained by the difference of sensitivity to elevated pCO_2. The short-term laboratory experiment reported that the fast calcifying hard corals were more sensitive to elevated pCO_2 and massive type corals were less affected (Comeau et al., 2014). The relative sensitivity to elevated pCO_2 may results in the survivability in pCO_2 vent in PNG. If such relative sensitivity determines the loser and winner ine $pCO_{2\,i}$nccrease, other organisms also can be a winner because there are many organisms in coral reefs.

1-2. Soft coral

1-2-1. Discovery of a new CO_2 seep and dense soft coral community in Iwotorishima Island

During expedition of Tokyo University marine expedition club, I discovered a new CO_2 seep in Iwotorishima Island, an uninhabited volcanic island of the Ryukyu Islands, Japan (Inoue et al., 2014, Figs. 1-1, 1-2). It is the second CO_2 seep found in coral reef after PNG (Fabricius et al., 2011). Iwotorishima Island is 3 km long (NNW–SSE) and 1 km wide (WSW–ENE). The island has an active volcano on its northwestern side and has been uninhabited since an eruption in 1967. Coral reef (approximately 200 m wide) fringes the eastern to southwestern coast of the island and hard coral is dominant in the coral reef other than the southeastern coast. The southeastern coast is characterized as a volcanically acidified area of seawater that extends for 200 m east–west and 400 m north–south. The reef crest is well developed in the area and is exposed above sea surface when the tide is low, which is different from PNG. Mushroom-shaped encrusting soft corals, dominated by *Sarcophyton elegans,* densely populate in the acidified areas (Fig. 1-2).

1-2-2. What is soft coral

"Soft coral" is a vernacular term of Alcyonacea, which belongs to Anthozoa. "Hard coral" is a vernacular term of Scleractinia, which also belongs to Anthozoa (Fig. 1-3). Both of hard and soft corals are composed colonies of polyps, in which photosynthesizing symbiotic algae, zooxanthella lives (Fig. 1-4). Hard corals have skeletons of aragonite, one of the calcium carbonate minerals and their soft body covers over the skeletons. In contrast, soft coral has tiny internal calcareous sclerites that is composed of high-Mg calcite (Mg content is 5-10 mol%, Rahman and Omori, 2008), less than 12%, which is known to be less soluble than the aragonite (Morse et al., 2007).

Soft coral is one candidate organisms which can be overriding instead of hard corals when ocean acidification prevailed. Soft coral lives same habitat and has spatial competition with hard coral (Fabricius and Alderslade, 2004). There is one report that soft coral cover increased from 20 % to 60 % when hard coral are stressed by plague of Acanthaster, which prefer eating hard coral over soft coral (Pratchett, 2010). Soft coral is important organism mutually-interfering with hard coral, though there are far less studies of soft coral than those of hard corals or than non-calcifying algae because its taxonomy and culturing is difficult.

1-3. The objective and the approach of this study

Observation at newly found CO_2 vent gives the hypothesis that soft coral can be a winner in coral reefs in the near future with the progress of ocean acidification. In this study the first objective is to reveal the difference in response to elevated pCO_2 between hard and soft corals. The second is to explore the possibility and conditions of community shift from hard to soft coral caused by ocean acidification in the future.

To approach these objectives, both field study and culture experiment were conducted. Field study gives important information on the effect of elevated pCO_2 in the long term, in the complex oceanographic conditions, and with complex relation of organisms. However, the complexity often makes it difficult to conclude the cause and effect. There can be unlimited possibilities. On the other hand, in laboratory, comparative experiments enables us to specify which factor is the cause, though there is a large gap between laboratory experiment and field observation for physical or chemical condition, time scale, relation with other organisms. Therefore, in this study, the response of coral reef community to elevated pCO_2 is observed in the field study and the cause and nature of the response is studied in the experiments.

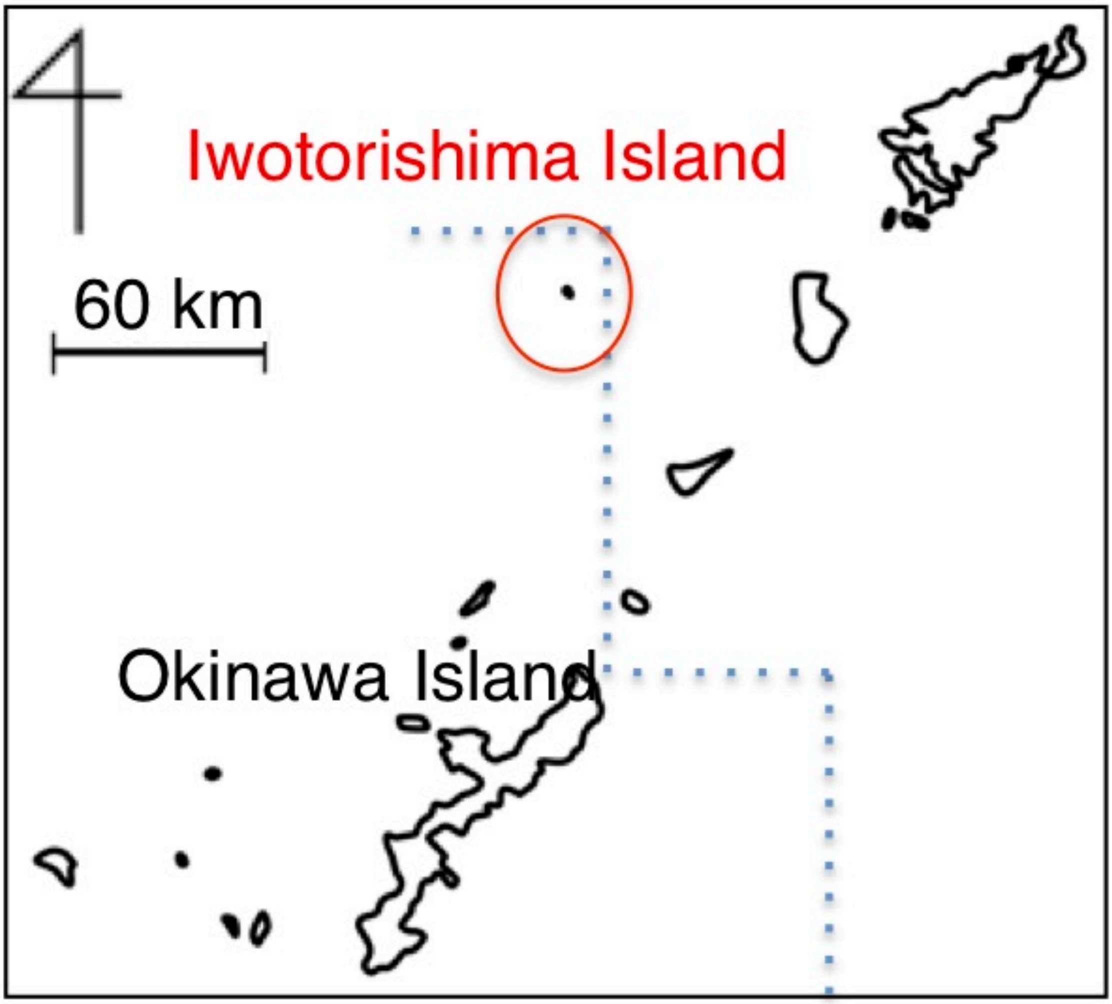

Fig. 1-1. The location of Iwotorishima Island. The dotted line is prefecture's border.

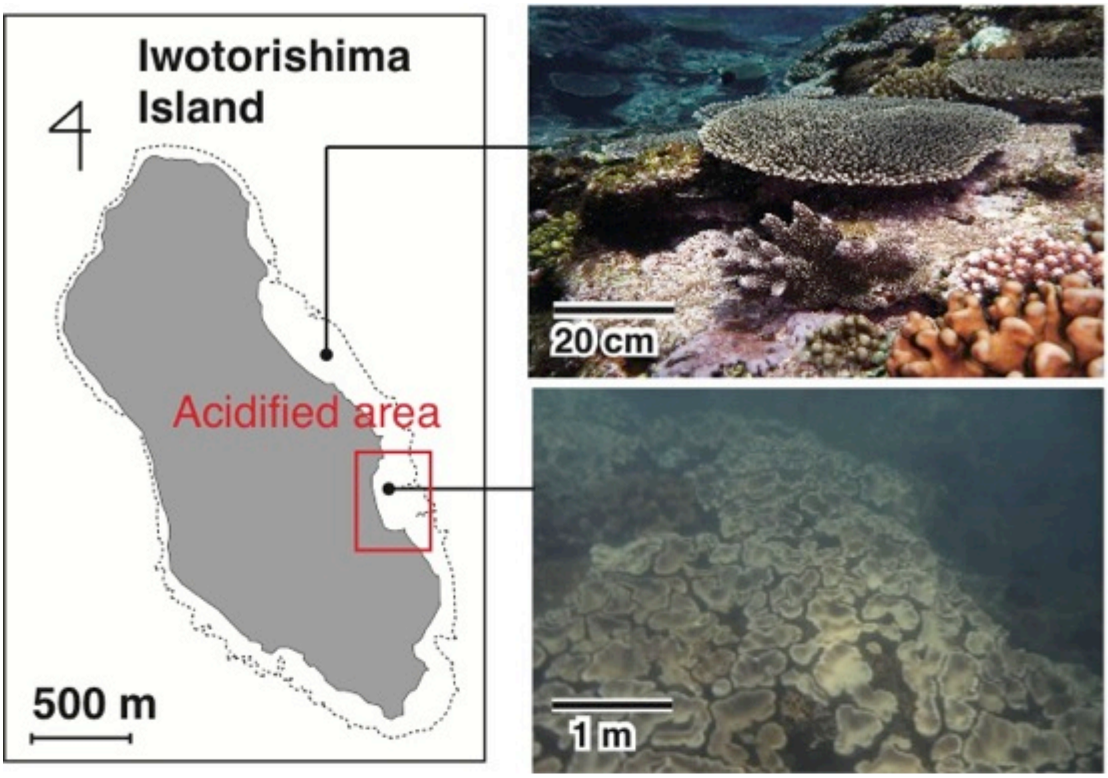

Fig. 1-2. The map of Iwotorishima Island (left). The dotted line around the island is the edge of the fringing reef and the view in ocean in the ambient pCO_2 area (upper right) and in the acidified area (bottom left). The area locally acidified with CO_2 seep is red squared.

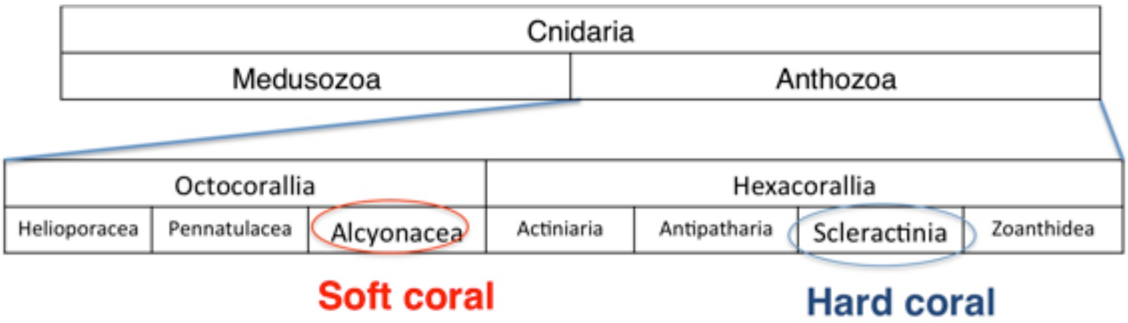

Fig 1-3. Taxonomy of hard coral and soft coral consulting with Fabricius and Alderslade (2004).

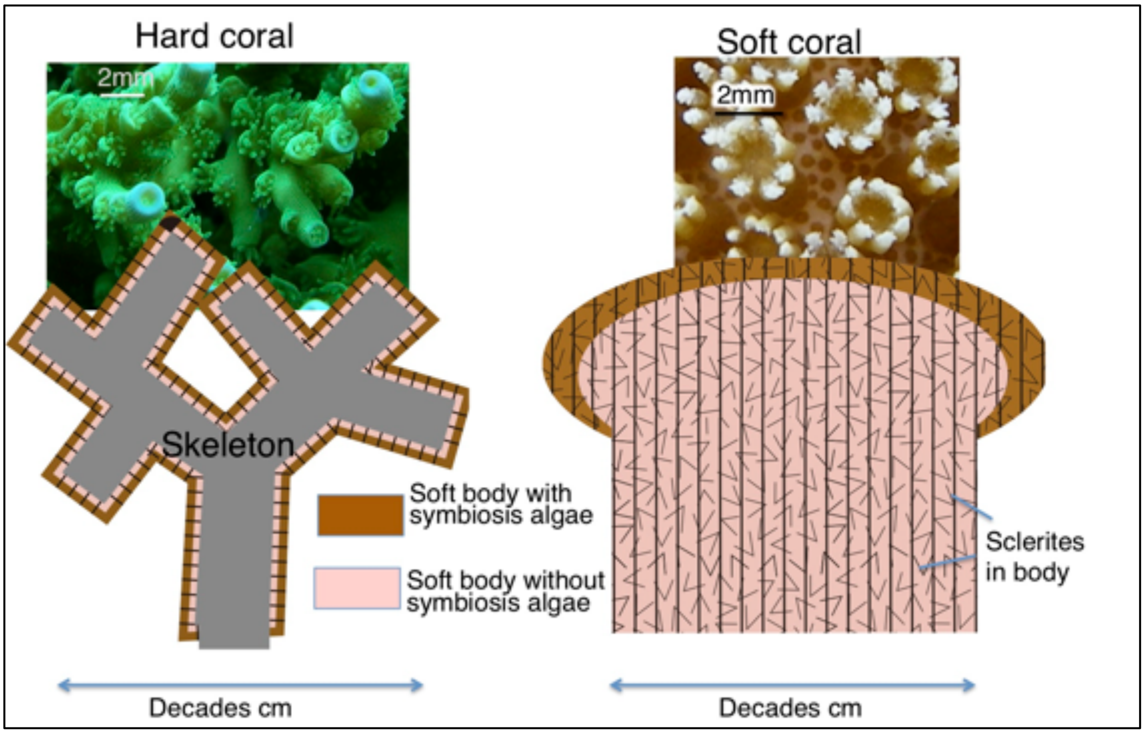

Fig. 1-4. The photos of polyps (upper) and the body structures (bottom) of each hard coral (left) and soft coral (right).

Chapter2 : Iwotorishima Island

2-1. Introduction

Observations of spatial community shifts under naturally high pCO_2 conditions can provide information on the relationship between community structure and pCO_2 levels. The distribution of organisms may be the result of effect of pCO_2 level.

The purpose of this study is to know how the ocean acidification occurs in Iwotorishima-Island and to check the tolerability of soft coral to high pCO_2 in long term.

2-2. Study site

Iwotorishima Island is an uninhabited volcanic island of the Ryukyu Islands, Japan located at 27° 52' 27" N and 128° 13' 35" E. The island has an active volcano on its northwestern side and has been uninhabited since an eruption in 1967. Coral reef (approximately 200 m wide) fringes the eastern to southwestern coast of the island and hard coral is dominant in the coral reef other than the southeastern coast (Fig. 2-1). The southeastern coast is characterized as a volcanically acidified area of seawater that extends for 200 m east–west and 400 m north–south.

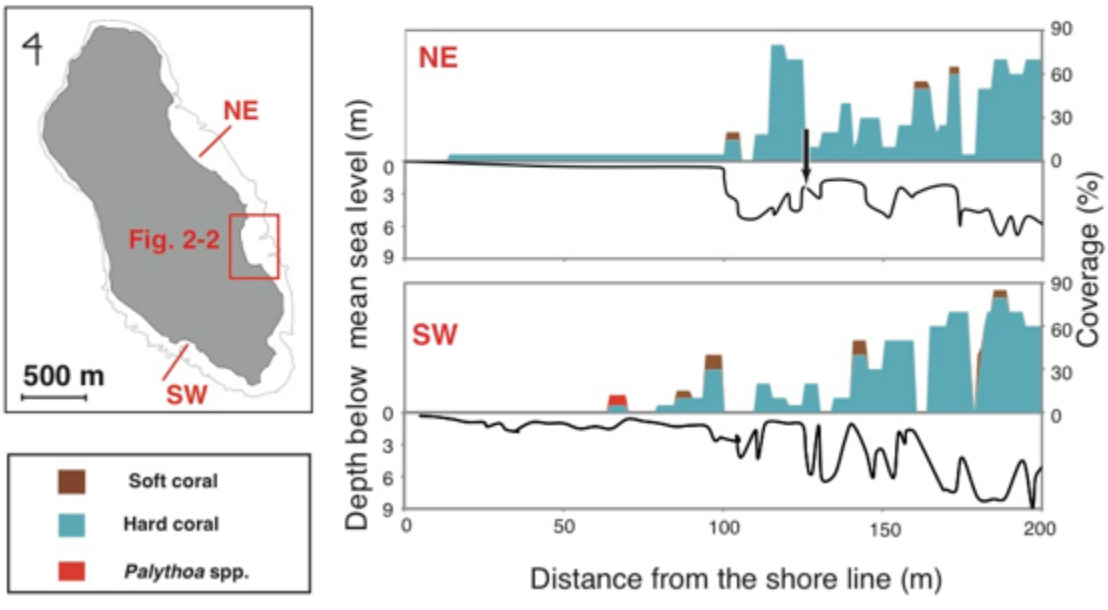

Fig. 2-1. The location of survey lines and cross sections with coral cover degree. Red lines in the map (left) are the transect survey lines of which benthos cover with cross-sections is shown in the right figures. Hard coral is dominant on both transects. The arrow on NE shows the point of the photo of hard coral dominated ocean view (Fig. 1-2, upper left) and seawater sample for nutrient analysis were taken (Fig. S1, from Inoue et al., 2013)

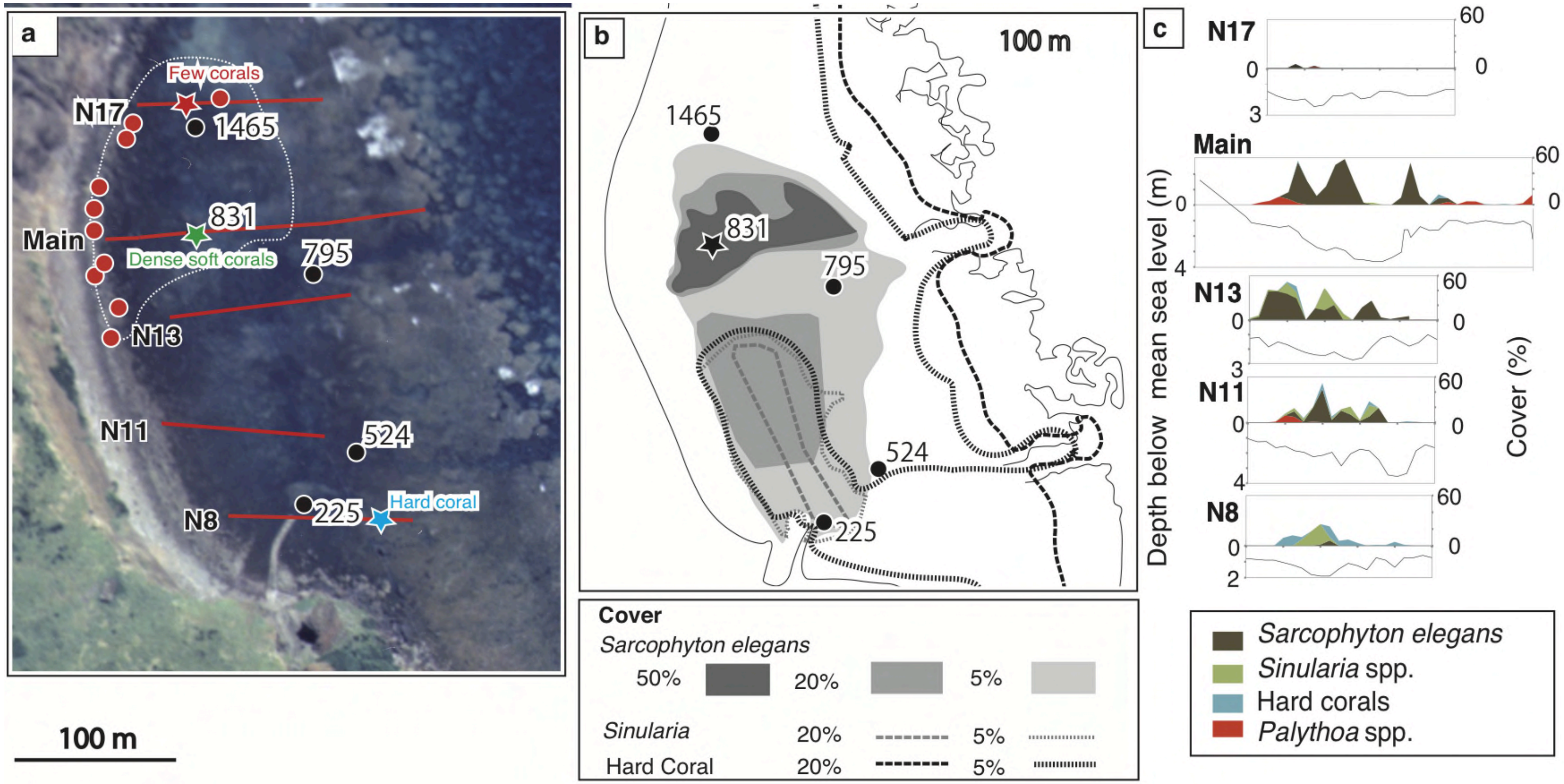

Fig. 2-2. Aerial photograph, map of the acidified area (red rectangle, Fig. 2-1), and transect profiles of the acidified area. **a,** Spring locations (red circles), areas with gas (white dotted line), pCO_2 levels and survey transects (red solid lines). Value of pCO_2 (μatm) at five locations (black dots and green star) represent measurements at spring low tide, 14th July 2010 (14:30-16:00). Red, green and blue star indicates the "Few corals", "Dense soft coral" and "Hard coral" point respectively, where multi-parameter water quality meters were placed. **b,** Distribution of primary benthic cover in acidified area. **c,** Cross-sections of survey transects showing benthos cover. (Fig. 1 from Inoue et al., 2013)

Fig. 2-3. The view from the start point of the survey line “Main” at low tide. Coral reef crest gets above sea level (embosomed with dotted red line) and channel (red arrow).

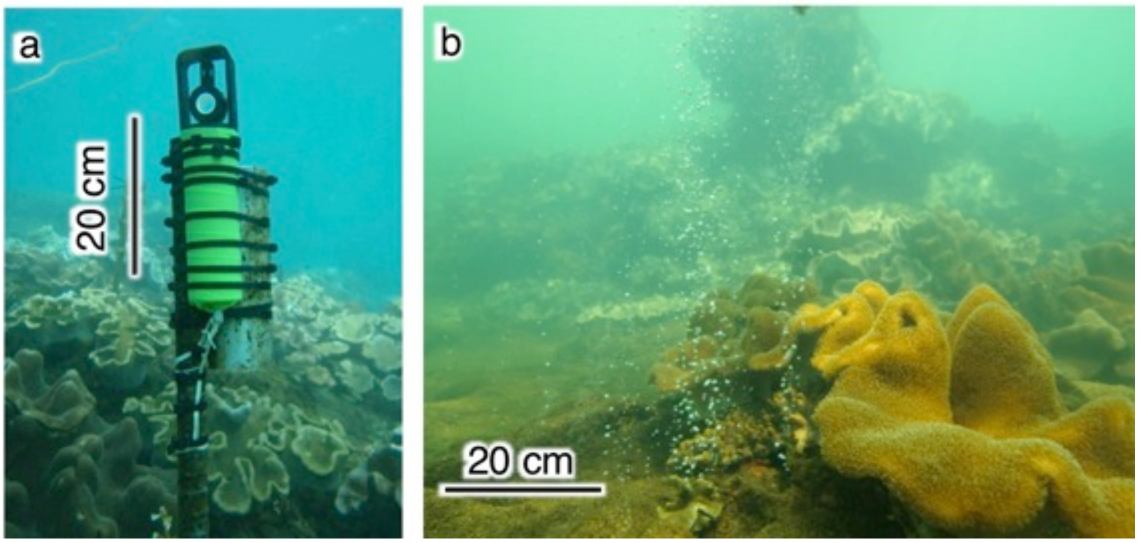

Fig. 2-4. View around “Dense soft coral” point. a: multi-parameter water quality meters, a quantameter, a wavemeter, and a current meter placed in the dense soft coral communities area. b: CO_2 venting by the soft coral.

2-3. Field survey and laboratory analysis

2-3-1. Reef morphology

Cross-sections transects from the shore to the edge of the reef crest were made along two lines in the ambient pCO_2 area (NE and SW; Fig. 2-1) and five lines in acidified area with CO_2 seeps (N17, Main, N13, N11 and N8; Fig. 2-2) on 13th August 2011 and 12th July 2010 respectively. Depth below mean sea level was calculated with the tide prediction at Naze (28° 22' 42" N, 129° 29' 42" E), the nearest monitoring point by Japan Meteorological Agency (http://www.jma.go.jp/jma/index.html).

2-3-2. CO_2 chemistry

Gas venting from the seafloor was collected around "Dense soft coral" point (Fig. 2-2) with an infundibulum into a gas washing bottle rubber closured. Then airtec tube was connected to the rubber closure and were measured the concentrations of CO_2 (range 5%–40%, No. 2HH, Gastec), SO_2 (range 0.2-5ppm, No. 5Lb, Gastec), and H_2S (range 0.2–2.0 ppm, No. 4LT, Gastec).

Seawater samples (300 ml each) were collected by filling up the glass bottles, which are imperviable with CO_2 (Duran, Schott), for analyzing of CO_2 chemistry, and 0.2 ml of $HgCl_2$ was added for fixation. These samples were shipped and preserved at ambient temperatures. On 13th July 2010, bottom seawater samples were collected at the "Dense soft coral" point (Fig. 2-2) every 30 minutes from 10:30 to 12:00 and from 14:30 to 18:00. On 14th July 2010, bottom seawater was sampled from five points during low tide, from 15:00 to 16:15. On 14th and 15th July 2010, seawater of ocean approximately 200 off the end of the line "Main" (Fig. 2-2) was sampled at the depth 1m from the surface.

Seawater Total Alkalinity (TA) and Total Inorganic Carbon (IC) were analyzed using a flow-through CO_2 chemistry analyzer (Kimoto Electronic, Watanabe et al., 2004), with accuracy and precision determinations made by analyzing certified reference materials (CRMs) obtained from Dickson Laboratory (Scripps Institution of Oceanography). The analytical errors of TA and TIC measurements were within 3 μmol kg−1. Salinity was measured using a salinometer (Portasal 8410A, Guildeline Instruments, Ltd). The seawater carbonate chemistry of each pCO_2 condition was calculated using the CO_2sys program (Lewis and Wallace, 1998), using the dissociation constants K_1 and K_2 (Mehrbach, et al., 1973), and values for aragonite solubility (Mucci, 1983).

2-3-3. Physical and chemical conditions

At the center of the area of dense soft coral ("Dense soft coral" in Fig. 2-2), a multi-parameter water quality meter (pH in NBS scale, dissolved oxygen, salinity, turbidity, temperature; WQC-24, DKK-TOA, Tokyo, Japan) was deployed from 9 June to 12 July 2010, measuring and logging every 15 minutes. In addition, a quanta meter

(Alec CQ, JFE Advantech, Tokyo, Japan), a wave meter (Alec Infinity Series Version 0.64, JFE Advantech, Tokyo, Japan) and a current meter (Alec Infinity Series Version 0.70, JFE Advantech, Tokyo, Japan) were installed and performed measurements every 10 minutes. Also, in 2012, the same quanta meter, the wave meter and the current meter were again set at the same central point of dense soft coral "Dense soft coral", and also the multi-parameter water quality meters were set at the three points, in area with few corals ("Few corals" in Fig. 2-2), in area with dense soft corals ("Dense soft coral" in Fig. 2-2), and in area with hard corals ("Hard coral" in Fig. 2-2) from 12th to 25th August 2011 and 22th July from 24th August 2012 measuring and logging every 15 minutes.

2-3-4. Seawater chemistry

Samples of spring water from along the coastline were collected between 13:00 and 14:00 (local time) on 14th July 2010, and the concentrations of the main ions (sulphate, potassium, magnesium, calcium, sodium, and chlorine ions) were measured by ion chromatography (ICA-2000; DKK-TOA).

On 14th August, 2011 seawater of 10-ml were sampled for nutrient analysis during at low tide at the "Dense soft coral" point and in ambient CO_2 area (Fig. 2-1). After sampling, samples were in cold storage with refrigerant for 6 hours with shipping and then frozen at -20^{o} C. Concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+) and phosphate (PO_4^{3-}) were determined colorimetrically using an autoanalyzer (QuAAtro 2HR, BLTEC) at Tokyo Institute of Technology.

2-3-5. Biota

Biota survey was done in parallel with geomorphic investigation. Cross-sections transects from the shore to the edge of the reef crest were made along two lines in the ambient pCO_2 area (NE and SW; Fig. 2-1) and five lines in acidified area with CO_2 seeps (N17, Main, N13, N11 and N8; Fig. 2-2). The coverage in square of 1m^2 wide was observed by 5m along the transect line. Photo of hard coral was taken to identify the species (Appendix tab. A1-A3). To identify the soft coral, which dominates in the "Dense soft coral" area, five colonies of the dominant soft coral were collected and preserved in ethanol for the examination of sclerites. Their sclerites were washed with sodium hypochlorite and collected with stereomicroscope at Seto Marine Biological Laboratory and scanned electron microscope for the purpose of species identification at Ryukyu University. In addition to the coverage around the line, 50 quadrates of 1m^2 wide were measured by visual observation with snorkeling and the trajectory information recorded with GPS on 14th July 2010.

2-4. Result

2-4-1. Reef morphology

Coral reefs in Iwotorishima Island are approximately 200 m wide (Figs. 2-1 and Fig. 2-2). Coral crest is developed in the acidified area (Figs. 2-2 and 2-3). The average of low tide in spring tide in this area is 1.15 m under mean sea level, lower than the depth of coral reef crest, which is exposed and the seawater in the reef is separated from the outer ocean, resulting in a semi-closed system (Fig. 2-3).

2-4-2. CO_2 chemistry

Gas venting from the seafloor contains CO_2 of more than 40%, upper limit of detection but is devoid of SO_2 or H_2S, which are the typical compositions of volcanic gas than CO_2.

Variability of $p$$CO_2$ at "Dense Soft Coral" point is shown in Fig. 2-5. Generally, $p$$CO_2$ in coral reef lagoon decreases in sunny daytime because of CO_2 consumption with photosynthesis (Kayanne et al., 2000). However, in Iwotorishima Island, there is no decrease of $p$$CO_2$ during daytime and after the peak of photon flux density, seawater $p$$CO_2$ increased up to 800 μatm at the "Dense soft coral". In contrast, during night-time $p$$CO_2$ rose up to 1200μatm because of respiration, though the depth is higher than daytime (Fig. 2-5).

Distribution of $p$$CO_2$ at low tide in acidified area showed decrease from north (1465 μatm) to south (225μatm) (Fig. 2-2 and Tab. 2-3).

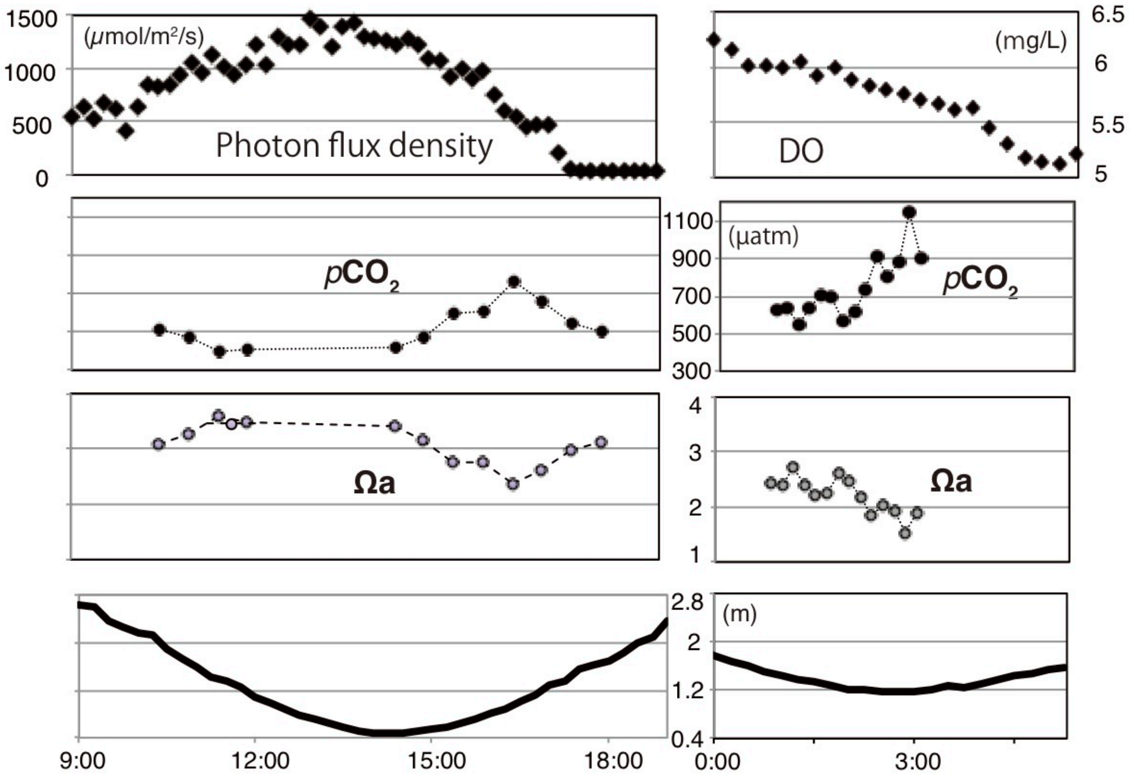

Fig. 2-5. Variability of pCO_2 in Iwotorishima Island during daytime on 13th July 2010 (The left figures from modified Fig. 2 of Inoue et al., 2013) and during night-time on 22th July 2012. In Iwotorishima Island, the coral crests emerge above sea level during low tide and make lagoon semi-closed.

Top panels: the photon flux density (dissolved oxygen was not measured on 13th July 2010) (right) and dissolved oxygen (left). The second panels: pCO_2 change. The third panels: aragonite saturation state. The bottom panels: depth below mean sea level. During daytime no decrease of pCO_2 was observed during high photon flux density because of photosynthesis and pCO_2 increased up to 800 μatm after the peak of photon flux density. In contrast, during night-time pCO_2 rose up to 1200μatm because of respiration, though the depth is larger than daytime. In summer, spring low tide occurs during daytime. In winter, spring low tide occurs during night-time and stronger acidification should occur.

2-4-3. Physical and chemical conditions

At the ”Dense Soft coral” point data from a one-month period (9 June to 12 July 2010) showed pH decreased at low tide, particularly during spring tides (Figs. 2-6 and 2-7). During neap tides, seawater pH reduction was less pronounced (Fig. 2-8).

The variabilities of pH at “Few coral”, “Dense soft coral”, and “Hard coral” points observed from 12th to 25th August 2011, showed synchronization with tidal change that the pH decreased when depth lowered (Fig. 2-9). The decrease of pH was the largest at “Few coral” and the smallest at “Hard coral”point. The average and standard deviation of pH during the logged term were 7.96 ± 0.14 at “Few corals”, 8.03 ± 0.20 at “Dense soft coral” and 8.21±0.10 at “Hard corals”.

From 22 July to 24 August 2012, during daytime, dissolved oxygen increased with photon flux density and pH at all points also increased, though pH at “Few corals” and “Dense soft coral” points showed small negative peak during low tide (Figs. 2-10 and 2-11). During night-time, pH showed synchronization with tidal change. The average of pH during observation was 7.80±0.14 at “Few corals”, 7.91±0.13 at “Dense soft coral” and 8.14±0.08 at “Hard corals”.

The maximum flow velocity observed at the “Dense soft coral” point was 9 cm/s, lower than the velocity at which the highest growth rate was found for soft coral *Sinularia flexibilis* (Khalesi et al., 2007). The minimum pH value was 7.4, and the highest values of seawater temperature and turbidity were 32°C and 100 NTU respectively, in 2010 (Fig. 2-6); these values are within the range of natural variations in lagoonal coral habitats (Oliver et al., 2011; Orpin et al., 2004) and temperatures were not significantly different in the north and south of the study area (Tab. 2-1).

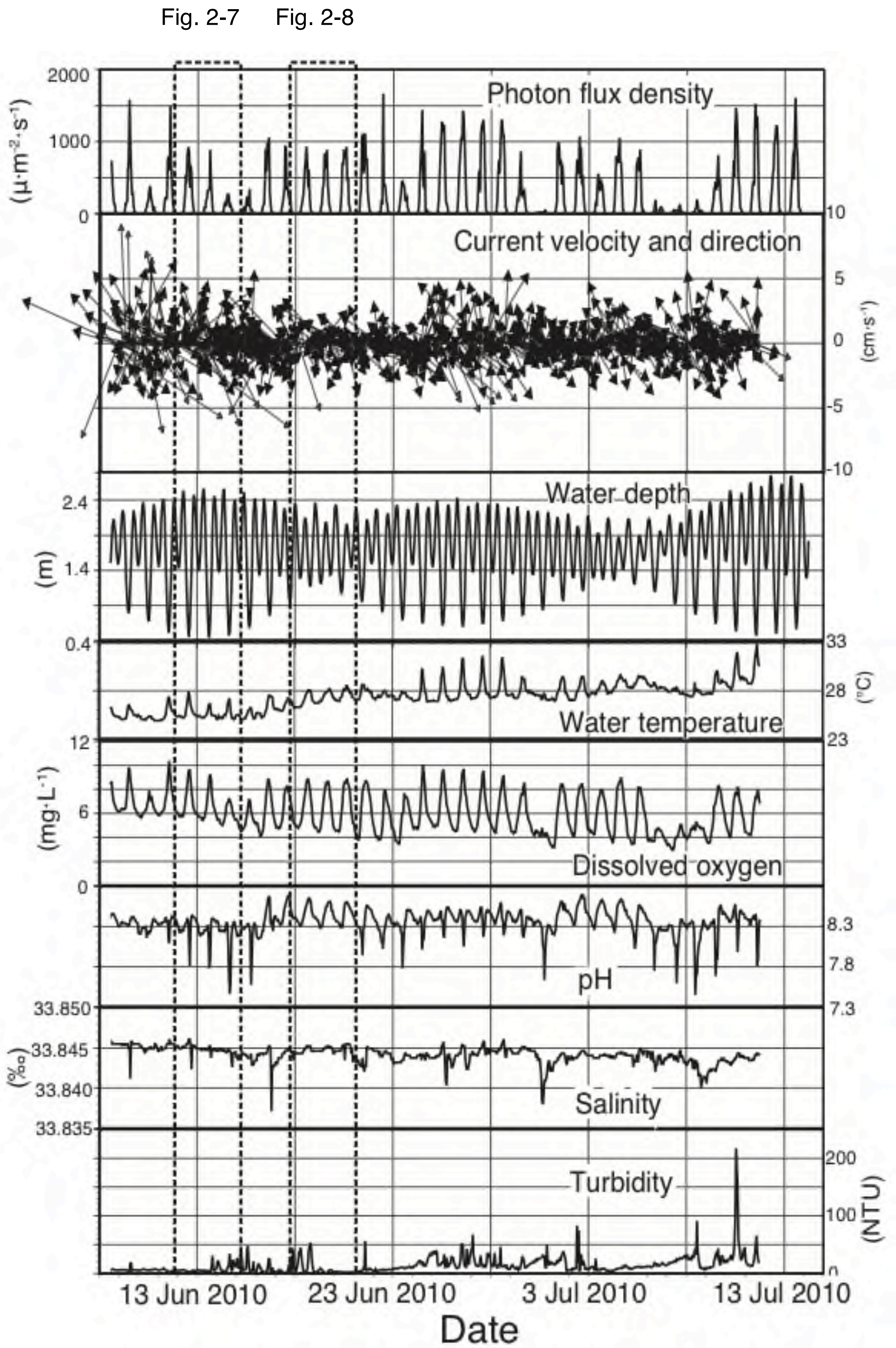

Fig. 2-7
Fig. 2-8
Photon flux density
(μ·m⁻²·s⁻¹)
2000
1000
0
Current velocity and direction
10
5
0
-5
-10
(cm·s⁻¹)
Water depth
(m)
2.4
1.4
0.4
Water temperature
33
28
23
(°C)
Dissolved oxygen
(mg·L⁻¹)
12
6
0
pH
8.3
7.8
7.3
Salinity
(‰)
33.850
33.845
33.840
33.835
Turbidity
200
100
0
(NTU)
13 Jun 2010
23 Jun 2010
3 Jul 2010
13 Jul 2010
Date

(The previous page) Fig. 2-6. Temporal changes in photon flux density, current velocity and direction, water depth, water temperature, dissolved oxygen, pH, salinity and turbidity at the "Dense soft coral" point (see Fig. 2-3) from 9 June to 11 July 2010. The periods of spring tide and neap tide (indicated by dotted rectangles) are enlarged in Figures 2-7 and 2-8, respectively (from Inoue et al., 2013).

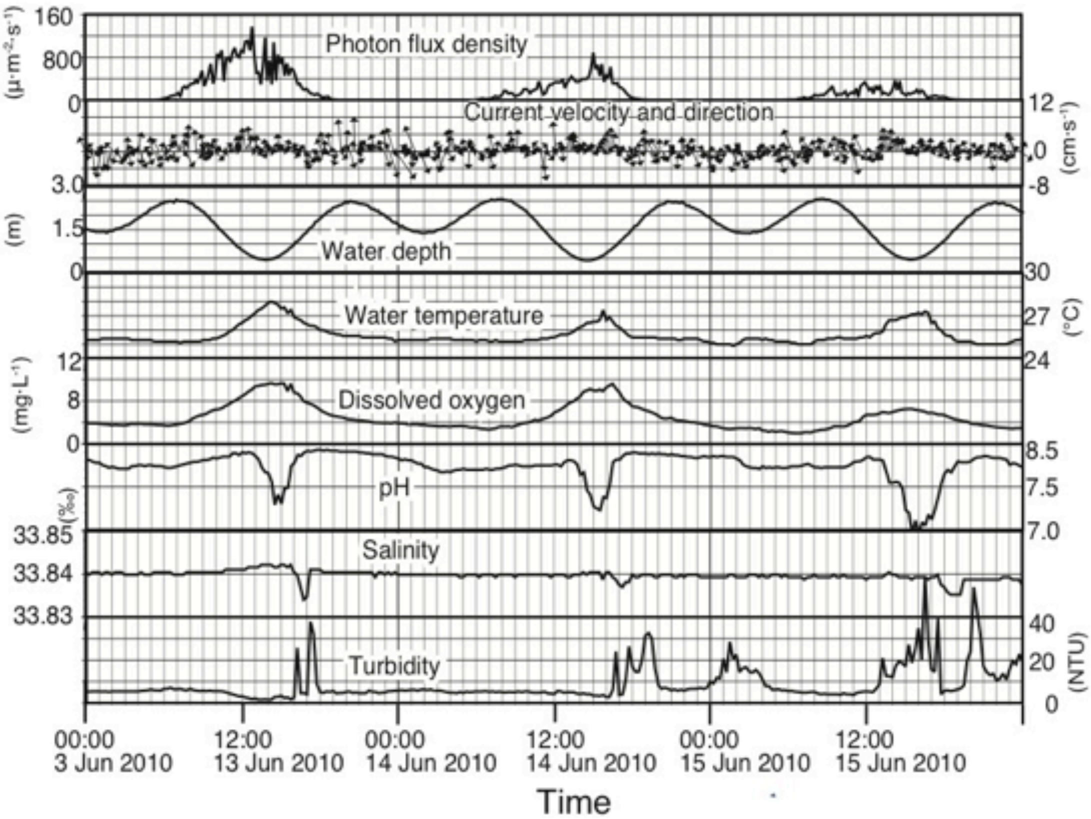

Fig. 2-7. Temporal changes in oceanographic parameters at the Main point during spring tide (see Fig. 2-6). The increase in dissolved oxygen with enhanced light volume was approximately synchronous with the decrease in pH at low tide.

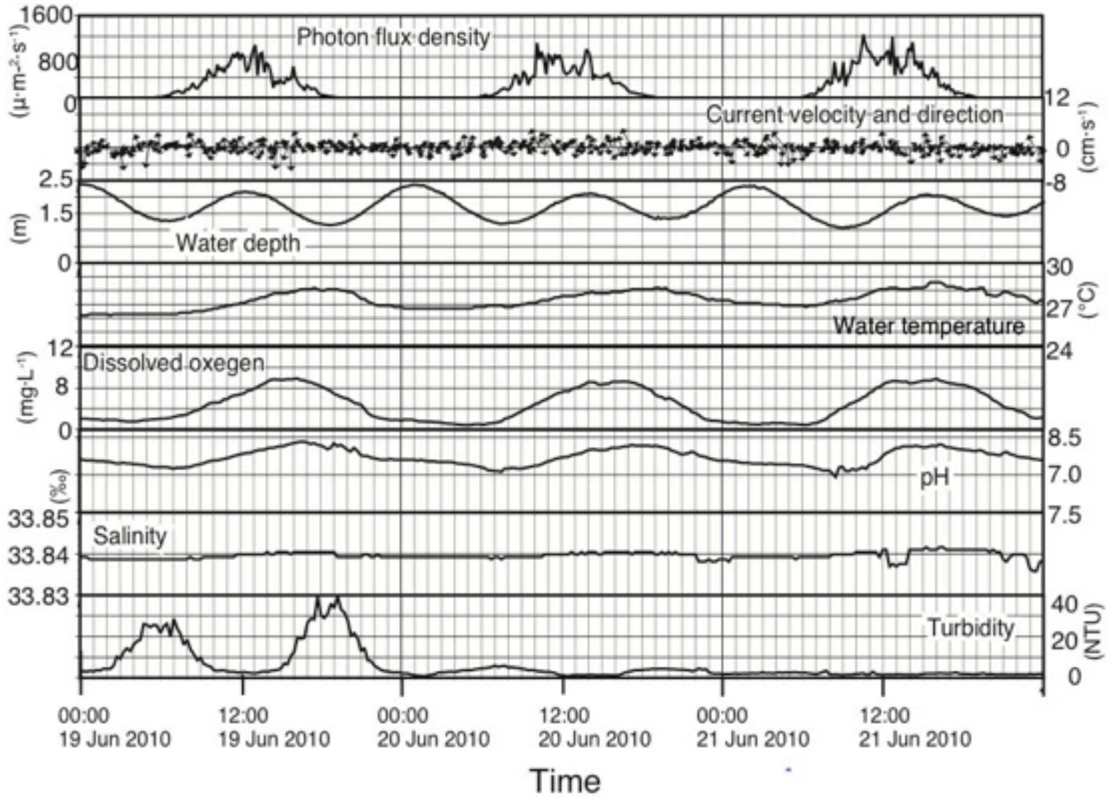

Fig. 2-8. Temporal changes in oceanographic parameters at the Main point during neap tide (see Fig. 2-6). Dissolved oxygen and pH are high in daytime, and a weak pH decrease occurs at low tide.

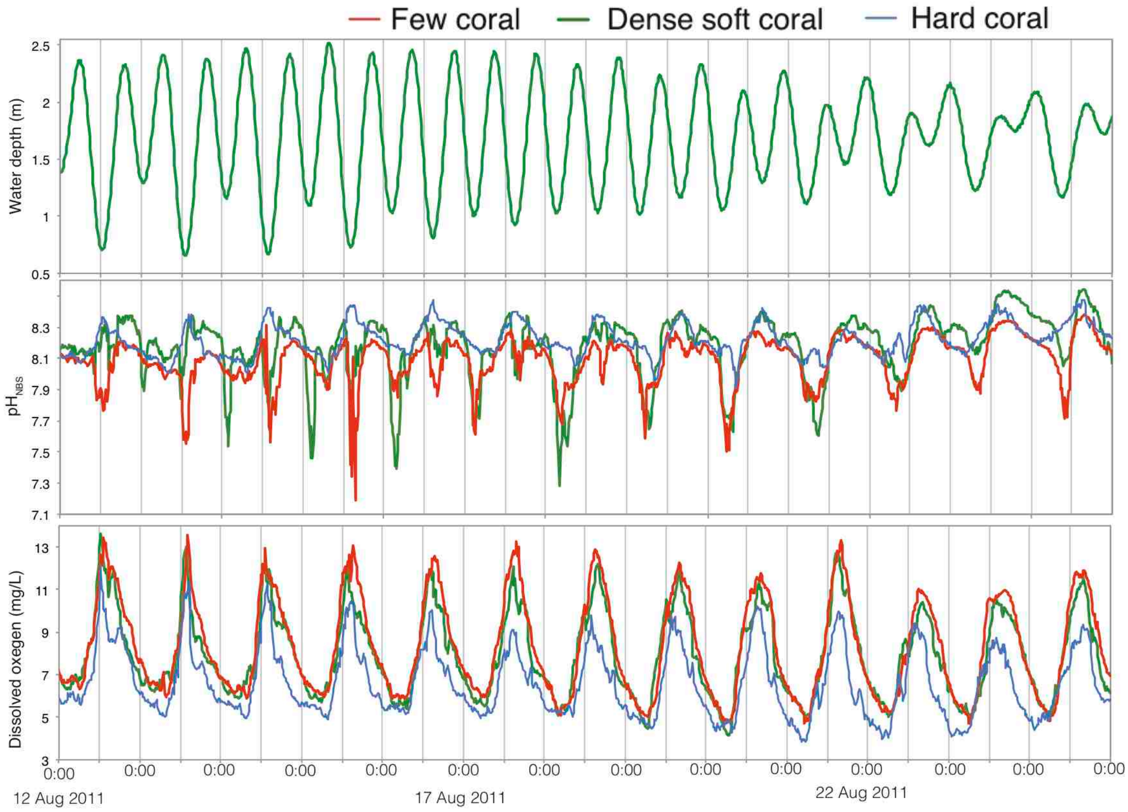

Fig. 2-9. The temporal changes of water depth, pH and dissolved oxygen from 12 to 25 Aug 2011. Water depth was measured at "Dense soft coral" point (Fig. 2-2, a) and pH and dissolved oxygen were measured at "Few corals" (red line), "Dense soft coral" (green line) and "Hard coral" (blue line) points. The average of pH during observation was 7.96 at "Few corals", 8.03 at "Dense soft coral" and 8.21 at "Hard corals" points. The lowest pH value was observed in the area with few corals. pH is in NBS scale.

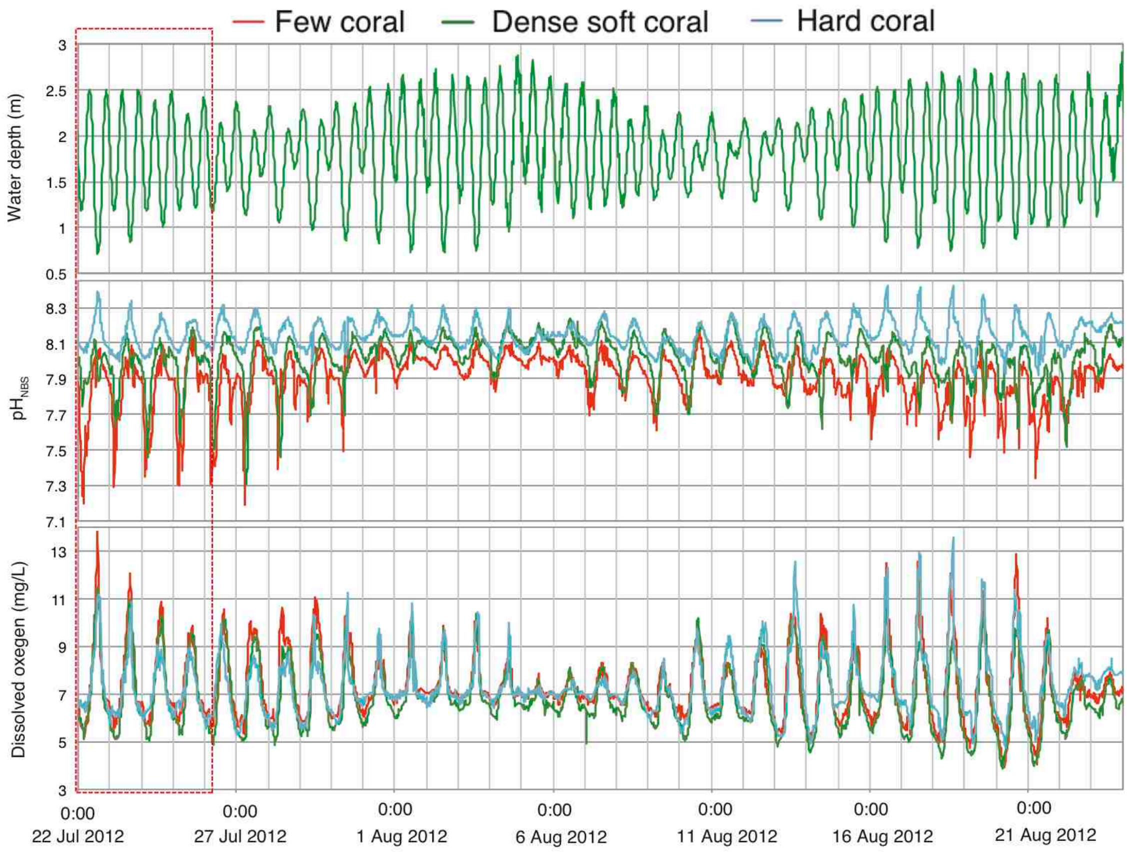

Fig. 2-10. The temporal changes of water depth, pH and dissolved oxygen from 22 July to 24 August 2012. Water depth was measured at "Dense soft coral" point (Fig. 2-2, a) and pH and dissolved oxygen were measured at "Few corals" (red line), "Dense soft coral" (green line) and "Hard coral" (blue line) points. The average of pH during observation was 7.81 at "Few corals", 7.91 at "Dense soft coral" and 8.14 at "Hard corals" points. The lowest pH value was observed in the area with few corals. pH is in NBS scale. The period of spring tide (indicated by red dotted rectangles) is enlarged in Figures 2-11.

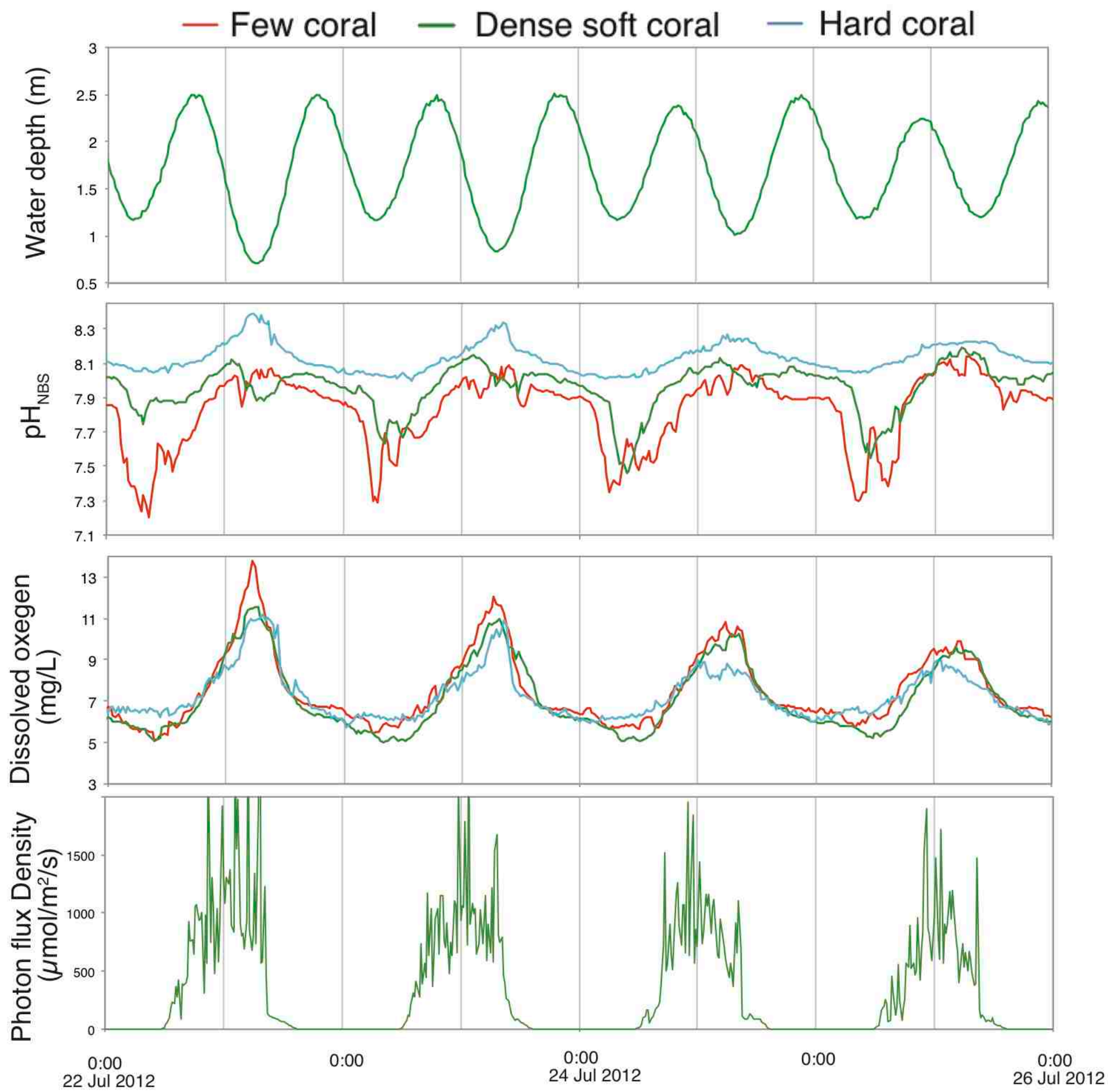

Fig. 2-11. The temporal changes of water depth, pH, dissolved oxygen (DO), and photon flux density from 22 to 25 July 2012. Water depth and photon flux density was measured at "Dense soft coral" point (Fig. 2-2, a) and pH and dissolved oxygen were measured at "Few corals" (red line), "Dense soft coral" (green line) and "Hard coral" (blue line) points. In this season, the peaks of DO and the low tide occur at the same time during daytime. The pH and DO increased at "Hard coral" point. At "Few corals" and "Dense soft coral" points, no pH negative peak occurs as shown in Fig. 2-7 because the acidification was offset by CO_2 consumption by photosynthesis.

2-4-4. Seawater chemistry

The spring water around the coast showed low salinity and low sulfate ion concentrations, though the ion concentrations at the main point were not different significantly from that of ocean (Fig. 2-12).

Nutrient concentrations in coral reef waters in Iwotorishima Island fall within the range of general coral reefs (Tanaka, 2012) and are not significantly different between acidified area and ambient pCO_2 area (Tab. 2-2).

Table 2-1. Average, minimum and maximum of temperature (°C) measured at the three points in eastern part of Iwotorishima Island (a. in Fig. 3), from 13 to 20 August 2011. Seawater variability is similar in three points (Table s2 of Inoue et al., 2013).

Measured point (see Fig. 2-3, a)	Average	Min	Max
Few corals	28.6	25.8	31.3
Dense soft corals	28.6	26.1	30.8
Hard corals	28.3	25.6	31.4

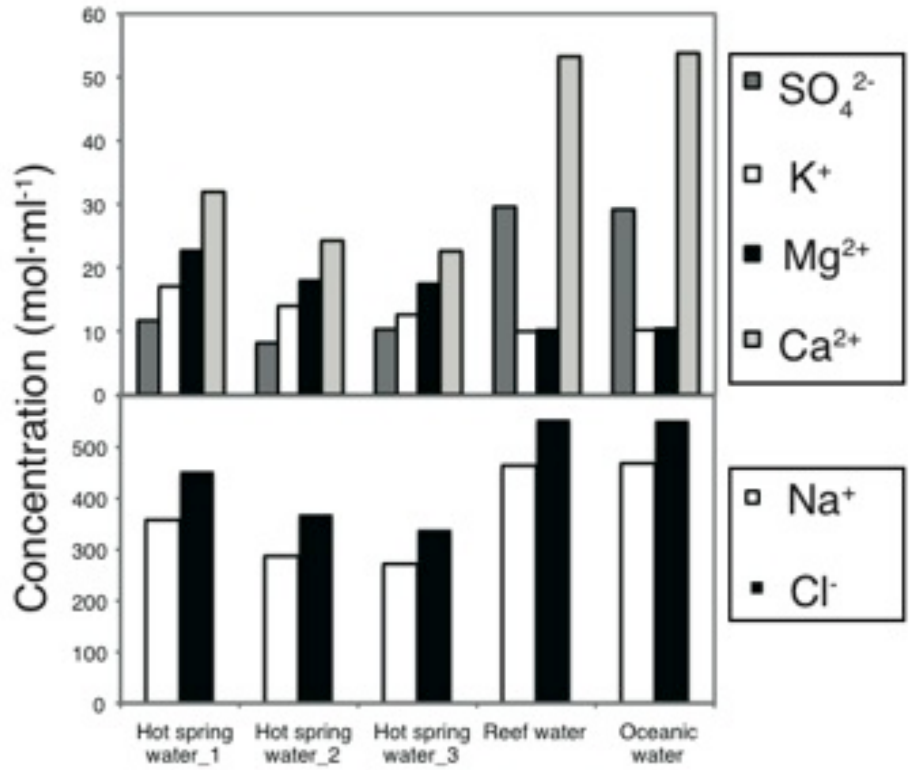

Fig. 2-12. Concentrations of the main ions in spring water and seawater collected at the "Dense soft coral" point (Fig. 2-3) at 13:00–14:00 (local time) on 14th July 2010. (Fig. of Inoue et al., 2013)

Table 2-2. Nutrient concentrations (average ± standard variation, n = 3) of coral reef seawater in acidified area (sampled at star in Fig. 2-2) and Ambient pCO_2 area (sampled at arrow on NE line in Fig. 2-1).

	Nutrient concentrations (μmol/L)			
Sampled area	NH_4^+	PO_4^{3-}	NO_3^-	NO_2^-
Acidified area	0.88 ±0.073	0.01 ±0.005	0.55 ± 0.0907	0.02 ±0.010
Ambient pCO_2 area	0.12 ±0.012	0	0.48 ±0.011	0.01 ±0.009

2-4-5. Biota

In the ambient pCO_2 area, on lines NE and SW, hard coral lives with high density in coverage up to 80% (Fig. 2-1) and more than 20 species were observed (See appendix Tables A1 and A2). In contrast, in the acidified area hard coral lives with a cover less than 20 % in lagoon. The acidified area is subdivided into four zones from north to south along the biota (Tab. 2-3 and Fig. 2-13). The first area is few hard or soft corals zone, where the cover of sessile aquatic invertebrates on rock beds to which invertebrates can easily attach is less than 5%. The second area is a zone with dense soft coral communities dominated by *Sarcophyton elegans,* where the substratum consists of sand and volcanic boulders, and the soft corals are attached to the boulders only. One to five meter wide patches of cohesive soft corals 20-30 cm in diameter are observed, with an average cover on the boulders of 50%. The soft corals are dominated by *S. elegans*, as identified by scanning electron microscope observations of the sclerites of five representative samples from "Dense soft coral" point (Fig. 2-14). However, morphological characteristics alone are not enough to identify the species *Sarcophyton* and *Lobophytum*, and it is possible that other *Sarcophyton* or *Lobophytum* species could also inhabit the *S. elegans* zone.

The third area is a zone with soft coral communities dominated by *Sinuraria* spp., with *S. elegans* (N13 transect in Fig. 2-2) whose abundance decreases from north to south, while the abundance of the branched soft coral *Sinularia* spp. increases. The fourth area is a zone dominated by hard corals dominated by *Montipora digitata*, which is typical dominant species in lagoonal coral reef in the Ryukyu Islands.

Non-calcifying coral *Palythoa* spp., which belongs to Zoanthidea, was observed in all the surveyed area, in a tidal zone of both ambient CO_2 area and acidified area. Non-calcifying algae and sea glass were not observed on all surveyed area.

The feature of each zone did not change during the three-year survey period (2010-2012).

Table 2-4. Information of subdivided zones in the study area (Table1 of Inoue et al., 2013)

Line included in the zone (Fig. 2-2)	Dominating benthos	Substratum	Cover (%) on hard substratum	pCO_2 (μatm) at the low tide on 12th July 2010
N 17	Few hard or soft corals	Coral reef	Less than 5	1,465
Main	Dense soft coral communities dominated by *S. elegans*	Boulders and sand	Average 50	831
N13-11	Soft corals dominated by *Sinularia* spp.	Boulders and sand	Average 20	795 - 524
N8	Hard corals (*Montipora digitata*) and few soft corals	Coral reef	Average 10	225

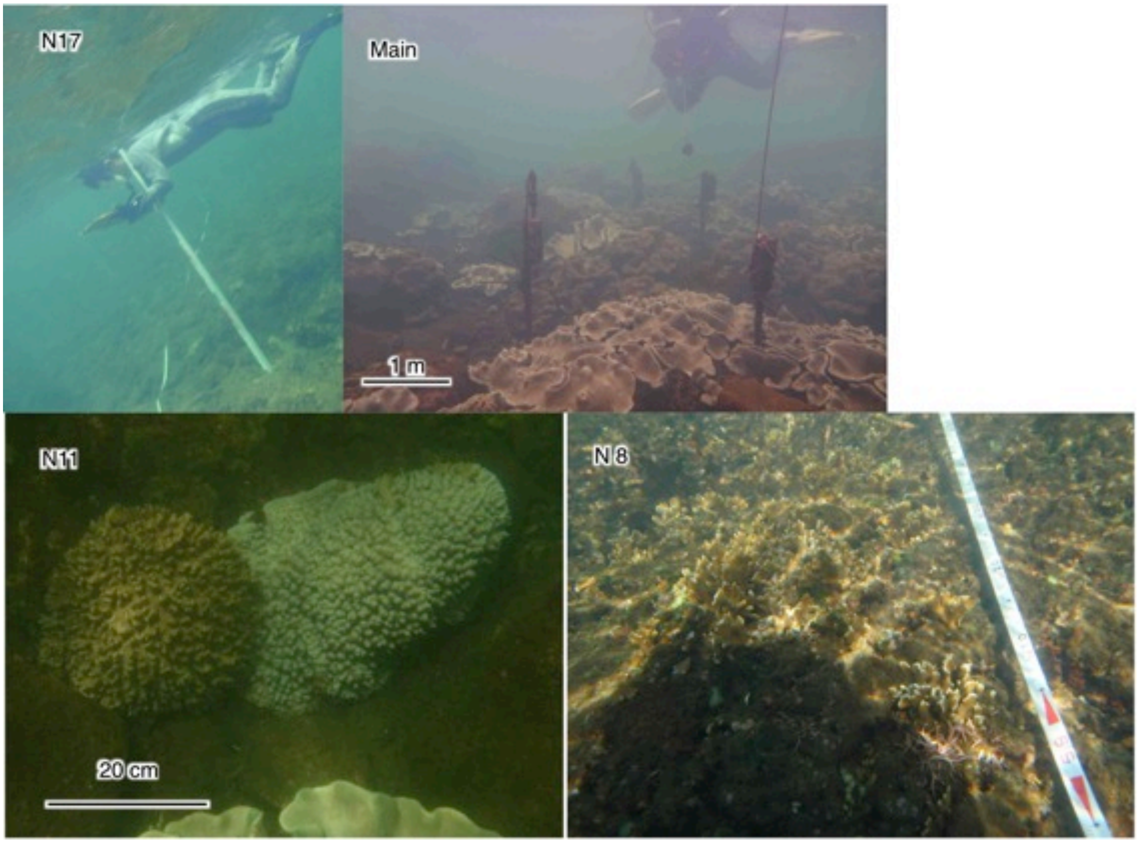

Fig. 2-13. Photos of each survey line (Table 2-4). N17: Few hard coral or soft coral lives and the substratum is coral reef. Main: multi-parameter water quality meters, a quantameter, a wavemeter, and a current meter placed in the dense soft coral communities area. N11: *Sinularia* spp. attached to the boulder. N8: *Montipora digitata* on the substratum of coral reef.

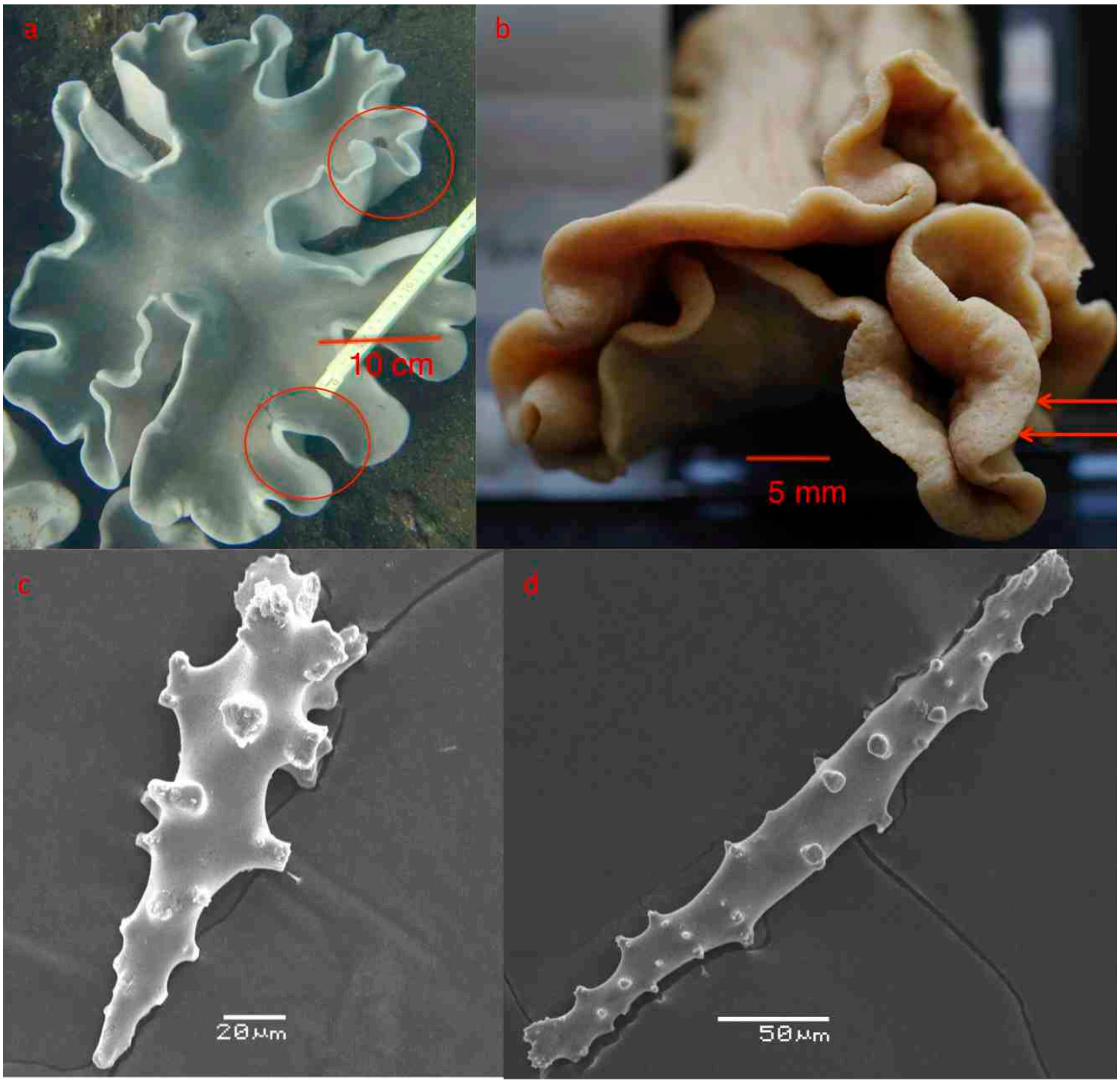

Fig. 2-14. Colony of *Sarcophyton elegans* in the sea (a), colony preserved in ethanol (b) and SEM photos of sclerites picked up from the coral colony (c, d). The features of the observed soft colony showed that the central part has a funnel shaped depression (a) and the margin extended distinctly beyond the stalk and sculptured with a number of semi-circular lobes (red circle in a). The edge was thin (a and b). The polyps were retracted and were apart 0.8 to 1.5 mm each other (b). The sclerites of surface layer with polyp were 90 to 300 μm (c and d); the majority was 100 to 200 μm. The head of sclerites type of “c” consisted of a number of warts. The sclerites in the stalks were up to 800 μm long. These features were concerted with the enrollment of *Sarcophyton elegans* (Moser, 1919).

2-5. Discussion

2-4-1. The cause of local acidification in Iwotorishima-Island

Local acidification in Iwotorishima Island is mainly due to CO_2 venting, which is the same causative molecule for the ocean acidification in global environmental problem. Local acidification is derived from two mechanisms observed so far. The one is gas venting such as in Papua New Guinea (Fabricius et al., 2011), which does not change the TA of seawater. The other is the mixing of low pH water reported in Mexico (Crook et al., 2011) and in Palau (Shamberger et al., 2014).

In Iwotorishima Island, both venting gas and spring water were observed along the coast. The most abundant gas typically released into the atmosphere from volcanic systems is H_2O, followed by CO_2 and SO_2. There was no SO_2 nor H_2S in the gas sampled in Iwotorishima Island. Therefore, the venting gas in Iwotorishima Island is composed of CO_2. The spring water along a cost consists of ratio of ions different from that of the ocean. However, the amount of spring water is too small to affect the ratio of ions at "Dense soft coral" point, which has the same ionic composition with that of oceanic water (Fig. 2-12). Also TA observed in Iwotorishima Island was in the range of typical coral reefs (Kayanne et al., 2005 and Watanabe et al., 2006, Fig. 2-12). The global ocean acidification is caused by human-induced CO_2 emission, which does not affect the amount of TA in seawater. The local acidification in Iwotorishima Island is close to the condition in the future ocean acidification. Also, the TA of acidified seawater in PNG is similar to that of control (Fig. 2-15).

2-5-2. CO_2 variability

The coral crest of reefs is developed in the acidified area in Iwotorishima Island and emerges above sea level at low tide (Fig. 2-2). The pCO_2 and pH varied in association with the tidal cycle at the "Dense soft coral" point, where dense soft coral populations occurred. During low tide, seawater pCO_2 increased to 831 μatm at the "Dense soft coral" point (Fig. 2-5). Also, data for a one-month period showed that pH decreased at low tide, particularly during spring tides. During neap tides, seawater pH reduction was less pronounced due to seawater exchange between the reef and the open ocean (Fig. 2-9). This indicates that the effect of volcanic acidification occurs during low tides when the reef crest is exposed and the seawater in the reef is separated from the outer ocean, resulting in a semi-closed system. The semi-closed system expands the acidified area over the lagoon. The pCO_2 distribution at low tide showed the acidification in this area was over 100×200 m^2 from line N17 to N11 (Fig. 2-2). The acidification was stronger in the north, where CO_2 seeps were densely distributed (Fig. 2-2, white dotted line in a). Line "N17" is more acidified than line "Main", because there is a channel 20 m to the south of the line "Main" (Fig. 2-3). The area around line N8 is with pCO_2 level of 225 μatm, even lowered than the ambient level by photosynthesis, unaffected by pCO_2 venting.

The average pH during observation also showed the stronger acidification in the north. The average pH was the lowest at the “Few coral” point on the line N17 and the highest at “Hard coral” point on the line N8.

In addition to the CO_2 venting, metabolism of living organisms also changes the CO_2 concentration. Photosynthesis consumes CO_2 and evolves O_2. Generally in coral reef lagoon, $p$$CO_2$ decreases during daytime because of photosynthesis (Kayanne et al., 2003). On the “Dense soft coral” point in Iwotorishima Island, $p$$CO_2$ decreased during high photon flux density followed by its increase up to 800 μatm (Fig. 2-5). CO_2 was consumed by photosynthesis of dense soft coral. The concentration of dissolved O_2 showed increase with high photon flux density (Figs. 2-7 and 2-11). Photosynthesis has the potential to reduce the acidification, but only with high photon flux density. On the other hand, O_2 decreased during the night-time (Figs. 2-7 and 2-11) because of the respiration of soft coral, which increases CO_2 and enhances the acidification.

Not only photosynthesis, but also calcification changes the carbon chemistry. Calcification decreases TA. As compared with Shiraho, the similar semi-closed coral reef with living hard coral, the variability of TA at “Dense soft coral” point was small (Fig. 2-8). It indicates that the amount of calcification of soft coral should be smaller than that of hard coral.

In summary, the feature of CO_2 variability in Iwotorishima Island is summarized as follows:

・The acidification occurs regularly in association with tidal cycle
・The acidification prevails over the area of about 100×200 m^2 in the lagoon
・The acidification is stronger in the northern part.

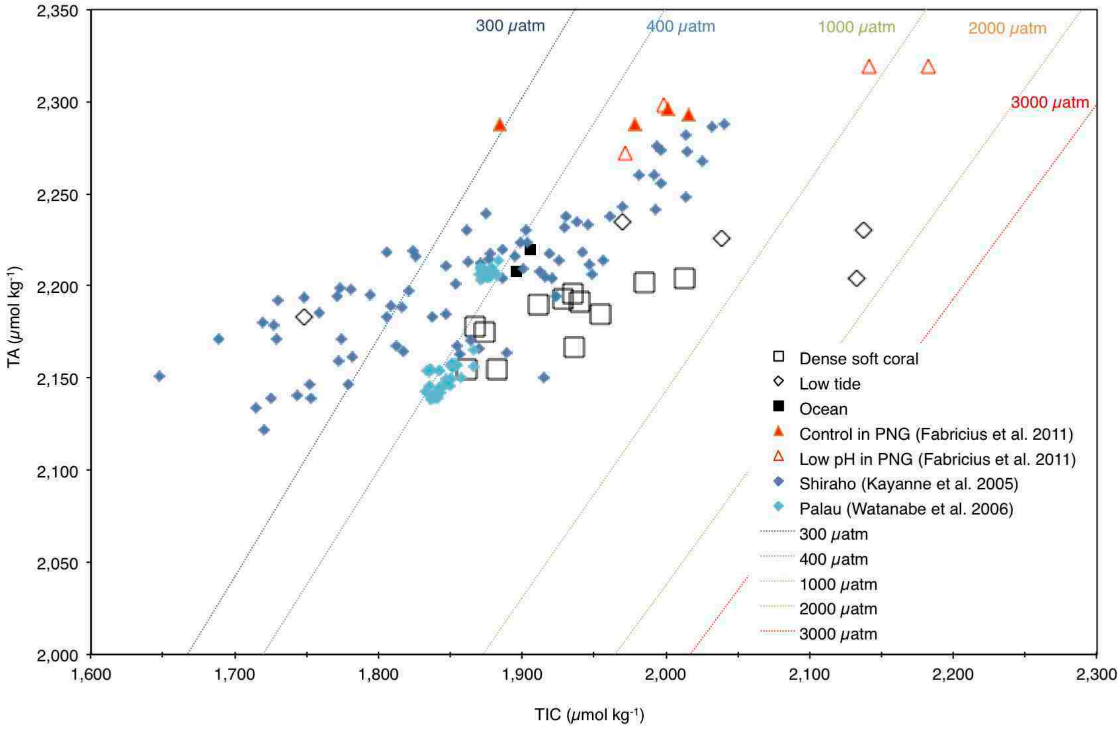

Fig. 2-15. The TA and TIC data of seawater in Iwotorishima Island sampled at "Dense soft coral" point (□, Fig. 2-6), in low tide (◇, Fig. 2-2, a) and open ocean 200 m off the end of the transect "main" (■, Fig. 2-2, a) and in PNG sampled in elevated pCO_2 area (△, Fabricius et al., 2011) and in ambient pCO_2 area (▲, Fabricius et al., 2011) Also, the data of the ambient CO_2 coral reefs of Shiraho, Ishigaki Island in Ryukyu Island (Kayanne et al., 2005) and of Palau (Watanabe, et al., 2006) are plotted. Isolines of pCO_2 at 300, 1,000, 2,000 and 3,000 μatm, when the salinity is 35‰ and the temperature is 30 ºC, are plotted. The black arrow indicates the direction of global ocean acidification, caused by CO_2 increase, having no effect on the amount of TA. The TA in Iwotorishima Island is in the range of that of natural coral reefs and only TIC is increased, which is close to the seawater in the future with ocean acidification.

2-4-3. CO_2 distribution and Biota

From the result it is clear that soft coral *Sarcophyton elegans* has a tolerance to live in elevated pCO_2 of around 831 (Fig. 2-2) at low-tide and in pH (average 7.91-8.03) for more than 3 years, as a community scale with high density.

The habitat of corals in the acidified area changes from south to north with increase of acidification; the amount of calcium carbonate in the body of the dominant species decreased. Hard coral, which lives only in the most south line N8, has skeletons on the order of centimetres. Those of *Sinularia,* which dominates N11 are on the order of millimeters, the sclerites of *Sarcophyton,* which dominates the line Main, are on the order of microns, and the most northern line N17, there were few corals.

Water temperature, salinity, turbidity were not different significantly among "Few coral", "Main" and "Hard coral" points. Also nutrient and TA were in the range of general coral reefs. Therefore, the difference of pCO_2 is considered as the principal factor for the change in dominant species from hard coral to soft corals (and from *Sinularia to Sarcophyton*). The distribution change of corals observed in the acidified area in Iwotorishima Island may be attributed to the differences in the tolerance capacities of hard versus soft corals to high pCO_2 levels.

2-4-4. Summary of Iwotorishima Island

The acidification in Iwotorishima Island is enhanced during low tides, and extends wide range of about $100 \times 200 m^2$.

Soft coral (*Sarcophyton elegans)* has a tolerance to live in elevated pCO_2 of around 831 during daytime, at low-tide and in pH (average 7.91-8.03) for more than 3 years, as a community scale with high density.

The distribution change of corals observed in the acidified area in Iwotorishima Island may be attributed to the differences in the tolerance capacities of hard versus soft corals to high pCO_2 levels.

Chapter 3: Culture experiment

3-1. Introduction

The study of Iwotorishima Island showed that soft coral *Sarcophyton* has higher tolerance to elevated pCO_2 than hard coral. To evaluate its metabolic superiority over hard coral, culture experiments to examine the metabolic response of soft and hard coral to high pCO_2 was conducted. The response of soft coral to pCO_2 increase has never been examined. Therefore experimental design of this study drew on experiments for hard coral, because soft coral has polyp in which zooxanthella cohabits in common with hard coral and it lives in the same habitat with hard coral. Comparison of metabolic responses to elevated pCO_2 between soft and hard corals would be applied to the spatial shift from hard to soft corals observed in the field.

The effects of ocean acidification on hard coral have been mainly studied in tank experiments (Kroker et al., 2013). In general, experimental studies have reported negative impacts of high pCO_2 on hard coral species. However, the impacts vary by degree (Pandolfi et al., 2011). These discrepancies among studies may be partly attributed to the differences in experimental design, as well as to species-specific responses to ocean acidification.

In this study, the metabolic reaction to ocean acidification of soft and hard coral in situ, and therefore the experiment was conducted under natural light to make experimental condition as natural as possible, though the volume of natural light was not controlled.

3-2. Method

3-2-1. Experimental outlines

The experiment was done with soft coral for 35 days in August 2010 and hard coral for 17 days in September 2011. We tested the effects of high pCO_2 levels on the photosynthesis and calcification rates of both corals. The soft coral *Sarcophyton elegans* was experimentally cultured for 35 days under control (392 ± 14 μatm), medium (1,020 ± 43 μatm), and high (2,361 ± 148 μatm) pCO_2 conditions and hard coral *Montipora digitifera* was cultured for 17days under control (394 ± 54μatm), medium (1664 ± 281 μatm) and high (2754 ± 476 μatm) pCO_2 conditions in outdoor tanks under natural summer-time temperature and light regimes (Fig. 3-1).

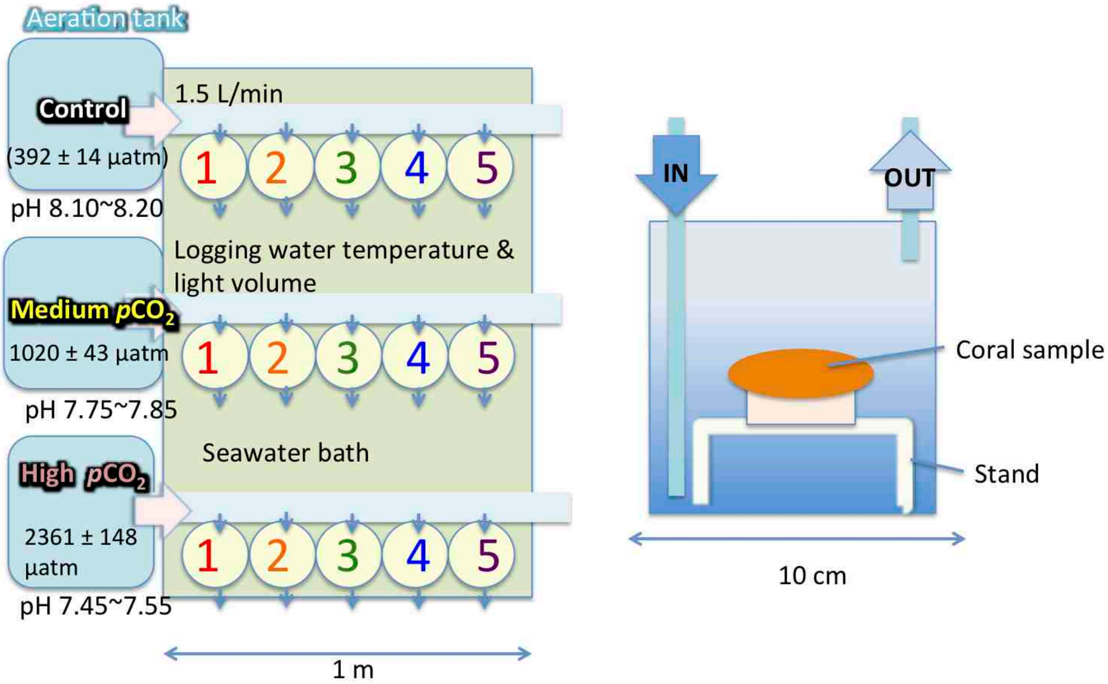

Figure 3-1. Setting up of the culture experiment. The left figure: the over view of water tank. Single soft coral nubbins from each of the five colonies were placed in separate culture chambers (820 ml), and were supplied with three different seawater $p\mathrm{CO_2}$ conditions at a flow rate of 150–180 ml/min under control (392 ± 14 μatm), medium (1,020 ± 43 μatm), and high (2,361 ± 148 μatm) $p\mathrm{CO_2}$ conditions.
The right figure: Seawater temperature of the culturing chambers was maintained to be similar with the field seawater temperature by placing them in a water bath (140 × 100 × 40 cm) that continuously received seawater pumped from the front of the station.

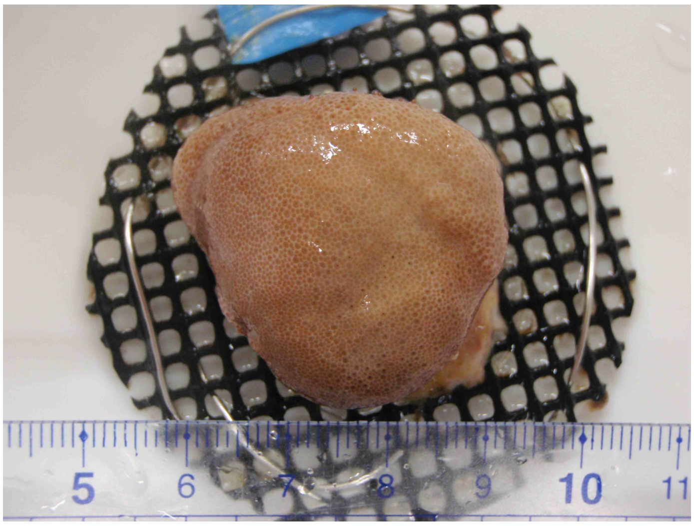

Fig. 3-2. A soft coral sample of the culture experiment. The colony sticks on the black net, repairing the damage of cutting for over a month.

3-2-2. Soft coral

3-2-2-1. Sampling and setting

Five colonies of the soft coral *S. elegans* were sampled on 9 June 2010 from "Dense soft coral" point at Iwotorishima Island (Fig. 2-2). The soft corals were shipped to the Tropical Biosphere Research Center, Sesoko Station, Sesoko Island, Okinawa, Japan, and kept in an outdoor flow-through tank, receiving a continuous supply of seawater pumped from a depth of 5 m in front of the station, before the start of experiment. Three soft coral nubbins of similar size (approximately 3 cm^2) were cut from each of the five colonies and fixed on plastic nets with spontaneous recovery. They were kept in the flow tank for 2 weeks before start of the experiment (Fig. 3-2)

3-2-2-2. Culture experiment set up

The culture experiment was run for 5 weeks, from 31 August to 5 October 2010. Single soft coral nubbins from each of the five colonies were placed in separate culture chambers (820 ml), and were supplied with three different seawater pCO_2 conditions at a flow rate of 150–180 ml/min under control (392 ± 14 μatm), medium (1,020 ± 43 μatm), and high (2,361 ± 148 μatm) pCO_2 conditions.

Seawater pCO_2 was controlled by bubbling air or CO_2 enriched air through the bubbling tank (20 L). The CO_2 enriched air gas was obtained by mixing air and pure CO_2 using a mass flow controller (HORIBASTEC, SEC-40, Japan). The experimental tank was placed under outdoor natural light conditions. Seawater temperature of the culturing chambers was maintained to be similar with the field seawater temperature by placing them in a water bath (140 × 100 × 40 cm) that continuously received seawater pumped from the front of the station. The pH (NBS scale) of each tank was measured daily at 10:00 am using a pH electrode (Mettler Toledo, MP125, USA). Temperature was measured using a water temperature data logger (Hobo U22-001, Onset Corp., USA) and the photon flux density was measured using an illuminometer (MDS-MkV/L, JFE, Japan). The water temperature and light intensity (photon flux density) were logged every 10 min (Fig. 3-3). The temperature data logger and illuminometer were set on the center of the water bath. Seawater samples (300 ml each) were collected by filling up the glass bottles, which are imperviable with CO_2 (Duran, Schott), for analyzing CO_2 chemistry and salinity, and 0.2 ml of $HgCl_2$ was added for fixation. These samples were shipped and preserved at ambient temperatures. The samples were taken one bottle per each chamber on the 1st, 15th, 21th and 35th day of the experiment. Seawater salinity was measured using a salinometer (Portasal 8410, Guildeline Instruments) and TA and TIC were measured with a flow-through CO_2 chemistry analyser (Kimoto Electronic, Watanabe et al., 2004), respectively. The seawater carbonate chemistry of each $p\mathrm{CO_2}$ condition was calculated using the CO_2sys program (Lewis & Wallace, 1998) (Table 3-1), using the dissociation constants K_1 and K_2 (Lewis, E. & Wallace, 1998) and values for aragonite solubility (Mucci, 1983).

Table 3-1. Average seawater carbonate chemistry for the three pCO_2 conditions during the five-week culture experiment period of soft coral. Values are means ± SD.

	Control	Medium pCO_2	High pCO_2
Salinity (ppt)	34.30 ± 0.14	34.41 ± 0.08	34.38 ± 0.19
TA (μmol $kgSW^{-1}$)	2,242 ± 7	2,248 ± 8	2,248 ± 5.6
DIC (μmol $kgSW^{-1}$)	1,981 ± 10	2,144 ± 6	2,255 ± 8
pH	8.17 ± 0.013	7.81 ± 0.018	7.47 ± 0.026
pCO_2 (μatm)	392 ± 14	1,020 ± 43	2,361 ± 148
HCO_3^- (μmol $kgSW^{-1}$)	1,782 ± 12	2,020 ± 7	2,138 ± 6
CO_3 (μmol $kgSW^{-1}$)	18 6± 4	92.5 ± 4	45 ± 2.7
CO_2 (μmol $kgSW^{-1}$)	12.2 ± 0.43	31.6 ± 1.44	73.18 ± 4.64

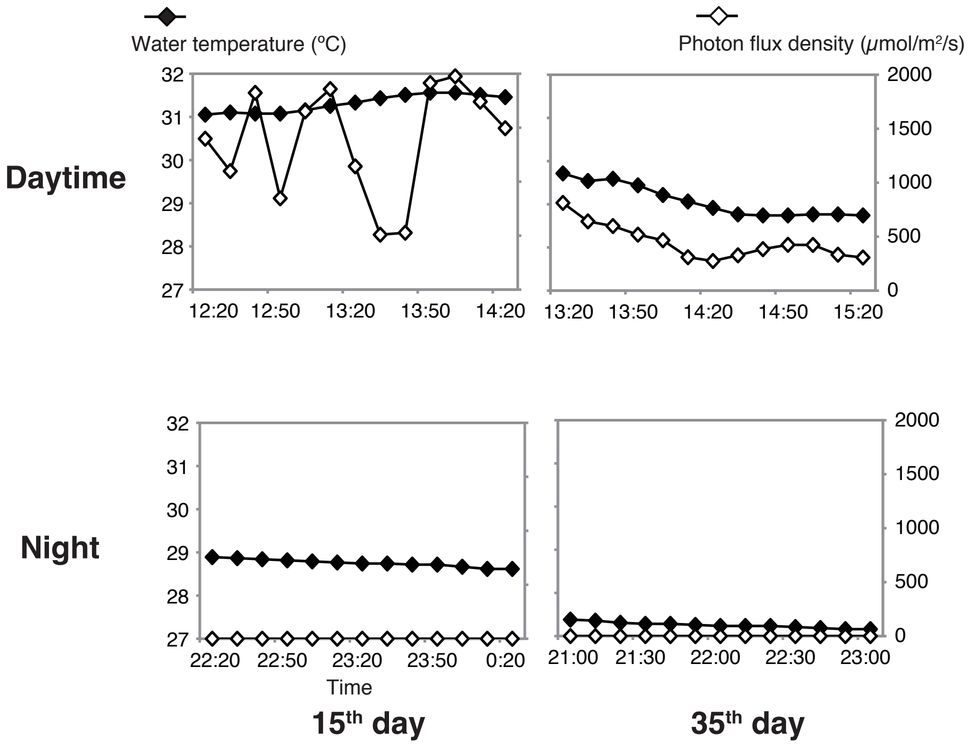

Fig. 3-3. Photon flux density and water temperature during the 2-h culture experiment on the day on the 15th and 35th days after the start bubbling of CO_2-enriched air experiment. The upper panels show daytime data and the lower panels show night-time data. The all data during the culture experiment is shown in appendix.

3-2-2-3. Total number of zooxanthellae

In this study, the total number of zooxanthellae was used for standardization of the net photosynthesis and net calcification rates. No previous study has compared the photosynthesis rates between soft and hard corals. Because the body structure differs between the two corals, the current measures for standardization such as surface area or dry weight is not appropriate to compare between these two corals. Generally, the weight including fresh body and sclerites is used for standardization of metabolism rate of soft coral (Tentori, 2006), which cannot be applied to hard coral as it contains hard skeletal part. In contrast, surface area is used for hard coral (e.g. Takahashi and Kurihara, 2012), which cannot be applied to soft coral as it changes with time. For comparing the photosynthesis rates of soft and hard corals in same dimension, the total number of zooxanthella is selected for standardization in this study because zooxanthella in polyps is the common feature of both soft and hard corals. The relations between the weight and the total number of zooxanthella were reported in appendix. In order to determine the total number of zooxanthella in the soft coral tissue, zooxanthella density and surface area of polyps were measured.

After the experiment, an equal area of surface polyp tissue of each group (0.5 mm^2) was cut from the center of each colony and homogenized in tubes containing filtered (0.25 μm) seawater (FSW). The homogenate was filtered (180 μm) and centrifugalised with 2400 rpm mixing speed for 4minitus at 25 ºC. Then filtered (40 μm) again and centrifugalised with 1500 rpm mixing speed for 4min at 25 ºC and repeat for three times. Next, supernatant liquid of the tube was tip off and added 10 mL FSW and vortexed. The 0.01 mL of vortexed seawater was taken on a Thoma - Zeiss hemocytometer and number of zooxanthella was counted for three times per one sample and calculated the number of zooxanthella per 0.5 m^2. All five individuals from control and medium pCO_2 conditions were examined, while only three individuals were studied for high pCO_2 condition because two individuals died on the 17th day and one on the 22nd day, respectively. The average value of zooxanthella density of the all colonies other than the two individuals was used for the dead two colonies because there was no significant difference in density of zooxanthella by pCO_2 condition (Appendix)

Surface area of polyp tissue was measured using the aluminium foil technique (Marsh 1970). Soft coral sample was taken from water and put on dry Kimtowel for 5 minutes for removal of water. Then aluminium foil was covered over the soft body, only brown part, which consists of polyps and the weight of the aluminum was measured. This operation was done for three times per one colony. The standard curve (weight-area) was made from the weight of the aluminium foil cut in known area (0.5, 1, 2, 3, 4 and 5 cm^2).

3-2-2-4. Calcification, photosynthesis and respiration

The calcification and net photosynthesis rates of the soft corals were measured using the TA anomaly technique (Smith and Key, 1975) and the dissolved inorganic carbon (DIC)-TA technique (Gattuso et al., 1996), respectively. Photosynthesis and calcification alter the DIC and TA values such that when 1 mol of organic carbon is produced, the DIC decreases by 1 mol, whereas TA is unchanged. When 1 mol of $CaCO_3$ is produced, the TA decreases by 2 mol and the DIC by 1 mol. From these relationships, TA and DIC measurements allow us to evaluate photosynthesis and calcification rates (Gattuso et al., 1996). Light and dark calcification were evaluated by measuring seawater TA changes during the 2 h incubation during the daytime and night time, respectively. Net photosynthesis (organic carbon production rate) was evaluated as the DIC change minus the calcification-induced alkalinity change during the 2 h culture period during the daytime. Respiration during night-time was evaluated as minus of net photosynthesis rate. The incubation was conducted under outdoor natural light condition on the day before bubbling and the 15^{th} and 35^{th} days after the start of the experiment. The initial seawater sample (300 ml) was obtained from the flowing seawater in each incubation chamber. Immediately after the incubation chambers were re-filled with new seawater, the chamber was sealed airtight without head space, and incubated for 2 h under natural light conditions. The chamber was continuously stirred with a rotator during incubation. At the end of the 2-h incubation, 300 ml of seawater was sampled from each chamber. All seawater samples were fixed with 0.2 ml of $HgCl_2$. Immediately after sampling and kept until TA and TIC analysis. Seawater TA and DIC were analysed using a flow-through CO_2 chemistry analyser (Kimoto Electronic; Watanabe et al., 2004), with accuracy and precision determinations made by analyzing certified reference materials (CRMs) obtained from Dickson Laboratory (Scripps Institution of Oceanography). The analytical errors of TA and DIC measurements were within 3 μmol kg^{-1}. Salinity was measured using a salinometer (Portasal 8410A, Guildeline Instruments, Ltd).

Net calcification rates (G) were calculated according to:

$$G = \Delta TA/2 \times \mathbf{V} \times D_{sw} \times t^{-1} \times W^{-1}$$

where ΔTA is the difference between the TA at the start and end of the culture experiment, V is the weight of seawater of each container minus the displacement weight of the colony, D_{sw} is the density of seawater, t is the duration of the culture experiment (2 h), and W is the total number of zooxanthella of each colony, as was mentioned in the previous paragraph.

Net photosynthesis rates during daytime (P) were calculated according to:

$$P = (\Delta DIC - \Delta TA/2) \times V \times D_{sw}\, t^{-1} \times W^{-1}$$

where ΔDIC is the difference between the DIC measured at the start and end of the culture experiment.

Net respiration rates during night-ime (R) were calculated according to:

$$R = -(\Delta DIC - \Delta TA/2) \times V \times D_{sw}\ t^{-1} \times W^{-1}$$

3-2-2-5. Statistical analysis

Statistical analyses were conducted with R, a free software environment for statistical computing (http://www.r-project.org/).

Data averages obtained during the experiments, for the photosynthesis and calcification rates, carbonate ion concentrations, and photon flux density were used in the statistical analysis.

Previous studies, which examined photosynthesis or calcification rates from the changes in TIC and TA, adopted pCO_2 condition only at the start of the experiment (ex. Schneider and Jonathan 2006, Chauvin et al., 2011; Takahashi and Kurihara et al., 2013). However, because the conditions changed by the metabolism of corals during the experiment and considering only the condition at the start is not appropriate.

In this study, data averages were used for discussion. The changes of pCO_2 of control conditions changed within a range of 200 μatm, which is observed in situ during daytime and nighttime in coral reefs (Kayanne et al., 2005). It indicates that average data is appropriate for the discussion in a time scale of half a day, though not appropriate to consider the momentary change of metabolism rate.

3-2-3. Hard coral

3-2-3-1. Sampling and setting

Four colonies of the hard coral *Montipora digitata* were sampled on 21 July 2011 from "Hard coral" point at Iwotorishima Island (Fig. 2-2). Same as soft coral, the hard corals were also shipped to the Tropical Biosphere Research Center, Sesoko Station and kept in an outdoor flow-through tank, before the start of experiment. Three hard coral nubbins of similar size (approximately 3 cm in diameter) were taken from each of the four colonies and kept in the flow tank for 2 weeks to recover from handling before start of the experiment.

3-2-3-2. Culture experiment set up

The culture experiment was run for 17 days, from 22 September to 8 October 2011. Single hard coral nubbins from each of the four colonies were placed in separate culture chambers (820 ml), and were supplied with three different seawater pCO_2 conditions at a flow rate of 150–180 ml/min under control (392 ± 14 μatm), medium (1,020 ± 43 μatm), and high (2,361 ± 148 μatm) pCO_2 (Fig. 3-1 and Table 3-2) conditions. Seawater pCO_2 control and measuring parameters (photon flux density, temperature,) were done in same way as the soft coral culture experiment. Seawater samples for the analysis of seawater salinity and TA were taken on the first, 7th and 15th day of the experiment.

Fig. 3-4. A hard coral sample of the culture experiment. The colony sticks on the stand be attached with an adhesive.

Table 3-2. Average seawater carbonate chemistry for the three pCO_2 conditions during the 17 days culture experiment period for hard coral. Values are means ± SD.

	Control	Medium pCO_2	High pCO_2
Salinity (ppt)	34.65 ± 0.025	34.66 ± 0.02	34.66 ± 0.027
TA (μmol $kgSW^{-1}$)	2,253.50 ± 8	2,256 ± 6	2,241 ± 11
DIC (μmol $kgSW^{-1}$)	1,986 ± 30	2,206 ± 9	2,286 ± 6
pH	8.18 ± 0.055	7.652 ± 0.035	7.36 ± 0.024
pCO_2 (μatm)	394 ± 56	1,529 ± 134	3,098 ± 160
HCO_3^- (μmol $kgSW^{-1}$)	1,784 ± 47	2,092 ± 10	2,155 ± 8
CO_3 (μmol $kgSW^{-1}$)	189 ± 20	66.47 ± 5	34.9 ± 2.0
CO_2 (μmol $kgSW^{-1}$)	12.2 ± 1.76	47.50 ± 4.14	95.9 ± 4.83

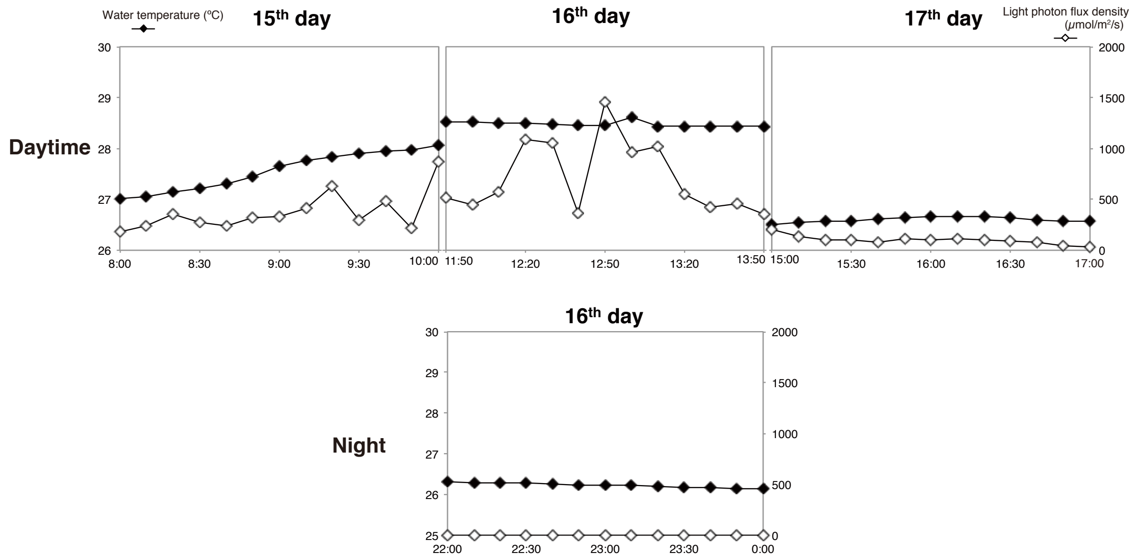

Fig. 3-5. Photon flux density and water temperature during the 2-h culture experiment on the day on the 15th, 16th and 17th days after the start bubbling of CO_2-enriched air. The upper panels show daytime data and the lower panel shows night-time data. The all data during the culture experiment are shown in appendix.

3-2-3-3. The total amount of zooxanthella

All zooxanthella of each hard coral sample colony were sampled with water pick and filtered, centrifugalised, vortexed and counted in the same way as soft coral.

3-2-3-4. Calcification, photosynthesis and respiration

Sampling seawater for measuring calcification and photosynthesis during daytime was done on the day before bubbling CO_2, 15, 16 and 17^{th} day and calcification and respiration during night-time was done on 16^{th} day of the experiment for hard coral. To obtain the data under different light intensity, sampling time were changed between the day as 8:00-10:00 on 15^{th}, 11:50-13:50 on 16^{th} and 15:00-17:00 on 17^{th} (Fig. 3-5). The samples were analysed in the same way with mentioned above.

3-2-3-5. Statistical analysis

Statistical analysis was done in the same way as that for soft coral.

3-3. Results

Two of the soft coral cultured in high pCO_2 condition died. They were detached from the net on 17th (sample IS: high_2) and 22th (sample ID: high_4) day, respectively, and got bleached and partially melted on the next day. No hard coral died during the experiment.

3-3-1. Light Calcification

The net light calcification of soft coral (*Sarcophyton elegans)* was $5.04 \pm 5.82 \times 10^{-9}$ μmol/ h (average± standard deviation), far less than that of hard coral ($42.16 \pm 4.80 \times 10^{-9}$ μmol/ h).

Fig. 3-6 shows the relations of Ω_a and the light calcification rates standardized with the total number of zooxanthellae of the colony of soft and hard corals. Each color shows different sampling day, with different photon flux density. The bars in the horizontal direction are the pCO_2 ranges from the start to the end of chamber isolation. The error bars of the calcification rates in the vertical direction were calculated from the standard variations of the zooxanthella density and the surface area using the error propagation method. The results of the light calcification experiment show that soft coral calcification is not significantly affected by elevated pCO_2 ($p = 0.400$), whereas hard coral is ($p = 0.0017$).

Fig. 3-7 shows the relations of average net light calcification rates and the average photon flux densities during closing chambers. The light calcification rates of soft ($p = 0.829$) and hard ($p = 0.581$) corals were not influenced significantly by photon flux densities.

3-3-2. Dark Calcification

Fig. 3-8 shows the relations of Ω_a and the net calcification rate during night-time (dark calcification) rates standardized with the total number of zooxanthella of the colony of soft and hard corals. Significantly large decalcification of both soft and hard coral occurred with $\Omega_a < 1$.

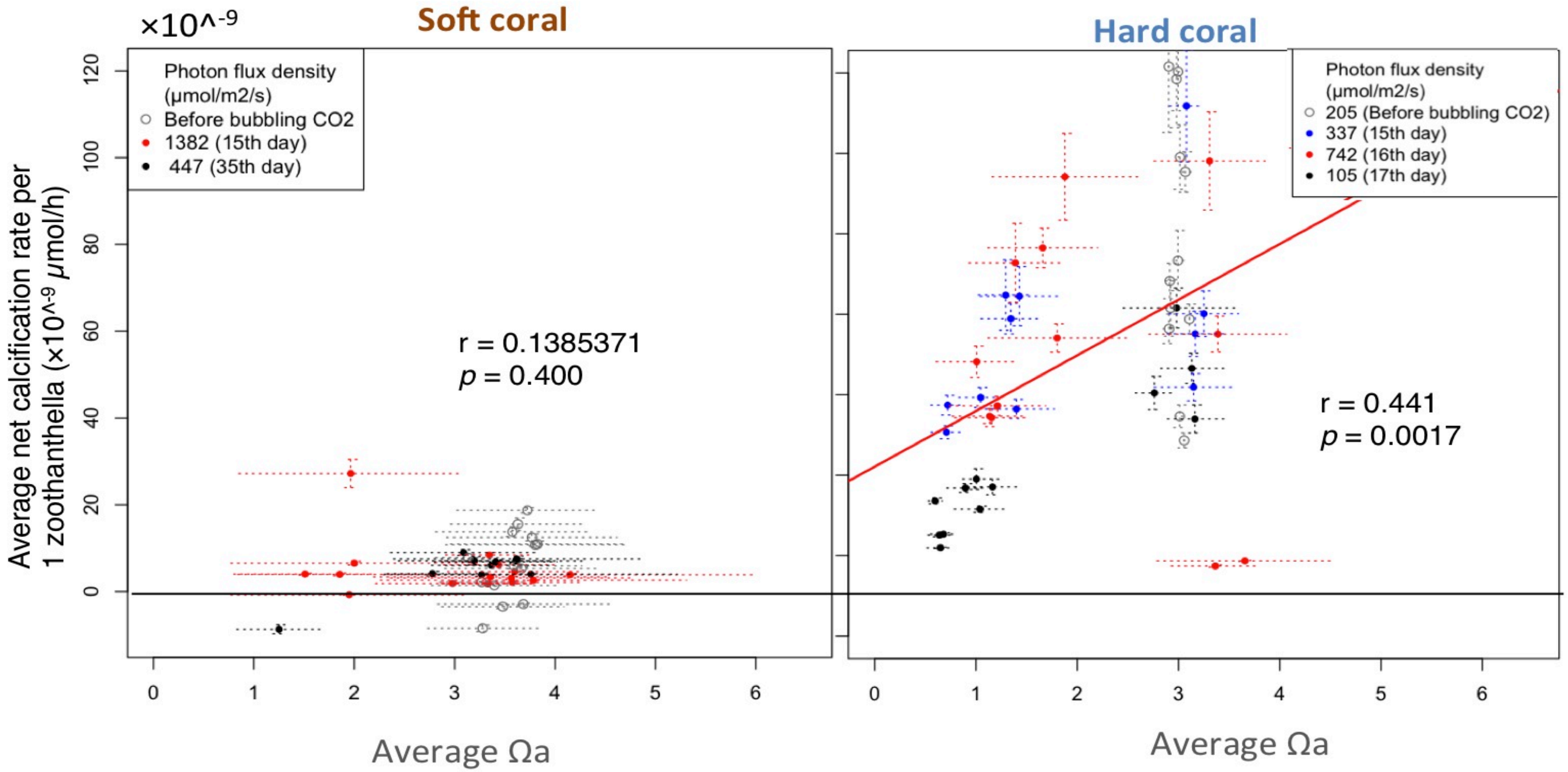

Fig. 3-6. The average Ω_a and the average net light calcification rate during chamber isolation of soft (left) and hard coral (right). Each color shows different sampling day, with different photon flux density. The bars in the horizontal direction are the pCO_2 ranges from the start to the end of chamber isolation. The error bars of the calcification rates in the vertical direction were calculated from the standard variations of the zooxanthella density and the surface area using the error propagation method.
Coefficients of correlation (r) and the significance of the relationships according to simple linear regression (p) are also shown. Light calcification of soft coral was not significantly influenced by pCO_2, but that of hard coral was inhibited by pCO_2. The red line is the regression line.

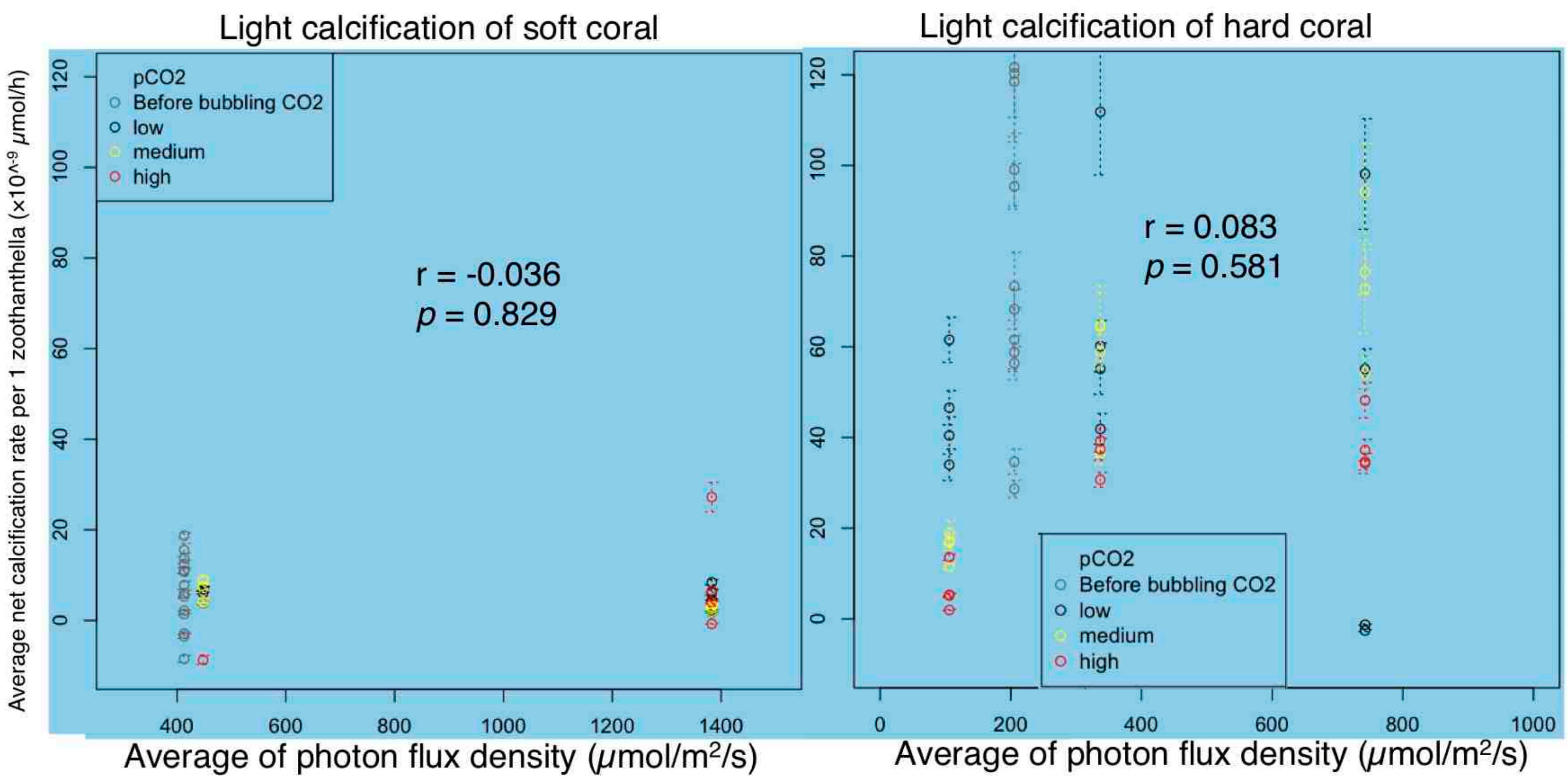

Fig. 3-7. The average photon flux densities and average net light calcification rates during chambers isolation of soft and hard corals. Each color shows different pCO_2 condition. The error bars of the calcification rates in the vertical direction were calculated from the standard variations of the zooxanthella density and surface area using the error propagation method.

Coefficient of correlation (r) and the significance of the relationships according to simple linear regression (p) are also shown.

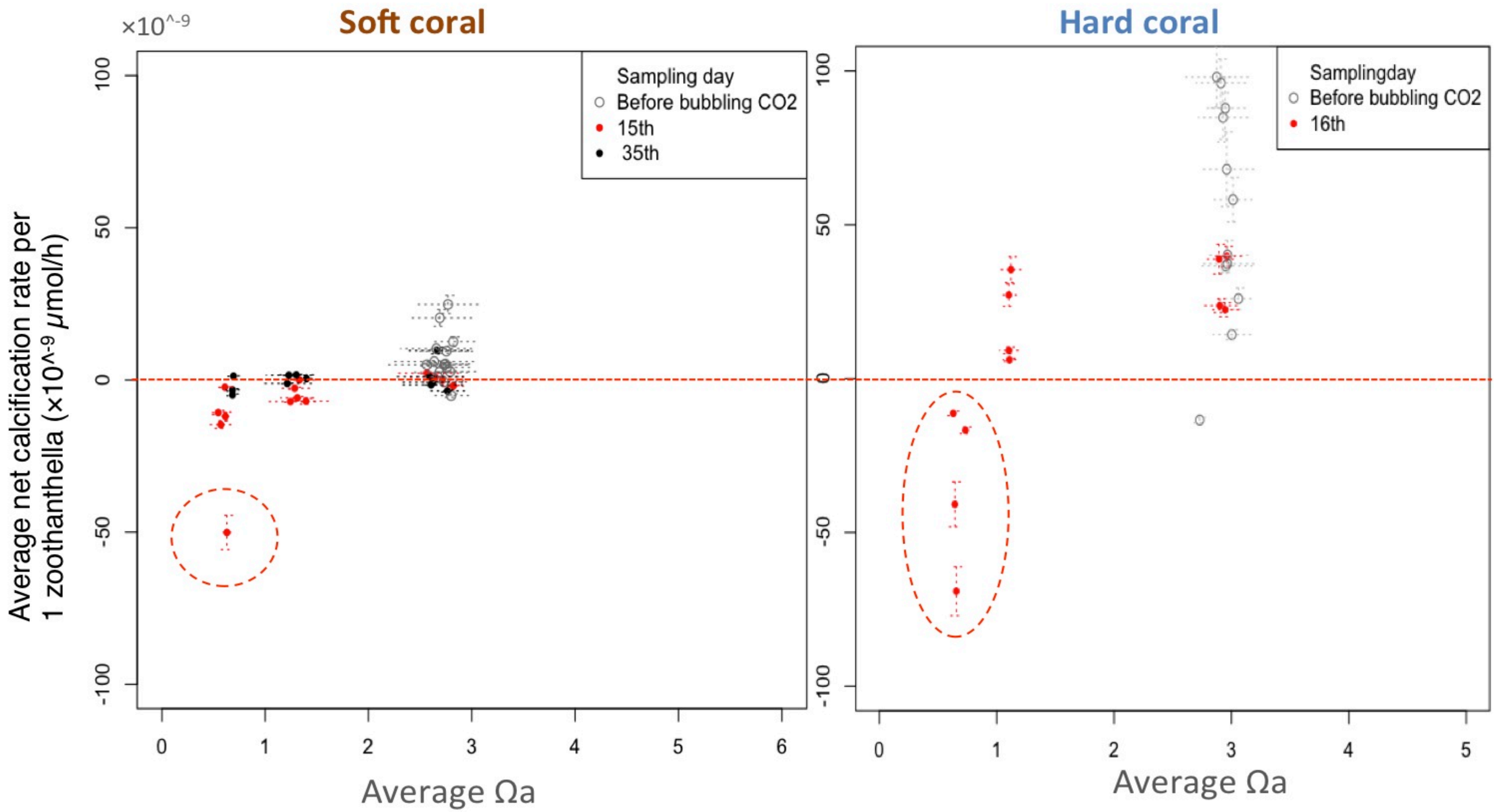

Fig. 3-8. AverageΩ_a and average net dark calcification rate during chamber isolation experiments of soft coral (left) and hard (right) coral. Each color shows a different sampling day. The bars in horizontal direction are the $p$$CO_2$ range from the start to the end of chambers isolation. The error bars of the calcification rates in the vertical direction were calculated from the standard variations of the zooxanthella density and surface area using the error propagation method.

Significantly large decalcification of both soft and hard coral occurred with Ω_a<1 (dotted red circle).

3-3-3. Light Photosynthesis

Fig. 3-9 shows the relations of the net photosynthesis rates during daytime (light photosynthesis) standardized with the total number of zooxanthella of the colony and pCO_2 of soft and hard corals. The light photosynthesis rate of soft coral was significantly correlated with pCO_2 ($r = 0.575$, $p = 1.3\times10^{-4}$, with simple linear regression). In contrast, that of hard coral was not correlated with pCO_2 significantly, but it seems to have weak negative correlation ($r = -0.280$, $p = 0.06$, with simple linear regression).

Fig. 3-10 shows the relations of photon flux density and light photosynthesis rates. The light photosynthesis rates and photon flux density were obtained as the average during chamber isolation. The light photosynthesis rate of soft coral was positively correlated with photon flux density ($r = 0.357$, $p = 0.026$ with simple linear regression). However, from the point diagram of Fig. 3-9, it is noticeable that photosynthesis rate for the only samples in low pCO_2 (before bubbling pCO_2 and low pCO_2 condition) only, had no positive correlation with photon flux density ($r = -0.327$, $p = 0.059$, with simple linear regression)(Fig. 3-10).

The light photosynthesis rate of hard coral had positive correlation with photon flux density ($r = 0.0773$, $p = 1.80\times10^{-10}$ with simple linear regression)(Fig. 3-9).

3-3-4. Dark respiration

Fig. 3-11 shows the relation between pCO_2 and the net respiration in night-time (dark respiration) rate standardized with the total number of zooxanthella of the colony of both soft and hard corals. The dark net respiration rates fell within a similar range and the rate of soft coral of 90.12×10^{-9} (μmol/h) was larger than that of hard coral of 66.29×10^{-9} (μmol/h) (One-way analysis of variance, $p = 0.0144$).

The dark respiration of soft coral was not influenced by pCO_2 ($r = 0.041$, $p = 0.798$, with simple liner regression), though that of hard coral decreased with increasing pCO_2 ($r = -0.548$, $p = 0.006$, with simple liner regression).

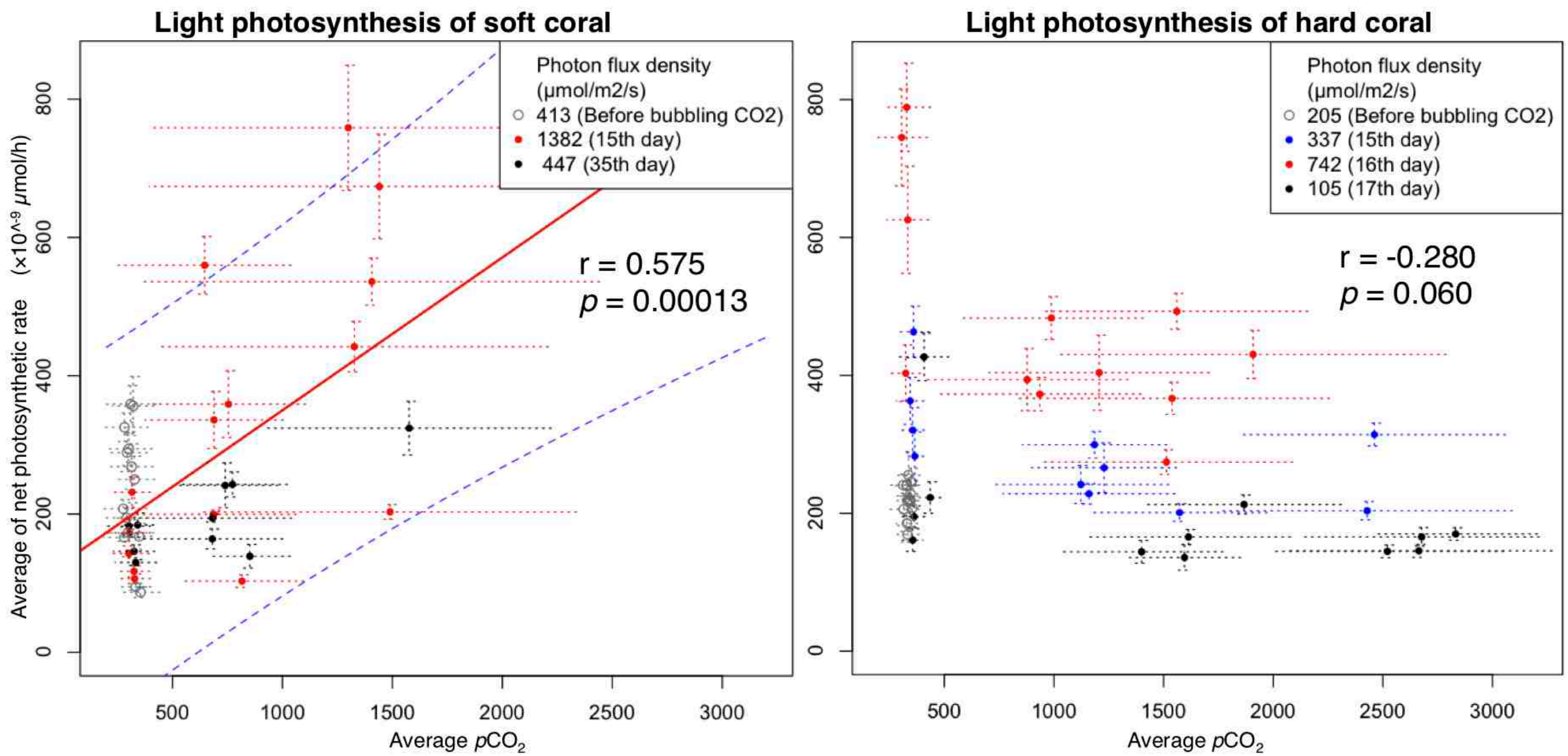

Fig. 3-9. Average pCO_2 and average net photosynthesis rate during daytime chamber isolation experiments of soft and hard coral. Each color shows a different sampling day with different photon flux density. The bars in the horizontal direction are the pCO_2 ranges from the start to the end of chamber isolation. The error bars of the photosynthesis rates were calculated from the standard variations of the zooxanthella density and surface area using the error propagation method.

Coefficients of correlation (r) and the significance of the relationships according to simple linear regression (p) are shown. Light photosynthesis of soft coral was significantly enhanced but that of hard coral was not influenced by pCO_2. The red line is the regression line, and the dotted blue lines show the 95 % confidence interval.

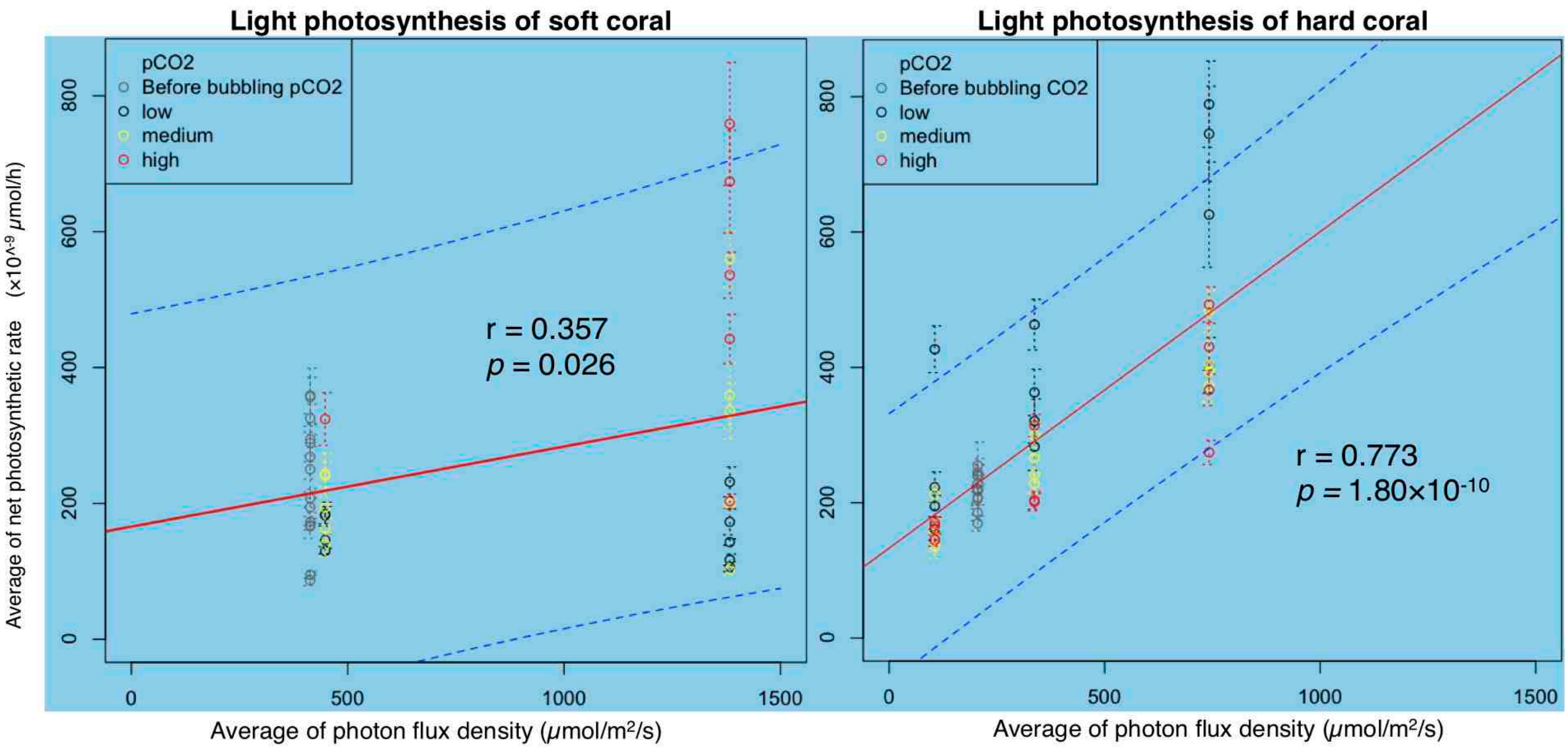

Fig. 3-10. Average photon flux density and average net light photosynthesis rate during chamber closing of soft and hard corals. Each color shows a different pCO_2 condition. The error bars of the calcification rates in vertical direction were calculated from the standard variations of the zooxanthella density and surface area using the error propagation method.

Both soft and hard coral photosynthesis was enhanced with increasing photon flux density.

The red line is the regression line and the dotted blue lines show the 95% confidence interval. Coefficients of correlation (r) and the significance of the relationships according to simple liner regression (*p*) are shown.

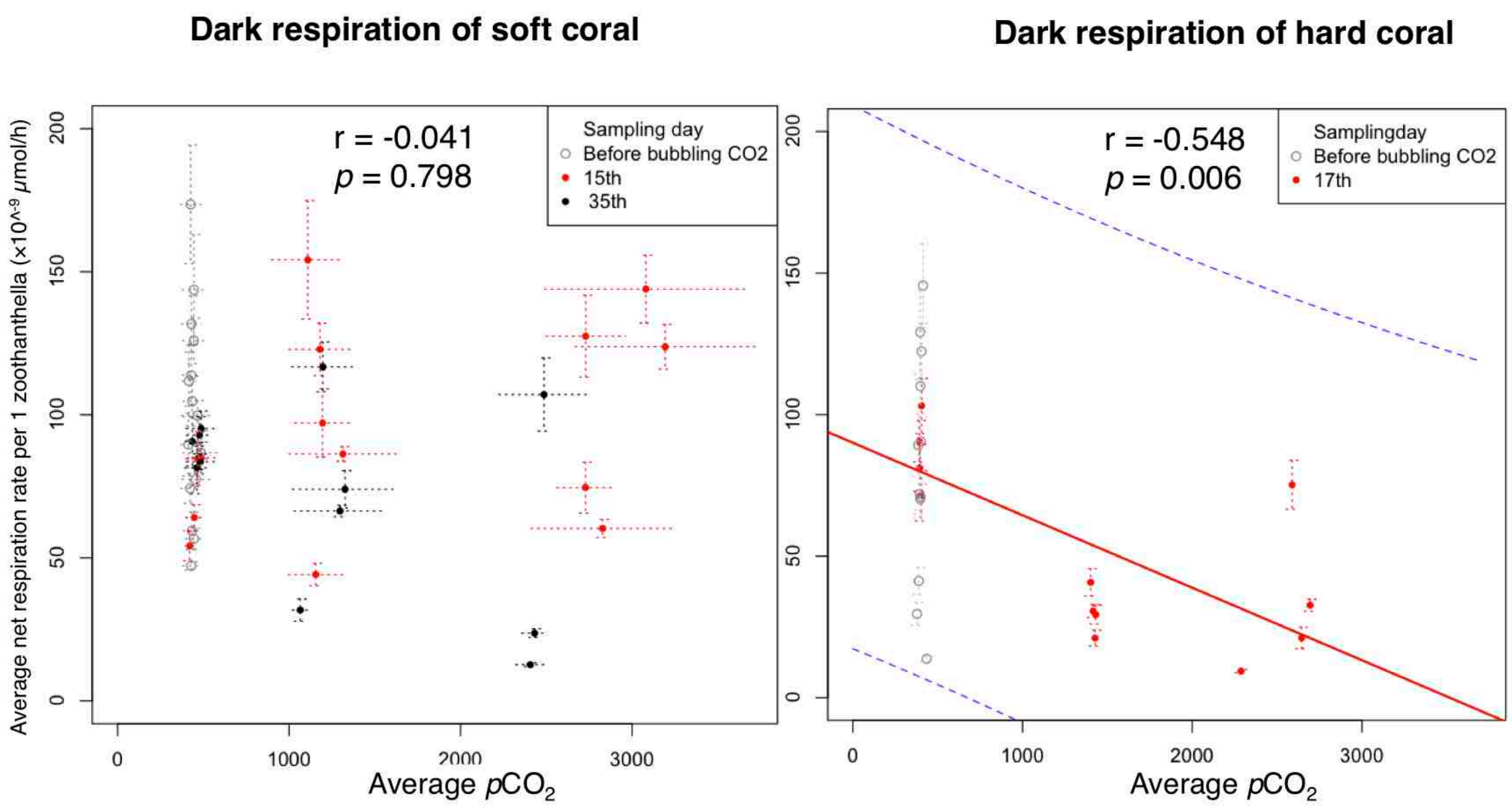

Fig. 3-11. Average pCO_2 and average dark net respiration rate during chamber isolation experiments of soft and hard corals. The bars in the horizontal direction are the pCO_2 range from the start to the end of chamber isolation. The error bars of the calcification in vertical direction are calculated from the standard variations of the zooxanthella density and surface area using the error propagation method. The respiration rates of soft coral of 90.12×10^{-9} (μmol/h) was larger than that of hard coral of 66.29×10^{-9} (μmol/h) (One-way analysis of variance, $p = 0.0144$).

The dark respiration of soft coral was not influenced by pCO_2 but that of hard coral decreased with increasing pCO_2.

The red line is the regression line and the dotted blue lines show the 95% confidence interval.

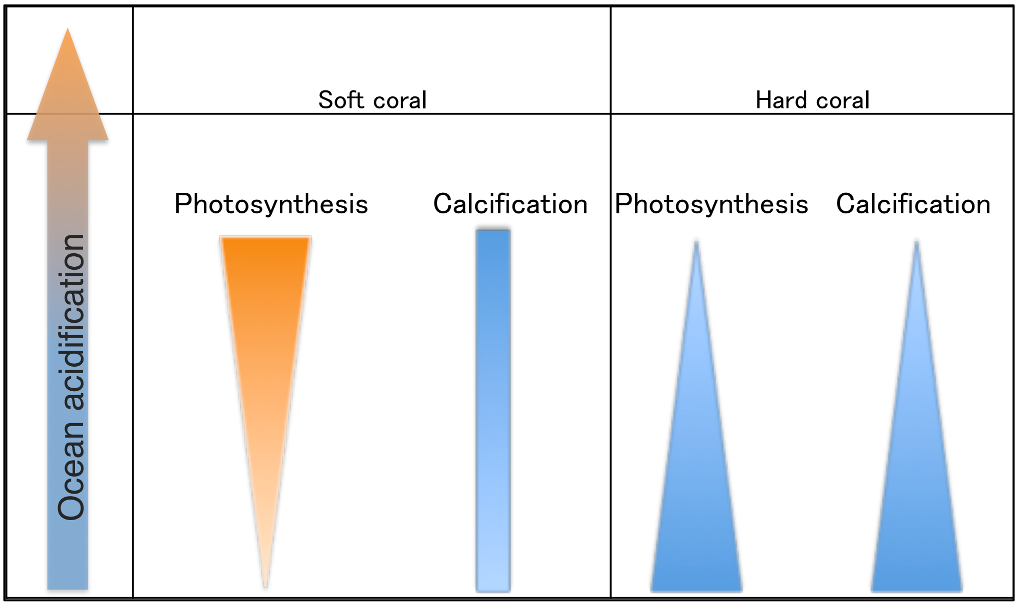

Fig. 3-12. Conceptual diagram of the daytime metabolic response of soft and hard corals derived from the results of the experiments of this study.

3-4. Discussion

Fig. 3-12 shows the summary of metabolic response during daytime. The results indicate that soft coral (*Sarcophyton elegans*) has higher tolerance to ocean acidification than hard coral (*Montipora digitata*). The results of the light calcification experiment show that soft coral calcification is not negatively affected by elevated pCO_2, which suppressed hard coral calcification. Light photosynthesis of soft coral was enhanced by pCO_2 increase. In contrast, that of hard coral was not affected by pCO_2. These results indicate that soft coral is not negatively affected by pCO_2 increase.

However, during night-time, both soft and hard coral calcification was inhibited by pCO_2 increase. In addition, two soft coral colonies cultured under high pCO_2 condition died during the experiment. This result indicates that high pCO_2 also has negative effect on soft coral.

3-4-1. Light photosynthesis and CO_2

Light photosynthesis of soft coral was enhanced by pCO_2 increase. In contrast, that of hard coral was not affected, or weakly inhibited by pCO_2 rising, which indicates that soft coral is better adapted to an elevated pCO_2 environment. A multiple linear regression model was established for the photosynthesis rate, which had the explanatory variables of pCO_2 and photon flux density (Fig. 3-13). At a photon flux density of 428 $\mu mol/m^2/s$, the average at Iwotorishima Island from 22 Jul to 25 Aug 2012, the photosynthesis rate of soft coral rose above that of hard coral at the pCO_2 levels within the range of 800-1300 μatm in confidence at 95%.

A possible explanation for the difference in the photosynthesis responses between the two corals to increased pCO_2 is that, because the zooxanthella living in soft coral can obtain more nutrient than that living in hard coral, and therefore the rate-limiting factor of photosynthesis are different between soft and hard coral

The zooxanthella living in hard coral is known to be nutrient-starved. Its C:N:P ratio is larger than the Redfield ratio, with smaller N and P (Tanaka, 2012). Chauvin et al. (2011) reported that hard coral photosynthesis increased under elevated pCO_2 when nutrient was added under high light intensity. These facts suggest the possibility that in ambient sea, hard coral photosynthesis is controlled by nutrient levels and not by CO_2.

The body structure of soft coral has a larger body volume ratio to polyp surface area than hard coral (Fig. 1-5), and the dark respiration per 1 zooxanthella is greater than that of hard coral (Fig. 3-12). This suggests that zooxanthella may obtain much nutrient from metabolite excretion. Soft coral photosynthesis is controlled by CO_2 and photon flux density, rather than by nutrient availability. However, this remains to be proven as currently no C:N:P ratio of zooxanthella in soft coral has been obtained.

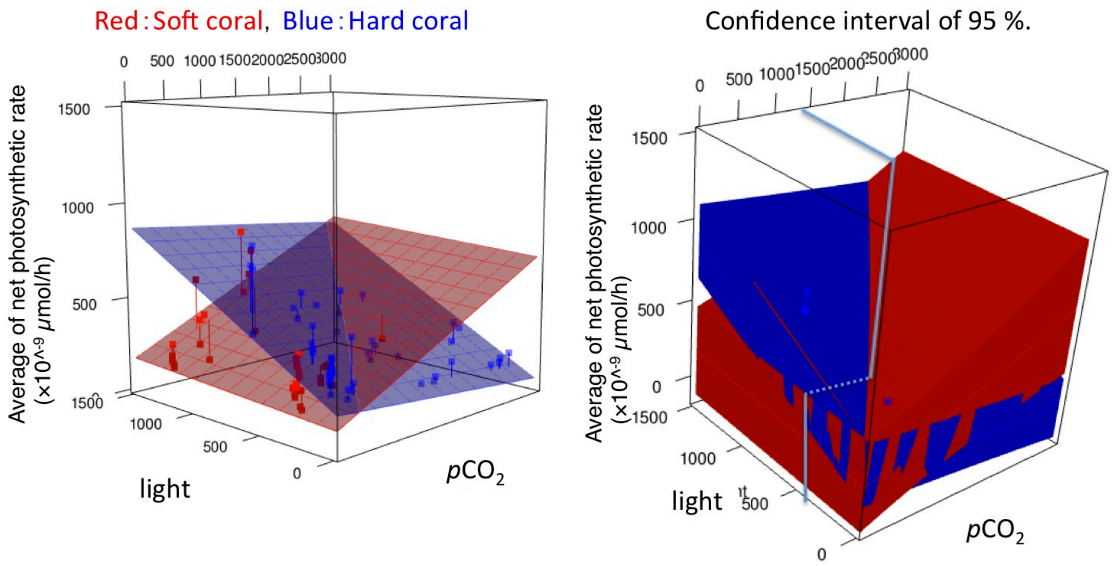

Fig. 3-13. Multiple linear regression models for photosynthesis rate, with the explanatory variables of pCO_2 and photon flux density. The data is presented in Table 3-3. The right figure shows the results of the 95% confidence interval. The red area indicates the soft coral model and the blue area indicates the hard coral model. At a photon flux density of 428 μmol/m^2/s, the average at Iwotorishima Island from 22 Jul to 25 Aug 2012, the photosynthesis rate of soft coral rises above that of hard coral at pCO_2 levels within the range of 800-1300 μatm (Ω_a: 2.19-1.54 at 30ºC) at the 95% confidence interval.

Table 3-3. The statistical results of multiple linear regressions.

Soft coral	Estimate	Std. Error	t value	Pr(>ltl)
(Intercept)	107.83558	44.41200	2.428	0.02031 *
pCO_2	0.19926	0.05778	3.448	0.00145 **
Photon flux denisty	0.04271	0.04968	0.860	0.39563

Hard coral	Estimate	Std. Error	t value	Pr(>ltl)
(Intercept)	177.50852	28.63543	6.199	1.58e-07 ***
pCO_2	-0.04080	0.01586	-2.572	0.0135 *
Photon flux denisty	0.45909	0.05344	8.591	4.83e-11 ***

3-4-2. Light Calcification and CO_2

The results of the light calcification experiment show that soft coral calcification is not significantly affected by elevated pCO_2, which inhibited hard coral calcification.

The light calcification rates of soft coral were only one tenth of that of hard coral. The light calcification rates of soft coral showed no significant correlation with either pCO_2 nor photon flux density. In contrast, the light calcification of hard coral was significantly inhibited by pCO_2 (Fig. 3-6). The decrease in the calcification rate of hard coral with pCO_2 increase of hard coral is in agreement with the result of Langdon and Atkinaon (2005), who investigated the same genus using HCl for acidification rather than aeration with CO_2.

The contrasting effects of high pCO_2 on the calcification rates of soft and hard corals are explained by their different skeletal structures. Soft corals have tiny internal calcareous sclerites composed of high-Mg calcite (Mg content, 5-10 mol%) (Rahman and Omori, 2005), which is known to have similar or less solubility than aragonite (Morse et al., 2007) that is utilized in most hard coral skeletons. The sclerites of soft corals are buried within the tissue. In contrast, hard coral accretes exoskeletons below its tissue layer (Cohen et al., 2003). Since tissues and external organic layers of temporal coral have been shown to protect skeletons against high pCO_2 levels (Ries et al., 2009), this may explain the lower sensitivity of the soft corals to high pCO_2 compared to hard corals.

Furthermore, the increase of the photosynthesis rate at elevated pCO_2 levels has been suggested to compensate for the effect of pCO_2 on light calcification, since photosynthesis is known to enhance calcification (Al-Horani et al., 2002, Tentori, 2006). Similar compensation for photosynthesis enhancement might occur in hard corals to some extent. However, it is considered that the aragonite skeletons of hard corals are more severely affected by pCO_2 than the Mg-calcite sclerites of soft corals, and more exposed to seawater, because it is only covered by a thin soft body. Therefore, light calcification of hard corals would not fully compensate for the enhancement by photosynthesis.

3-4-3. Dark calcification

Dark calcification was negatively affected by high pCO_2 levels in both soft and hard corals. Increases of pCO_2 levels resulted in decrease of dark calcification in both soft and hard corals. Night decalcification (negative net calcification) was observed for both soft and hard corals at high pCO_2 levels. A similar result was reported for hard coral in previous studies (Pandolfi, 2011). The decrease of calcification at night underlines the importance of calcification enhancement by photosynthesis in daytime.

Even though the light calcification of soft coral was not affected by pCO_2 increase, net calcification over a 24-h cycle may turn negative at certain pCO_2 levels, when nighttime decalcification exceeds daytime calcification. As the sclerites of octocorals provide a physical deterrence to predation (Fabricius and Klummp, 1995), an increase in pCO_2 to such a level may be detrimental to soft corals.

3-4-4. Summary

The present study reports the metabolic response of soft coral to elevated pCO_2 for the first time. The results of the dark calcification experiment indicate that elevated pCO_2 levels negatively affect the calcification rate of both soft and hard corals. The difference in calcification rates between the two corals appears during the daytime.

The light condition experiment results show that soft coral is more tolerant to ocean acidification than hard coral. This tolerance is explained by the body structure of soft coral: the sclerites of soft coral are buried inside the fresh body, which limits their exposure to seawater. This enables soft corals to compensate for the negative effect on calcification under elevated pCO_2 levels through calcification enhancement by photosynthesis.

This study was a short-term study without investigation for the complete range of the natural conditions of light intensity and pCO_2, which restricts the interpretation of the results. The observation of long-term effects of elevated pCO_2 under in situ conditions at Iwotorishima Island should provide important information for the prediction of the coral community response to ocean acidification with consideration of metabolic response as a basic index of tolerance.

Chapter 4: General discussion

4-1. Comparison between field observation and culture experiment

At Iwotorishima Island, few soft or hard corals live at the site with high pCO_2 (point A in Fig. 4-1), dense soft coral and little hard coral lives at the site with medium pCO_2 (B in Fig. 4-1), and few soft corals and dominant hard corals inhabit the site with ambient pCO_2 (C in Fig. 4-1). In the experimental part of this study, metabolic responses to elevated pCO_2 during daytime indicated advantages for soft coral in terms of living with elevated pCO_2. However, at nighttime, both soft and hard coral decalcified under conditions of Ω_a <1. A comparison of the field survey at Iwotorishima Island with the culture experiment showed that the results of the experiment corresponded to the in situ distributions of corals. This result indicates that the difference in metabolic response to elevated pCO_2 between soft and hard coral is an important contributor to community shift with ocean acidification.

Dense soft corals, but few hard corals, live at the medium-pCO_2 area (B in Fig. 4-2); in contrast, hard corals dominate, and few soft corals live at the ambient-pCO_2 area (C in Fig. 4-1). The metabolic response to elevated pCO_2 indicates that soft coral has an advantage compared with hard coral under conditions of ocean acidification. During daytime, the photosynthesis rate of soft coral was increased by increased pCO_2, whereas the calcification rate was not affected. The results indicate that soft coral is not harmed and soft coral photosynthesis is enhanced by ocean acidification. In contrast, photosynthesis and calcification rates of hard coral decreased with elevated pCO_2, indicating that hard coral is harmed by ocean acidification. Photosynthesis and calcification sustain the growth of the fresh body and the calcium carbonate of the skeleton or sclerites, respectively. Therefore, soft coral has an advantage in spatial competition with hard coral at elevated pCO_2. The difference in the metabolic response of corals during daytime to elevated pCO_2 contributes to the dense community of soft coral at medium pCO_2.

Few soft or hard corals lived at the high-pCO_2 location (A in Fig. 4-1). Considering the results of the culture experiments, the absence of the two corals in the high-pCO_2 area may be the result of dark decalcification. In the culture experiment, under the condition of Ω_a <1, significant decalcification occurred for both soft and hard coral. During daytime, no decalcification was observed, even under Ω_a <1 conditions, because the Ω_a in the body of coral was increased by photosynthesis.

At Iwotorishima Island, the Ω_a <1 condition was actually observed during the observation period in summer, although not regularly. However, during winter, the seawater should attain lower Ω_a because the water temperature is lower, and a large low tide occurs during the night in winter.

Therefore, the frequency of Ω_a <1 conditions was estimated. The lowest pH was found to be associated with the lowest tide depth per night in the high- and medium-pCO_2 areas (A and B in Fig. 4-1) during the observation period from 22 Jul to 25 Aug 2012 (Figs. 4-2 and 4-3). Fig. 4-2 shows that Ω_a <1 occurred when the seawater depth was less than 1.07 m. The depth at Iwotorishima Island during 1–31 January 2013 was determined based on depth data at Naze from the Japan Meteorological Institute. The result showed that the high-pCO_2 area (A in Fig. 4-1) would have experienced nighttime Ω_a<1 (20:00~6:00) for 28 of the 31 days studied, and the average duration of Ω_a<1 per day should be 4.26 h. In contrast, in the medium-pCO_2 area (B in Fig. 4-1), Ω_a<1 was not estimated to have occurred regularly (Fig. 4-3). This suggests that hard coral would be absent in the medium-pCO_2 area (B in fig. 4-1) because hard coral is at a disadvantage in competition with soft coral, as mentioned above, rather than because hard coral is absolutely unable to survive at low Ω_a.

The relationship between the distributions of corals and pCO_2 conditions at Iwotorishima Island was consistent with the results of the culture experiment, which showed that metabolic responses are important factor to control the coral distribution at Iwotorishima Island. Additionally, both approaches suggested that ocean acidification would cause similar metabolic responses in soft coral and hard coral and would result in a community shift from hard to soft coral also in other coral reefs, in the future. Finally, both corals would be absent under certain conditions.

However, metabolic responses are not the sole cause of the community shift. The photosynthesis rate result indicated that the photosynthesis rate of soft coral should exceed that of hard coral at Ω_a = 2.19–1.54 (at average temperature and photon flux density from 22 July to Aug 2012 at Iwotorishima Island; Fig. 3-13), which is lower than the average Ω_a at the medium-pCO_2 area, where soft coral is dominant. There are several possible reasons for the gap between the Ω_a estimated based on the photosynthesis rate and the Ω_a value measured in situ. These include the calcification rate decrease (Fig. 3-6), the relationship with animals other than soft coral, which were difficult to examine in the culture experiment, and the effect during the most fragile larval stage, which is important for affiliation (Suwa et al., 2010).

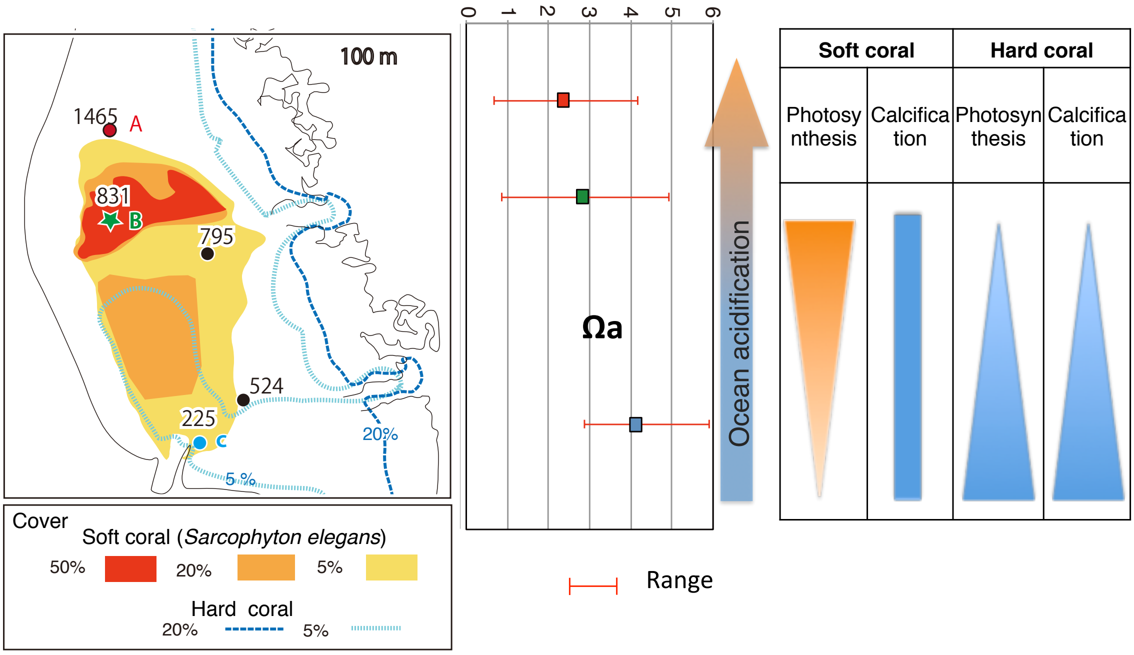

Figure 4-1. Distribution of corals and pCO_2, Ωa, and a conceptual figure of the metabolic responses of coral during daytime. Left figure: Distribution of soft and hard coral and pCO_2 level at low tide on 12 Jul 2010. Middle figure: Average (red square: A, green square: B, blue square: C in the left figure) and range of Ω_a from 22 Jul to 25 Aug 2012. Right figure: Conceptual illustration of the metabolic responses of soft and hard coral drawn based on the result of the experiment in Chapter 3.

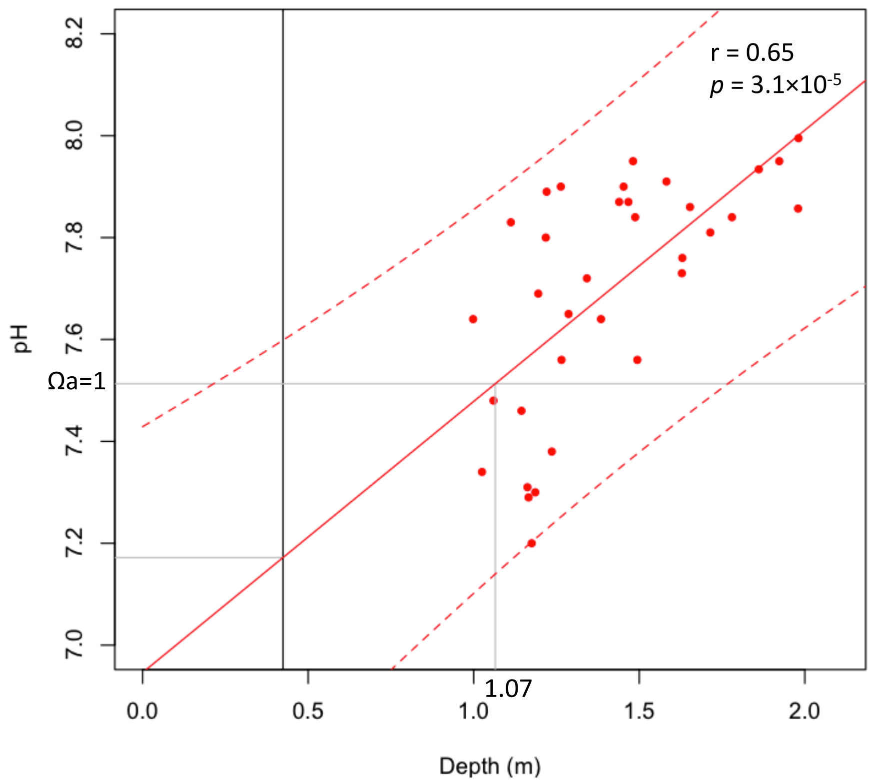

Fig. 4-2. Plot of the lowest depth and the lowest pH at site A in Fig. 4-1 per night from 22 Jul to 25 Aug 2012. $\Omega_a = 1$ is calculated with the temperature of 20.7ºC, the average for January 2011 at B in Fig. 4-1. The depth when $\Omega_a = 1$ is 1.07 m.

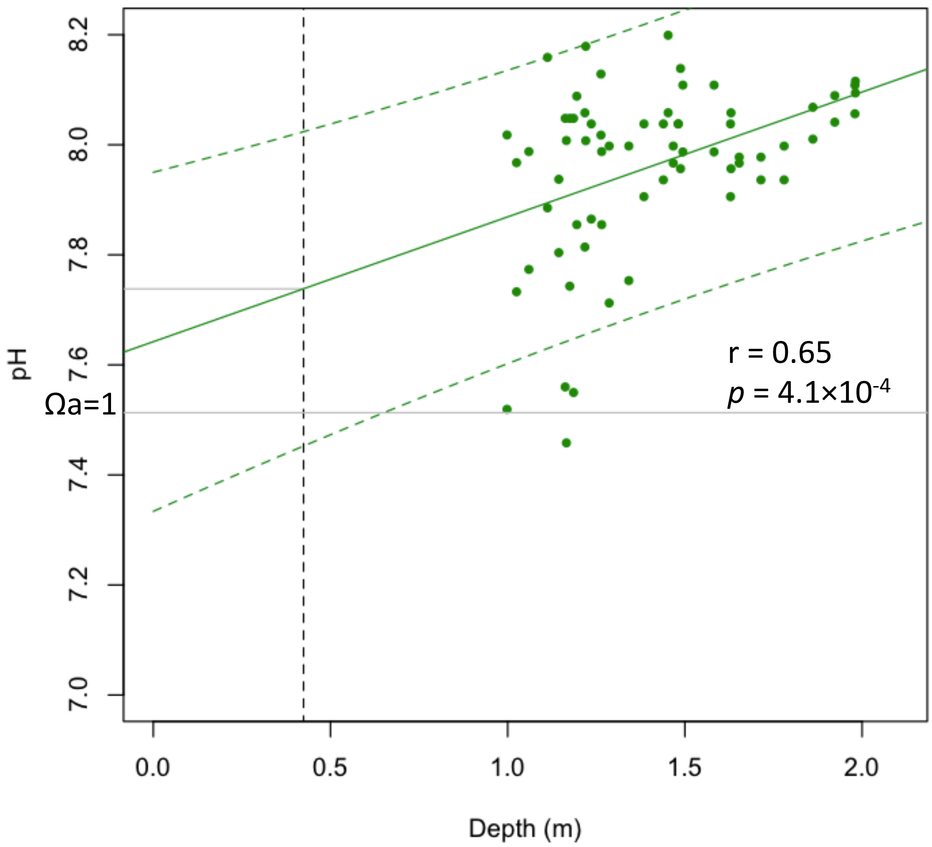

Fig. 4-3. Plot of the lowest depth and the lowest pH at B in Fig. 4-1 per night from 22 Jul to 25 Aug 2012. $\Omega_a = 1$ is calculated with the temperature of 20.7ºC, the average for January 2011 at B in Fig. 4-1. The coefficient of correlation (r) and the significance of the relationships with simple linear regression (p) are also shown.

4-2. Comparison of Iwotorishima Island with other locally acidified coral reef sites

Table 4-1 shows a comparison of Iwotorishima Island and other locally acidified coral reef areas. Four in situ locally acidified sites had been reported previously: PNG (Fabricius et al., 2011), Galapagos (Manzello, 2010), Mexico (Crook et al., 2011), and Palau (Shamberger et al., 2010). The acidification occurring at Iwotorishima Island and PNG is caused by CO_2 gas bubbling. However, the other 3 sites are acidified by upwelling or terrestrial water, so the seawater has characteristics such as low temperature and high nutrients (Galapagos), low salinity and high nutrients (Mexico), and low salinity (Palau), which are not accompanied by ocean acidification.

The biota at these locally acidified sites are divided into three phases (Fig. 4-4). The first phase is reported only at Palau (Shamberger et al., 2010), where hard coral diversity, cover, and calcification rates are maintained. The second phase is reported at all sites other than Palau. In this phase, branching-type hard corals, which are the main builders of reefs, decrease or are absent, and less-calcifying corals, including soft corals, are dominant. The third phase, which is reported at the high-pCO_2 area at Iwotorishima Island and at PNG, has no corals, i.e., neither soft corals nor massive-type corals.

A previous field survey of CO_2 gas vents in the Mediterranean Sea revealed that under reduced pH conditions the abundance of calcifying organisms decreased and the abundances of macroalgae and seagrasses increased (Hall-Spencer et al., 2008), as did the recruitment of algae (Kroeker et al., 2009), suggesting a shift from a calcareous to a non-calcareous algae-dominated community (O. Hoegh-Guldberg et al., 2007). However, in the case of coral reefs, non-calcifying algae may not be the dominant organism with ocean acidification. All sites except for PNG showed no increase in non-calcifying algae at low Ωa. Even at PNG, the coverage of non-calcifying algae was 17% at most, which is not high enough to regard non-calcifying algae as dominant species, although the abundance of non-calcifying algae did increase under low-Ω_a conditions. This may be due to the fact that algae abundance is affected by feeding fish (McIntosh et al., 1996).

The average Ω_a was similar among these sites, with values in the range Ω_a = 2–3 (Fig. 4-4). It is suggested that discussing coral abundance with average Ω_a is not appropriate because biota change dramatically under similar Ω_a conditions, although the value is uncertain because the methods of observation differed among these studies. It is noteworthy that at Palau, which is the only site with high diversity of hard corals, the lowest Ω_a was higher than the values at other sites (Fig. 4-4). These points suggest that the frequency at which Ω_a falls below a certain threshold value, rather than the average Ω_a value, is important for the survivability of corals.

As is discussed previously, the threshold value of Ω_a for absence of coral is suggested to be Ω_a=1. At the high-pCO_2 area at Iwotorishima Island, where few corals live, the duration of Ω_a <1 was estimated to be 4.26 h/day in winter. In contrast, Ω_a <1 was estimated to not occur regularly at the medium-pCO_2 area at Iwotorishima Island. At the medium-pCO_2 area at PNG, the duration of Ω_a <1 was estimated to be 0.5 h/day, calculated using pH data for 3 days (Fabricius et al., 2013). There are no additional data that enable discussion of the duration of Ω_a <1 at other sites. It is suggested that the threshold duration of Ω_a <1 for corals to survive is 0.5~4.26 h/day.

Table 4-1. Catalog of locally acidified studies.

	Site		Features	Conditions that affect Ω_a	Average Ω_a	Observation time
This study	Iwotorishima Island	Medium pCO_2	Dense soft coral and little hard coral	CO_2 bubbling and low tide	2.83	pH was logged from 21 Jul to 25 Aug 2012.
		High pCO_2	Little soft coral or hard coral		2.36	
Fabricius et al., 2011, 2013	PNG	Medium pCO_2	Compared to the control site, the cover of massive *Porites* corals doubled, and that of branching corals decreased threefold.	CO_2 bubbling and wave currents	2.11	Seawater samples representing a range of tidal, irradiance, and wave conditions were taken from 2010 to 2012.
		High pCO_2	Absence of hard corals		2.28	
Manzello, 2010	Galapagos, which had the lowest reported Ω_a in the Eastern Pacific		Small size of coral reefs, discontinuous distribution, and low species diversity	Upwelling coupled with low temperature and high nutrient concentrations	2.46	Twenty-four samples were taken during daytime during 13–18 May 2003, when seawater temperature was low.
Crook et al., 2011	Mexico		Only three hard corals species, *Porites divaricate, Porites astreoides, and Siderastrea radians* occur.	Groundwater coupled with low salinity and high nutrient concentrations	0.32–0.99	Samples were taken in Jun and Nov 2009 and Aug 2010.
Shamberger et al., 2014	Palau		Coral cover, diversity, and richness were higher than at ambient-Ω_a sites.	Calcification and respiration of hard coral, groundwater coupled with low salinity	2.34	Samples were taken during wet (19–24 Sep 2011) and dry (25 Mar to 07 Apr 2012) seasons from sunrise to sunset.

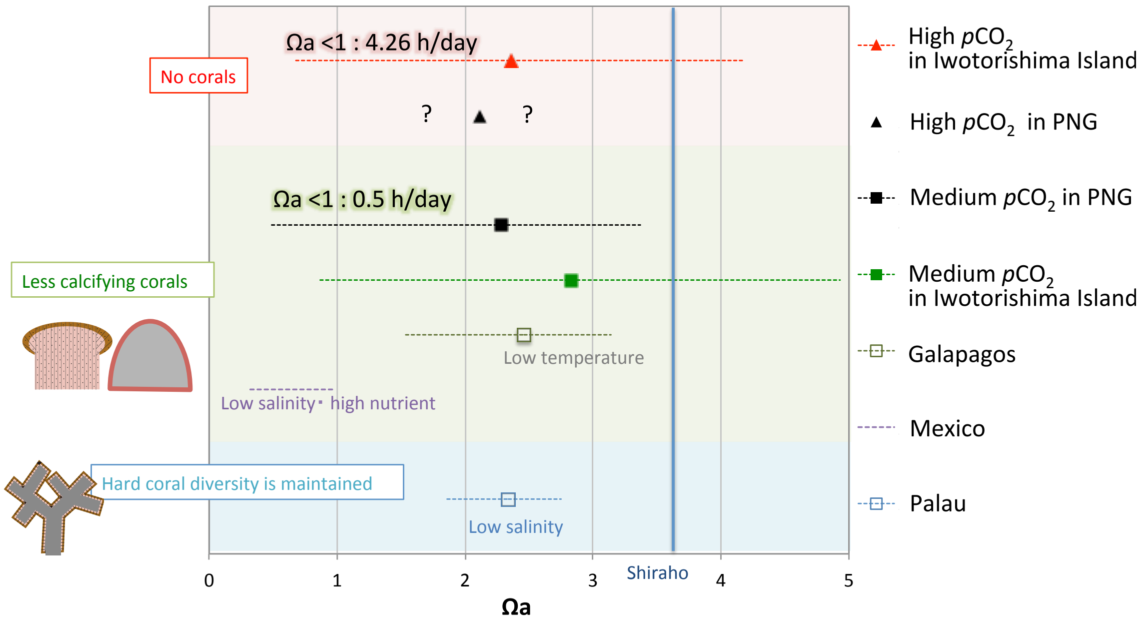

Fig. 4-4. Ω_a and biota at locally acidified sites. The square or triangle is the average at each site, and the bar is the observed range. The method of observation is described in Table 4-1. The blue bar is the average Ω_a (=3.56) at Shiraho, an ambient-pCO_2 coral reef (Kayanne et al., 2005). The biota at these locally acidified sites are divided into three phases. In the first phase, hard coral diversity, cover, and calcification rates are maintained (blue box). In the second phase, the main builder of reefs decreases or is absent, and less calcifying corals including soft corals, rather than branching-type corals, are dominant (green box). The third phase has no living corals, including soft corals and massive-type corals (red box). The features of the seawater are also mentioned beneath the symbols. The estimated duration of Ω_a <1 is shown above the symbols.

4-3. Discussion of community shifts in coral reefs in the future

As was discussed in the previous paragraph, how often Ω_a falls below $\Omega_a = 1$, rather than the average Ωa, is important for the survivability of corals. This indicates that coral colonies can live at lower Ω_a than was expected in previous studies. The limiting Ω_a for coral reef development has been suggested to be $\Omega_a = 3$ (Kleypas et al., 1999; Guinotte et al., 2003), and this value is used to estimate coral habitat expansion (Yara et al., 2012). This study concludes that the pCO_2 level uninhabitable for all corals (not about coral reefs) is about 2,000 μatm with Ω_a <1, although in reality, Ω_a does not fall below 1 because of the negative feedback of decalcification. However, Ω_a in semi-closed coral reef lagoons falls at nighttime because of the respiration of lagoon organisms; thus, the community may be affected before open coral reefs are.

Before corals disappear, the dominant species change from reef-building branching hard corals to less-calcifying corals, including soft corals, because the advantage for survival changes with increasing relative $p\mathrm{CO_2}$. In the case of coral reefs where soft corals live together with hard corals, rising $p\mathrm{CO_2}$ benefits soft corals, which become dominant species by at least a $p\mathrm{CO_2}$ level of 800–1300 μatm, when the photosynthesis rate of soft coral overtakes that of hard coral. At Okinawa Island, soft corals live together with hard corals. At more than 20% of coral reefs, the cover of soft corals is greater than 10% (Okinawa Prefecture Nature Conservation Division, 2010), and soft corals can be the dominant organisms in places with ocean acidification.

The present study suggests that ocean acidification induces shifts from hard-coral- to soft-coral-dominated communities. Some ecological models have predicted community shifts from hard to soft corals under locally stressed conditions (Norström, 2009). The present site is not affected by local anthropogenic stresses, suggesting that the impacts of ocean acidification may be even more severe in places that are thus affected by anthropogenic stresses.

It is also worth noting that soft coral may not always be the winner in acidified waters. As mentioned previously, massive-type hard corals have been reported to be dominant at low Ω_a at other locally acidified sites. What is important is that reef development by hard coral will be difficult because the main constituent of hard coral reefs decreases or disappears. Additionally, the fossil record indicates that rising $p\mathrm{CO_2}$ causes coral reef crises. The Paleocene–Eocene Thermal Maximum was characterized by a CO_2 increase similar in magnitude to the present one. It has been reported that a faunal shift from coral–algal reefs to reefs dominated by large benthic foraminifera occurred on continental carbonate platforms (Scheibner et al., 2008). Similar shift of reef builder may occur in the future.

The diminishment or absence of the main reef-building hard coral and the cessation of reef development may have deleterious ramifications for coral reef ecosystems. With ongoing ocean acidification, the ecosystem services supplied by existing coral reefs, such as habitats for many organisms and effective breakwaters, may be difficult to retain.

Chapter 5: Conclusions

Previous studies considered ocean acidification causes a shift from hard coral to non-calcareous algae or massive type coral dominated community in coral reef.

This study found a new CO_2 seep site and suggest a new community shift from hard to soft coral caused by ocean acidification. At Iwotorishima Island, few soft corals and dominant hard corals inhabit the site with ambient pCO_2 and dense soft coral and little hard coral lives at the site with medium pCO_2 (Average Ω_a=2.82) and few soft or hard corals live at the site with high pCO_2 (Average Ω_a=2.36).

In the experimental part of this study, metabolic responses of soft and hard corals to elevated pCO_2 were studied and the results explain the distribution change with pCO_2 in Iwotorishima Island. During daytime, the photosynthesis rate of soft coral was increased by increased pCO_2, whereas the calcification rate was not affected. The results indicate that soft coral is not harmed and soft coral photosynthesis is enhanced by ocean acidification. In contrast, photosynthesis and calcification rates of hard coral decreased with elevated pCO_2, indicating that hard coral is harmed by ocean acidification. The difference in the metabolic response of corals during daytime to elevated pCO_2 contributes to the dense community of soft coral at medium pCO_2.

The absence of the two corals in the high-pCO_2 area may be the result of dark decalcification. In the culture experiment, significant decalcification occurred for both soft and hard coral under the condition of Ω_a <1. The frequency of Ω_a <1 conditions was estimated as in the high-pCO_2 area, the average duration of Ω_a <1 per day should be 4.26 h.

Comparing Iwotorishima Island with other locally acidified sites, it is indicated that discussing coral abundance with average Ω_a is not appropriate because biota change dramatically under similar average Ω_a conditions. It is suggest that the frequency at which Ω_a falls below a certain threshold value is more important for the survivability of corals rather than the average Ω_a value. The threshold value of Ω_a for absence of coral is suggested to be Ω_a =1 with experimental night-time decalcification results.

This study concludes that the pCO_2 level uninhabitable for all corals is about 2,000 μatm with Ω_a<1, although in reality, Ω_a does not fall below 1 because of the negative feedback of decalcification. However, Ω_a in semi-closed coral reef lagoons falls at nighttime because of the respiration of lagoon organisms; thus, the community may be affected before open coral reefs are.

Before all corals disappear, the dominant species change from reef-building branching hard corals to less-calcifying corals, including soft corals, because the advantage for survival changes with increasing relative pCO_2. In the case of coral reefs where soft corals live together with hard corals, rising pCO_2 benefits soft corals, which become dominant species by at least a pCO_2 level of 800–1300 μatm, when the photosynthesis rate of soft coral overtakes that of hard coral.

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Appendix

Table A1

Hard coral species and number of colony on line NE (Fig. 2-1)

Species	Number of colony
Acropora digitifera	36
Acropora millepora	4
Acorpora aspera	2
Acropora gemmifera	1
Acropora hyacinthus	2
Acropora nasuta	3
Acropora solitayensis	1
Acropora striata	1
Acropora pulchra	1
Non-identifiable table form *Acropora*	34
Isopora palifera	1
Pocillopora meandrina	1
Montipora mollis	5
Montiopora turgescens	1
Montipora venosa	1
Non-identifiable covering form *Montipora*	1
Echinopora lamellosa	1
Gonisatrea retiformis	2
Favites abdita	1
Symphillia recta	2
Sum	101

Table A2

Hard coral species and number of colony on line SW (Fig. 2-1)

Species	Number of colony
Acropora digitifera	17
Acropora mileipora	7
Acorpora aspera	1
Acropora gemmifera	3
Acropora hyacinthus	3
Acropora nasuta	4
Acropora solitaryensis	1
Acropora dendrum	1
Non-identifiable table type Acropora	11
Isopora palifera	3
Astreopora myriophthalma	2
Pocillopora meandrina	1
Montipora mollis	2
Montiopora turgescens	6
Montipora peltiformis	3
Non-identifiable covering form *Montipora*	3
Psammocora cotigua	8
Pachyseris rugosa	1
Galaxea astreata	3
Turbinaria mesenterina	3
Symphyllia agaricia	1
Symphillia radians	1
Favites abdita	2
Favites cinensis	3
Favites halicora	1
Favia speciosa	3
Platygyra ryukyuensis	1
Platygyra sinensis	2
Cyphastrea serailia	1
Goniastrea aspera	3
Gonisatrea retiformis	3
Sum	104

Table A3

Hard coral species and number of colony on line Main in acidified area (Fig. 2-2)

Species	Number of colony
Montipora stellata	1
Porites sp	1
Psammocora contigua	1
Sum	3

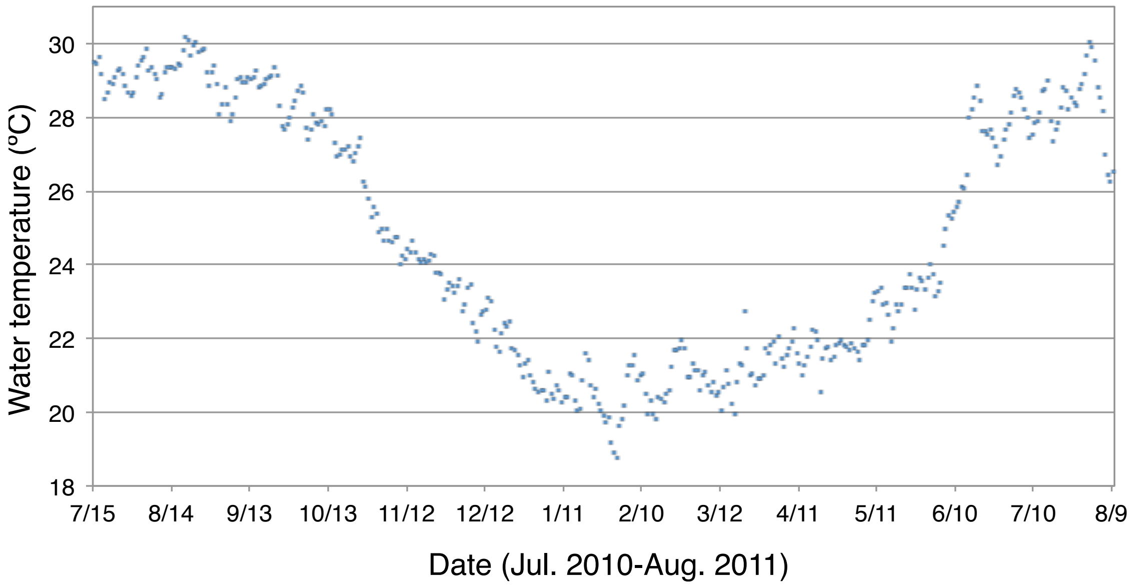

Fig. A1

The water temperature at “Dense soft coral” point from 15 Jul 2010 to 9 Aug 2011

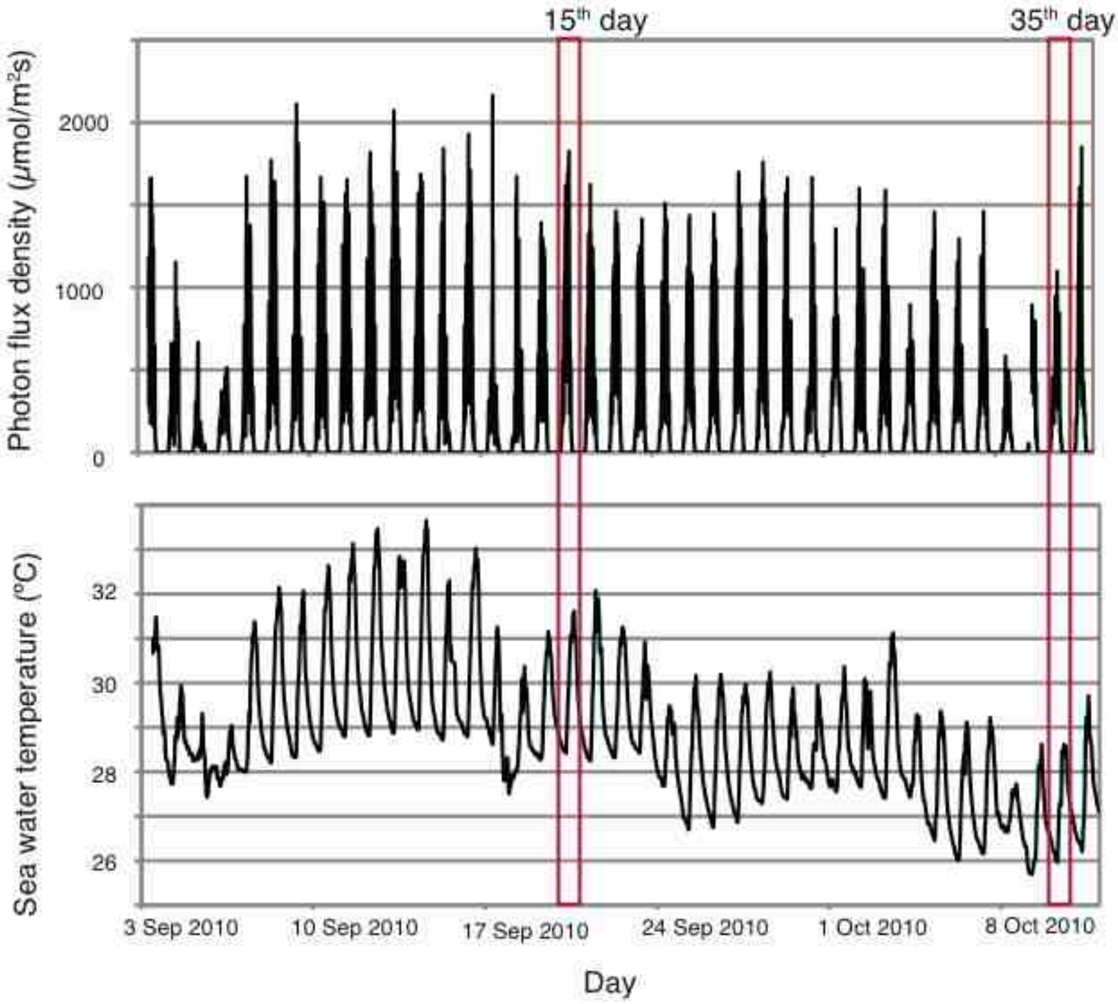

Figure A2

Photon flux density and seawater temperature during the 5-week soft coral culture experiment. The red boxes show light and temperature conditions for the days that the calcification and photosynthesis rate measurements were conducted.

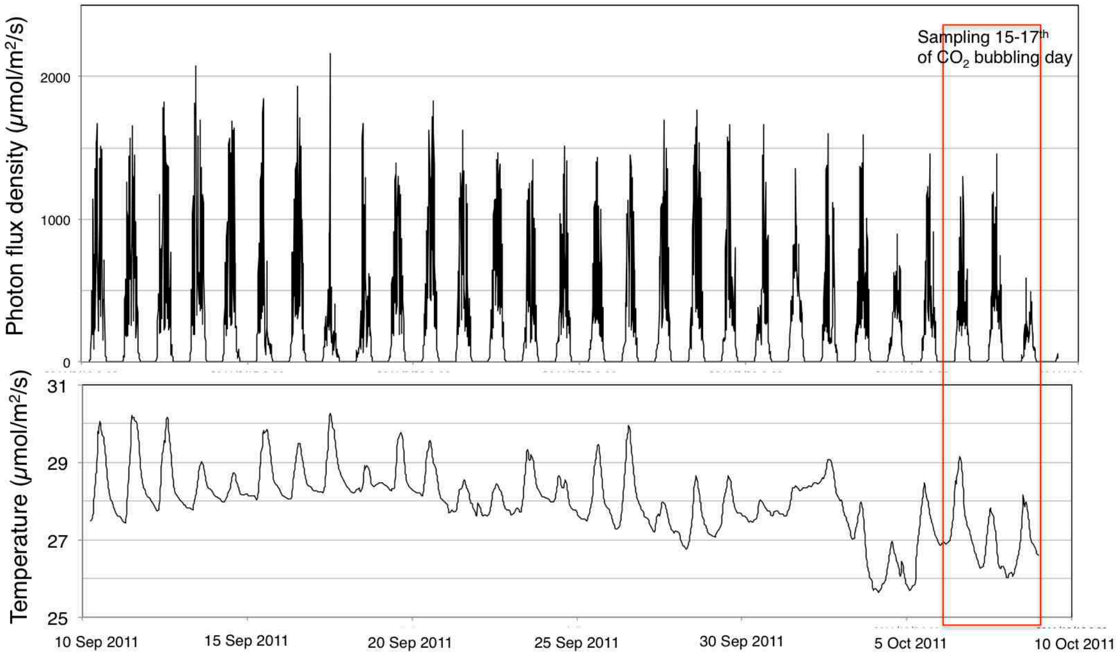

Fig. A3

Photon flux density and seawater temperature during the 2-week hard coral culture experiment. The red boxes show light and temperature conditions for the days that the calcification and photosynthesis rate measurements were conducted.

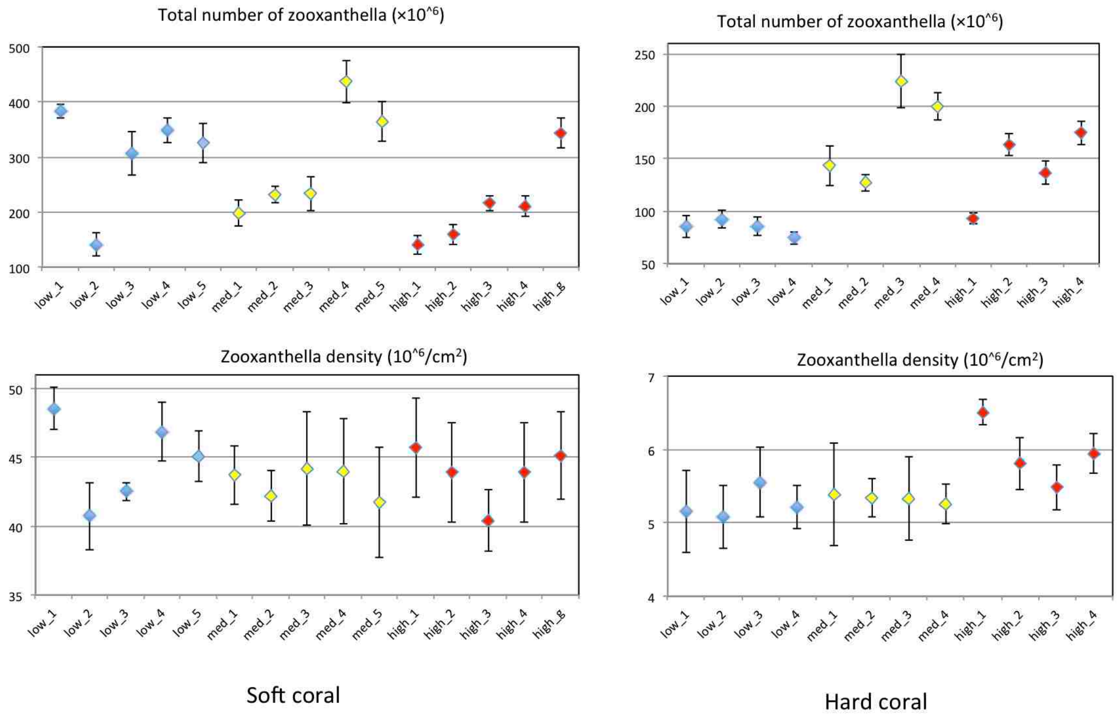

Fig. A4

The total number of zooxanthella of each colony and density.

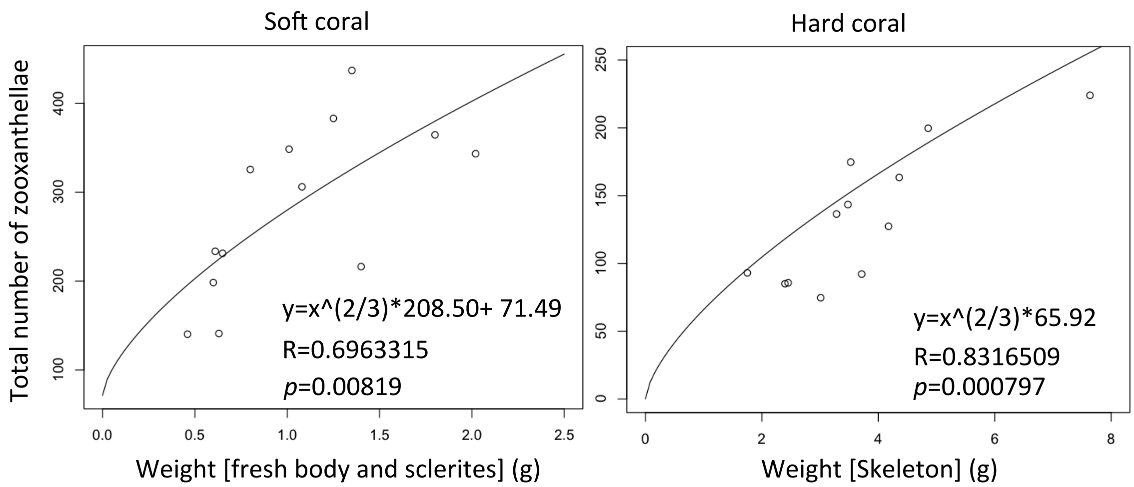

Fig. A5

The relation of the total number of zooxanthellae and weight including fresh body and sclerites of soft coral (left) and the relation of weight of skeleton and the total number of zooxanthellae of hard coral (right). There are significant correlations.

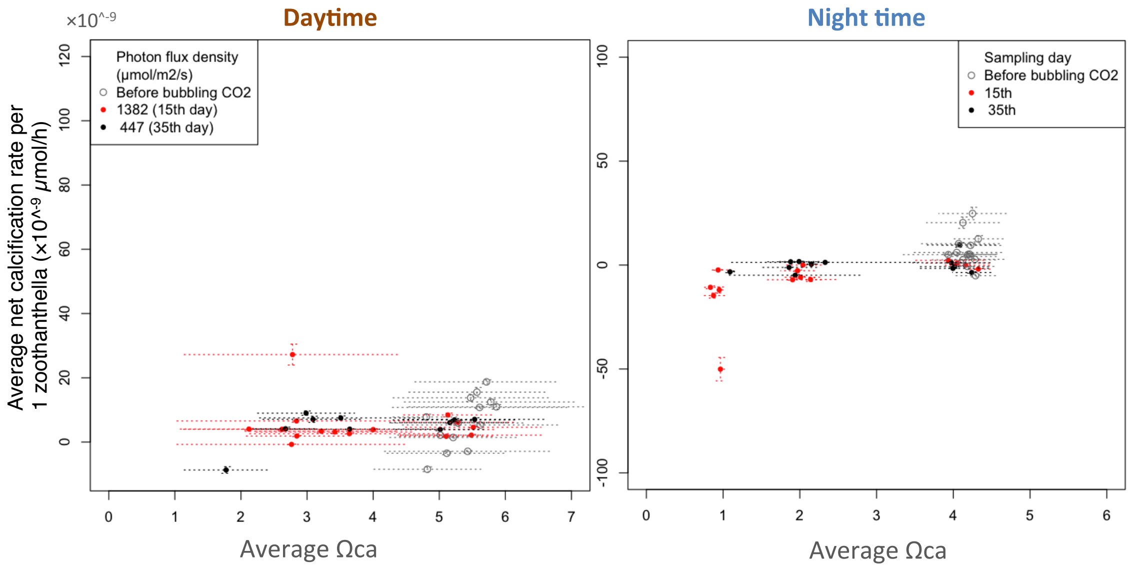

Fig. A6

Average Ωca and average net dark calcification rate during closing chamber of soft coral(left) and hard (right) coral. Each color shows different sampling day. The bars in horizontal direction are the pCO_2 range from the start to the end of closing chambers. The error bars in vertical direction of calcification rates are calculated from the standard variations of the zooxanthella density and surface area according to the system of propagation of error.

Table A5

The information of culture experiment (Soft coral, daytime)

Group	ID	class	Weight of seawater (g)	Photon flux density (μmol/m2/s)	Total number of zooxhanthella (×10^9)	measurement deviation	Start								
							TA (μmol/kg)	TA (μmol/kg)	pH (Total)	pCO2 (μatm)	HCO3 (μmol/kg)	CO3 (μmol/kg)	CO2 (μmol/kg)	Ωa	Ωca
A	1	low	800	1383	383.16	12.44	2242.7	1981.2	8.032	394.1	1782.6	186.4	12.2	2.93	4.49
A	2	low	809	1383	194.39	18.40	2240.6	1982.6	8.026	399.7	1786.3	183.9	12.4	2.89	4.43
A	3	low	803	1383	320.34	22.47	2240.8	1981.1	8.030	395.9	1783.7	185.2	12.3	2.91	4.46
A	4	low	804	1383	348.56	22.53	2236.5	1980.5	8.026	399.4	1785.4	182.7	12.4	2.87	4.4
A	5	low	807	1383	325.68	35.84	2237.7	1981.5	8.025	400.9	1786.2	182.9	12.5	2.87	4.41
B	1	med	810	1383	198.44	24.34	2236.7	2132.0	7.678	1003.2	2008.2	92.7	31.1	2.36	2.23
B	2	med	809	1383	180.10	13.42	2236.7	2137.7	7.663	1039.7	2015.7	89.6	32.3	2.29	2.16
B	3	med	810	1383	183.57	24.64	2235.5	2144.6	7.639	1105.2	2024.9	85.4	34.3	2.19	2.06
B	4	med	798	1383	437.11	38.53	2236.8	2144.2	7.643	1096.4	2023.9	86.4	33.9	2.21	2.08
B	5	med	791	1383	422.93	12.68	2237.3	2140.7	7.655	1062.1	2019.3	88.4	33.0	2.26	2.13
C	1	high	813	1383	140.32	16.76	2245.6	2244.4	7.361	2203.4	2128.6	47.5	68.3	0.85	1.14
C	2	high	810	1383	164.11	18.41	2244.3	2259.1	7.311	2487.5	2139.6	42.6	77.0	0.77	1.03
C	3	high	798	1383	216.35	13.69	2243.0	2257.0	7.319	2442.0	2138.4	42.6	76.0	0.77	1.03
C	4	high	807	1383	216.40	17.81	2241.5	2241.2	7.359	2208.6	2125.6	47.1	68.6	0.84	1.13
C	5	high	788	1383	361.18	18.98	2240.4	2247.5	7.336	2337.2	2130.3	44.7	72.5	0.8	1.08
A	1	low	800	448	383.16	12.44	2249.8	2003.8	8.003	427.7	1813.8	176.7	13.3	2.77	4.25
A	2	low	809	448	194.39	18.40	2246.8	1994.6	8.014	414.9	1801.2	180.5	12.9	2.83	4.34
A	3	low	803	448	320.34	22.47	2251.6	1992.5	8.027	400.6	1795.0	185.0	12.5	2.9	4.45
A	4	low	804	448	348.56	22.53	2245.7	1996.6	8.009	419.9	1805.0	178.5	13.0	2.8	4.3
B	1	med	810	448	198.44	24.34	2258.4	2155.6	7.669	1035.6	2031.3	92.2	32.1	2.33	2.22
B	2	med	809	448	180.10	13.42	2256.7	2151.8	7.674	1022.2	2026.9	93.3	31.6	2.36	2.25
B	3	med	810	448	183.57	24.64	2259.3	2148.0	7.691	977.9	2020.9	96.8	30.3	2.44	2.33
B	4	med	798	448	437.11	38.53	2257.4	2149.9	7.682	1001.6	2024.2	94.7	31.0	2.39	2.28
B	5	med	791	448	422.93	12.68	2250.0	2149.0	7.665	1042.1	2025.7	91.1	32.3	2.3	2.19
C	1	high	813	448	140.32	16.76	2248.3	2247.5	7.358	2221.8	2131.4	47.5	68.7	0.83	1.14
A	1	low	800	413	383.16	12.44	2247.6	1977.8	8.051	375.0	1774.1	192.0	11.7	3.02	4.63
A	2	low	809	413	194.39	18.40	2240.9	1976.6	8.041	384.6	1776.3	188.3	11.9	2.96	4.54
A	3	low	803	413	320.34	22.47	2234.9	1976.5	8.032	393.5	1780.0	184.3	12.2	2.9	4.44
A	4	low	804	413	348.56	22.53	2238.3	1973.9	8.041	383.9	1773.7	188.3	11.9	2.96	4.54
B	1	low	810	413	198.44	24.34	2222.6	1974.5	8.013	412.5	1784.3	177.4	12.8	2.91	4.28
B	2	low	809	413	180.10	13.42	2211.5	1970.2	7.999	425.5	1784.3	172.8	13.2	2.83	4.17
B	3	low	810	413	183.57	24.64	2216.2	1972.1	8.006	418.8	1784.4	174.7	13.0	2.87	4.21
B	4	low	798	413	437.11	38.53	2202.1	1970.7	7.984	442.1	1790.7	166.3	13.7	2.73	4.01
B	5	low	791	413	422.93	12.68	2227.6	1979.5	8.011	415.5	1789.0	177.6	12.9	2.91	4.29
C	1	low	813	413	140.32	16.76	2219.0	1972.9	8.009	415.9	1784.0	176.0	12.9	2.77	4.25
C	2	low	810	413	164.11	18.41	2219.7	1969.8	8.017	406.7	1778.7	178.4	12.6	2.81	4.3
C	3	low	798	413	216.35	13.69	2229.3	1969.0	8.034	390.2	1771.5	185.4	12.1	2.92	4.47
C	4	low	807	413	216.40	17.81	2223.4	1973.7	8.017	407.7	1782.6	178.4	12.7	2.81	4.3
C	5	low	788	413	361.18	18.98	2225.6	1971.8	8.023	401.7	1778.2	181.1	12.5	2.85	4.37

Group	ID	class	End								
			TA (µmol/kg)	TA (µmol/kg)	pH (Total)	pCO2 (µatm)	HCO3 (µmol/kg)	CO3 (µmol/kg)	CO2 (µmol/kg)	Ω a	Ω ca
A	1	low	2233.9	1839.8	8.254	207.1	1561.8	271.6	6.4	4.27	6.54
A	2	low	2234.6	1867.0	8.216	232.1	1605.4	254.4	7.2	3.99	6.13
A	3	low	2227.3	1880.5	8.184	253.9	1632.0	240.6	7.9	3.78	5.8
A	4	low	2233.4	1886.0	8.182	255.9	1636.8	241.2	7.9	3.79	5.81
A	5	low	2234.2	1839.0	8.255	206.4	1560.3	272.3	6.4	4.28	6.56
B	1	med	2233.6	1963.7	8.049	373.9	1760.7	191.4	11.6	4.77	4.61
B	2	med	2233.2	1883.9	8.186	252.9	1633.6	242.4	7.9	6.01	5.84
B	3	med	2232.4	1978.3	8.022	402.9	1784.4	181.3	12.5	4.52	4.37
B	4	med	2232.7	2029.5	7.918	535.3	1863.2	149.7	16.6	3.75	3.61
B	5	med	2231.8	1927.0	8.112	313.3	1703.6	213.7	9.7	5.31	5.15
C	1	high	2226.5	1968.7	8.029	394.8	1772.9	183.5	12.2	3.08	4.42
C	2	high	2244.9	1983.0	8.033	393.2	1784.0	186.8	12.2	3.13	4.5
C	3	high	2235.9	1963.5	8.052	371.0	1758.9	193.1	11.5	3.23	4.65
C	4	high	2237.2	1999.9	7.987	444.8	1815.2	170.9	13.8	2.87	4.11
C	5	high	2233.1	2060.4	7.850	641.6	1909.4	131.1	19.9	2.22	3.16
A	1	low	2238.2	1873.5	8.206	238.5	1613.7	252.4	7.4	3.96	6.07
A	2	low	2243.0	1903.2	8.167	268.5	1658.4	236.5	8.3	3.71	5.69
A	3	low	2240.3	1840.6	8.259	203.9	1559.0	275.3	6.3	4.32	6.62
A	4	low	2233.7	1863.3	8.217	230.9	1600.2	256.0	7.2	4.02	6.16
B	1	med	2254.3	2084.6	7.838	667.4	1934.0	130.0	20.6	3.23	3.13
B	2	med	2248.6	2038.4	7.929	522.7	1867.8	154.4	16.2	3.82	3.72
B	3	med	2252.8	2033.9	7.945	501.1	1858.5	159.9	15.5	3.95	3.85
B	4	med	2241.0	1962.4	8.063	360.5	1754.1	197.1	11.2	4.85	4.74
B	5	med	2241.5	1939.4	8.103	322.4	1717.3	212.1	10.0	5.22	5.1
C	1	high	2254.4	2136.8	7.710	930.5	2007.9	100.1	28.8	1.67	2.41
A	1	low	2211.7	1800.7	8.286	186.3	1513.1	281.9	5.8	4.43	6.8
A	2	low	2225.8	1828.7	8.262	201.4	1549.0	273.5	6.3	4.3	6.6
A	3	low	2226.4	1816.5	8.283	189.5	1528.8	281.8	5.9	4.43	6.8
A	4	low	2219.2	1783.4	8.321	168.2	1479.8	298.3	5.2	4.69	7.19
B	1	low	2216.7	1838.3	8.236	217.0	1570.8	260.8	6.7	4.27	6.29
B	2	low	2214.1	1809.9	8.274	193.4	1526.4	277.5	6.0	4.54	6.69
B	3	low	2219.4	1858.9	8.209	235.8	1602.2	249.4	7.3	4.09	6.02
B	4	low	2220.6	1884.9	8.167	266.3	1643.3	233.3	8.3	3.83	5.63
B	5	low	2204.8	1785.3	8.300	177.9	1492.7	287.1	5.5	4.7	6.93
C	1	low	2218.0	1847.4	8.223	225.6	1584.5	255.9	7.0	4.02	6.17
C	2	low	2213.3	1897.8	8.137	289.6	1668.8	220.0	9.0	3.46	5.31
C	3	low	2215.8	1786.2	8.311	172.8	1486.8	294.1	5.4	4.62	7.09
C	4	low	2208.5	1807.0	8.271	194.5	1525.4	275.6	6.0	4.34	6.65
C	5	low	2221.7	1884.2	8.171	263.5	1641.4	234.6	8.2	3.69	5.66

Table A6

The information of culture experiment (Soft coral, nighttime)

A	1	low	2251.7	2011.4	7.991	443.3	1824.3	173.3	13.7	2.72	4.17
A	2	low	2248.4	2029.4	7.949	495.8	1854.1	159.9	15.4	2.51	3.85
A	3	low	2244.1	2050.2	7.897	568.8	1888.3	144.2	17.7	2.26	3.47
A	4	low	2247.4	2043.1	7.918	538.2	1875.6	150.8	16.7	2.37	3.63
B	1	mid	2246.5	2182.3	7.559	1357.1	2067.6	72.7	42.1	1.14	1.75
B	2	mid	2254.4	2189.7	7.559	1362.3	2074.4	73.1	42.1	1.15	1.76
B	3	mid	2256.7	2187.9	7.572	1321	2071.9	75.1	40.9	1.18	1.81
B	4	mid	2251.8	2183.1	7.572	1316.6	2067.4	74.9	40.8	1.18	1.8
B	5	mid	2270.5	2228.4	7.489	1628.4	2114.5	63.5	50.5	1	1.53
C	1	high	2250.8	2286.22	7.2489	2895.8	2159.35	37.19157	89.67876	0.58	0.9
C	2	high	2291.1	2328.091	7.247	2961.3	2198.676	37.71186	91.70312	0.59	0.91
C	3	high	2254.7	2326.863	7.1446	3719.4	2182.076	29.55654	115.2308	0.46	0.71
C	4	high	2265.3	2334.944	7.152	3671.2	2191.031	30.24462	113.6681	0.47	0.73
C	5	high	2252.9	2304.789	7.2014	3246.4	2170.667	33.45355	100.6683	0.53	0.81
A	1	low	2252.8	2055.4	7.9	565.5	1891.3	146.6	17.5	2.3	3.53
A	2	low	2254.7	2026.5	7.966	474.3	1846.1	165.7	14.7	2.6	3.99
A	3	low	2252.1	2051.6	7.909	552.9	1886	148.5	17.2	2.33	3.57
A	4	low	2249.2	2055.7	7.893	576.2	1893.7	144.1	17.8	2.26	3.47
A	5	low	2239.1	2034.5	7.919	534	1867.4	150.6	16.6	2.36	3.62
B	1	mid	2248	2156.8	7.6388	1111	2036.4	85.8725	34.5279	1.35	2.07
B	2	mid	2248.5	2186	7.5524	1380.1	2071.331	71.93055	42.73845	1.13	1.73
B	4	mid	2251.4	2211.1	7.4846	1633.8	2098.223	62.21419	50.66306	0.98	1.5
B	5	mid	2246.2	2201	7.5005	1566.4	2088.217	64.11801	48.66483	1.01	1.54
C	1	high	2256.9	2284.7	7.271	2751.7	2160.2	39.3	85.1	0.62	1.03
C	3	high	2256.2	2271.4	7.309	2508.2	2151	42.8	77.6	0.67	2.8
C	5	high	2257.3	2271.9	7.312	2490.1	2151.8	42.9	77.3	0.67	3.55
A	1	low	2219	2020.2	7.915	536.8	538.6	1856.8	146.6923	2.31	3.54
A	2	low	2226.9	2007.3	7.957	481.8	483.4	1832.6	159.7589	2.51	3.85
A	3	low	2236	2013.8	7.962	477.4	479	1837.3	161.6813	2.54	3.9
A	4	low	2226.3	2011.4	7.948	492.7	494.4	1839.2	156.8158	2.47	3.79
A	5	low	2227	2006.1	7.954	483.9	485.5	1830.9	160.2463	2.52	3.85
B	1	mid	2223.3	1993.9	7.976	456.8	458.4	1813.9	165.8	2.61	4
B	2	mid	2227.8	2009.9	7.952	487.8	489.4	1836	158.8	2.5	3.83
B	3	mid	2215.2	2009.2	7.929	516.2	517.9	1842.1	151.1	2.38	3.65
B	4	mid	2222.9	2039.3	7.88	591.6	593.6	1883.3	137.6	2.17	3.32
B	5	mid	2225	2003.4	7.959	478.1	479.7	1827.6	161	2.54	3.89
C	1	high	2220.3	2003.8	7.952	486.72	488.3457	1831.016	157.6563	2.48	3.81
C	2	high	2230.6	1999.8	7.9776	455.97	457.4909	1818.752	166.9195	2.63	4.03
C	3	high	2223.7	2014.6	7.9358	509.32	511.0181	1845.581	153.1967	2.41	3.7
C	4	high	2229.9	2011.5	7.9521	488.61	490.2343	1837.188	159.1878	2.51	3.84
C	5	high	2223.9	2031.1	7.9011	558.84	560.6988	1870.544	143.1747	2.25	3.46

Group	ID	class	Total number of zooxanthella (×10^9)	measurement deviation	Start							
					TA (μmol/kg)	TA (μmol/kg)	pH (Total)	pCO2 (μatm)	HCO3 (μmol/kg)	CO3 (μmol/kg)	CO2 (μmol/kg)	Ωa
A	1	low	85.68	8.74	2254.63	2028.4	8.098	483.964	1849.106	164.33	14.96	2.57
A	2	low	74.69	6.04	2257.53	2024.5	8.276	294.872	1697.319	226.86	9.119	3.55
A	3	low	85.68	8.74	2252.83	1980.7	8.26	307.921	1709.278	220.1	9.526	3.45
A	4	low	74.69	6.04	2255.07	1986.3	8.259	308.876	1711.55	220.1	9.555	3.45
B	1	mid	143.39	19.36	2242.91	2219.1	7.571	1851.35	2106.264	55.432	57.4	0.87
B	2	mid	127.36	8.19	2240.03	2245.1	7.479	2310.75	2128.101	45.404	71.6	0.71
B	3	mid	223.97	25.59	2248.66	2218.6	7.591	1770.42	2105.751	58.007	54.84	0.91
B	4	mid	199.74	12.99	2241.14	2232.2	7.524	2078	2117.835	50.029	64.34	0.78
C	1	high	93.02	4.90	2235.71	2285.7	7.343	3205.02	2152.982	33.568	99.15	0.53
C	2	high	163.40	10.35	2233.77	2276.6	7.363	3050.92	2147.194	35.126	94.28	0.55
C	3	high	136.46	11.01	2232.94	2283.3	7.341	3213.35	2150.535	33.435	99.33	0.52
C	4	high	174.69	11.35	2231.06	2284.1	7.333	3272.06	2150.115	32.834	101.2	0.51
A	1	low	85.04	10.56	2265.61	2005.6	8.163	406.053	1807.057	185.95	12.59	2.91
A	2	low	92.17	8.69	2266.55	2007	8.162	407.4	1808.718	185.64	12.64	2.91
A	3	low	85.68	8.74	2261.58	2003.8	8.16	408.702	1806.717	184.4	12.69	2.89
A	4	low	74.69	6.04	2250.16	2004.6	8.139	431.374	1814.92	176.29	13.39	2.76
B	1	mid	143.39	19.36	2261.38	2214.3	7.644	1559.66	2100.562	65.326	48.41	1.02
B	2	mid	127.36	8.19	2262.83	2211.9	7.656	1514.38	2097.829	67.025	47.05	1.05
B	3	mid	223.97	25.59	2260.57	2210.6	7.653	1523.52	2096.726	66.547	47.33	1.04
B	4	mid	199.74	12.99	2257.48	2210.2	7.646	1551.32	2096.704	65.296	48.2	1.02
C	1	high	93.02	4.90	2254.51	2297.7	7.365	3059.06	2167.26	35.444	95	0.56
C	2	high	163.40	10.35	2254.88	2238.7	7.547	1971.45	2124.612	52.894	61.19	0.83
C		high	136.46	11.01	2255.89	2307.8	7.34	3250.49	2173.371	33.539	100.9	0.53
C	4	high	174.69	11.35	2260.21	2304.5	7.362	3090.76	2173.301	35.328	95.87	0.55
A	1	low	85.04	10.56	2247.76	2002.1	8.141	428.246	1812.516	176.25	13.33	2.76
A	2	low	92.17	8.69	2248.58	2000.1	8.146	422.811	1808.884	178.07	13.15	2.79
A	3	low	85.68	8.74	2243.07	1980.3	8.173	390.88	1781.226	186.92	12.15	2.93
A	4	low	74.69	6.04	2238.64	1997.6	8.132	437.429	1811.022	172.99	13.59	2.71
B	1	mid	143.39	19.36	2244.29	2210.4	7.604	1708.47	2097.933	59.368	53.1	0.93
B	2	mid	127.36	8.19	2260.64	2200.1	7.686	1406.49	2085.127	71.287	43.69	1.12
B	3	mid	223.97	25.59	2254.23	2187.4	7.705	1337.3	2071.831	74.045	41.52	1.16
B	4	mid	199.74	12.99	2253.51	2192.4	7.687	1396.93	2077.673	71.37	43.36	1.12
C	1	high	93.02	4.90	2259.46	2255.1	7.511	2157.96	2139.183	48.872	67.05	0.77
C	2	high	163.40	10.35	2259.28	2260.7	7.493	2256.05	2143.652	46.981	70.07	0.74
C	3	high	136.46	11.01	2259.67	2289.7	7.405	2788.69	2164.355	38.742	86.6	0.61
C	4	high	174.69	11.35	2261.24	2252.5	7.525	2089.52	2137.192	50.408	64.9	0.79
A	1	low	85.68	8.74	2264.55	1984	8.199	367.661	1773.683	198.95	11.37	2.99
A	2	low	74.69	6.04	2267.26	1990.6	8.192	375.429	1782.38	196.61	11.61	3.01
A	3	low	85.68	8.74	2264.97	1987.8	8.193	373.853	1779.401	196.84	11.56	3.05
A	4	low	74.69	6.04	2271.53	1994.9	8.19	377.464	1786.544	196.69	11.67	3.08
B	1	mid	143.39	19.36	2257.02	1989.5	8.177	389.54	1787.061	190.39	12.05	2.9
B	2	mid	127.36	8.19	2244.16	1972.5	8.187	377.01	1768.249	192.59	11.66	2.87
B	3	mid	223.97	25.59	2252.38	1983.1	8.181	384.49	1779.826	191.38	11.89	2.81
B	4	mid	199.74	12.99	2251.98	1985.1	8.177	388.041	1783.274	189.8	12.03	2.85
C	1	high	93.02	4.90	2260.8	1987.4	8.187	379.708	1781.332	194.33	11.74	3.12
C	2	high	163.40	10.35	2263.9	1989.1	8.188	378.346	1782.143	195.26	11.7	3.11
C	3	high	136.46	11.01	2262.15	1991.3	8.183	384.354	1786.623	192.79	11.89	3.05
C	4	high	174.69	11.35	2265.8	1994.5	8.183	385.03	1789.387	193.21	11.9	3.05

Table A7

The information of culture experiment (Hard coral, daytime)

Group	ID	class	photo	Total number of zooxhanthella	measurement deviation	Start							
						TA (μmol/kg)	TA (μmol/kg)	pH (Total)	pCO_2 (μatm)	HCO_3 (μmol/kg)	CO_3 (μmol/kg)	CO_2 (μmol/kg)	Ωa
A	1	low	106	85.68	8.74	2254.63	2028.4	8.098	483.964	1849.1	164.3	15	2.573
A	2	low	106	74.69	6.04	2257.53	2024.5	8.276	294.872	1697.3	226.9	9.12	3.554
A	3	low	106	85.68	8.74	2252.83	1980.7	8.26	307.921	1709.3	220.1	9.53	3.449
A	4	low	106	74.69	6.04	2255.07	1986.3	8.259	308.876	1711.6	220.1	9.56	3.448
B	1	mid	106	143.39	19.36	2242.91	2219.1	7.571	1851.349	2106.3	55.43	57.4	0.868
B	2	mid	106	127.36	8.19	2240.03	2245.1	7.479	2310.751	2128.1	45.4	71.6	0.711
B	3	mid	106	223.97	25.59	2248.66	2218.6	7.591	1770.417	2105.8	58.01	54.8	0.909
B	4	mid	106	199.74	12.99	2241.14	2232.2	7.524	2078	2117.8	50.03	64.3	0.784
C	1	high	106	93.02	4.90	2235.71	2285.7	7.343	3205.017	2153	33.57	99.2	0.526
C	2	high	106	163.40	10.35	2233.77	2276.6	7.363	3050.916	2147.2	35.13	94.3	0.55
C	3	high	106	136.46	11.01	2232.94	2283.3	7.341	3213.348	2150.5	33.44	99.3	0.524
C	4	high	106	174.69	11.35	2231.06	2284.1	7.333	3272.06	2150.1	32.83	101	0.514
A	1	low	337	85.04	10.56	2265.61	2005.6	8.163	406.053	1807.1	186	12.6	2.911
A	2	low	337	92.17	8.69	2266.55	2007	8.162	407.4	1808.7	185.6	12.6	2.906
A	3	low	337	85.68	8.74	2261.58	2003.8	8.16	408.702	1806.7	184.4	12.7	2.887
A	4	low	337	74.69	6.04	2250.16	2004.6	8.139	431.374	1814.9	176.3	13.4	2.76
B	1	mid	337	143.39	19.36	2261.38	2214.3	7.644	1559.659	2100.6	65.33	48.4	1.023
B	2	mid	337	127.36	8.19	2262.83	2211.9	7.656	1514.383	2097.8	67.03	47	1.049
B	3	mid	337	223.97	25.59	2260.57	2210.6	7.653	1523.522	2096.7	66.55	47.3	1.042
B	4	mid	337	199.74	12.99	2257.48	2210.2	7.646	1551.316	2096.7	65.3	48.2	1.022
C	1	high	337	93.02	4.90	2254.51	2297.7	7.365	3059.058	2167.3	35.44	95	0.555
C	2	high	337	163.40	10.35	2254.88	2238.7	7.547	1971.454	2124.6	52.89	61.2	0.828
C		high	337	136.46	11.01	2255.89	2307.8	7.34	3250.493	2173.4	33.54	101	0.525
C	4	high	337	174.69	11.35	2260.21	2304.5	7.362	3090.76	2173.3	35.33	95.9	0.553
A	1	low	742	85.04	10.56	2247.76	2002.1	8.141	428.246	1812.5	176.3	13.3	2.759
A	2	low	742	92.17	8.69	2248.58	2000.1	8.146	422.811	1808.9	178.1	13.2	2.787
A	3	low	742	85.68	8.74	2243.07	1980.3	8.173	390.88	1781.2	186.9	12.1	2.927
A	4	low	742	74.69	6.04	2238.64	1997.6	8.132	437.429	1811	173	13.6	2.707
B	1	mid	742	143.39	19.36	2244.29	2210.4	7.604	1708.467	2097.9	59.37	53.1	0.929
B	2	mid	742	127.36	8.19	2260.64	2200.1	7.686	1406.492	2085.1	71.29	43.7	1.116
B	3	mid	742	223.97	25.59	2254.23	2187.4	7.705	1337.295	2071.8	74.05	41.5	1.159
B	4	mid	742	199.74	12.99	2253.51	2192.4	7.687	1396.929	2077.7	71.37	43.4	1.117
C	1	high	742	93.02	4.90	2259.46	2255.1	7.511	2157.958	2139.2	48.87	67	0.765
C	2	high	742	163.40	10.35	2259.28	2260.7	7.493	2256.047	2143.7	46.98	70.1	0.736
C	3	high	742	136.46	11.01	2259.67	2289.7	7.405	2788.694	2164.4	38.74	86.6	0.607
C	4	high	742	174.69	11.35	2261.24	2252.5	7.525	2089.517	2137.2	50.41	64.9	0.789
A	1	low	205	85.68	8.74	2264.55	1984	8.199	367.661	1773.7	198.9	11.4	2.985
A	2	low	205	74.69	6.04	2267.26	1990.6	8.192	375.429	1782.4	196.6	11.6	3.012
A	3	low	205	85.68	8.74	2264.97	1987.8	8.193	373.853	1779.4	196.8	11.6	3.054
A	4	low	205	74.69	6.04	2271.53	1994.9	8.19	377.464	1786.5	196.7	11.7	3.079
B	1	mid	205	143.39	19.36	2257.02	1989.5	8.177	389.54	1787.1	190.4	12.1	2.897
B	2	mid	205	127.36	8.19	2244.16	1972.5	8.187	377.01	1768.2	192.6	11.7	2.869
B	3	mid	205	223.97	25.59	2252.38	1983.1	8.181	384.49	1779.8	191.4	11.9	2.807
B	4	mid	205	199.74	12.99	2251.98	1985.1	8.177	388.041	1783.3	189.8	12	2.847
C	1	high	205	93.02	4.90	2260.8	1987.4	8.187	379.708	1781.3	194.3	11.7	3.124
C	2	high	205	163.40	10.35	2263.9	1989.1	8.188	378.346	1782.1	195.3	11.7	3.105
C	3	high	205	136.46	11.01	2262.15	1991.3	8.183	384.354	1786.6	192.8	11.9	3.047
C	4	high	205	174.69	11.35	2265.8	1994.5	8.183	385.03	1789.4	193.2	11.9	3.05

			End							
Group	ID	class	TA (μmol/kg)	TA (μmol/kg)	pH (Total)	pCO2 (μatm)	HCO3 (μmol/kg)	CO3 (μmol/kg)	CO2 (μmol /kg)	Ω a
A	1	low	2237.3	1972	8.177	386.589	1771.652	188.389	12	2.952
A	2	low	2234.54	1933.3	8.068	519.932	1854.732	153.686	16.1	2.407
A	3	low	2238.27	1938.9	8.162	402.682	1784.703	183.541	12.5	2.876
A	4	low	2237.68	1941.2	8.151	415.802	1793.826	179.613	12.9	2.814
B	1	mid	2229.25	2163.6	7.7	1339.289	2049.295	72.876	41.4	1.141
B	2	mid	2229.32	2172	7.676	1423.146	2058.895	69.05	44.1	1.082
B	3	mid	2229.53	2128.3	7.805	1028.562	2005.849	90.594	31.9	1.419
B	4	mid	2229.6	2143.7	7.762	1148.699	2025.41	82.708	35.6	1.296
C	1	high	2229.38	2243	7.451	2459.366	2124.281	42.593	76.1	0.667
C	2	high	2229.41	2215.3	7.539	1991.177	2102.115	51.567	61.6	0.807
C	3	high	2229.44	2225	7.509	2140.843	2110.49	48.213	66.3	0.755
C	4	high	2229.34	2219.7	7.526	2057.067	2106.038	49.978	63.7	0.783
A	1	low	2218.07	1921.7	8.236	324.253	1704.345	207.301	10.1	3.245
A	2	low	2238.83	1909.5	8.289	281.134	1671.35	229.428	8.72	3.594
A	3	low	2237.95	1923.3	8.265	301.663	1694.054	219.89	9.36	3.444
A	4	low	2234.52	1910.3	8.281	287.272	1675.481	225.911	8.91	3.538
B	1	mid	2214.93	2095.7	7.856	897.247	1968.04	99.852	27.8	1.564
B	2	mid	2225.31	2097.8	7.877	855.076	1966.603	104.697	26.5	1.64
B	3	mid	2188.34	2039.1	7.936	720.969	1900.789	115.967	22.3	1.816
B	4	mid	2221.07	2078	7.918	767.898	1940.82	113.362	23.8	1.775
C	1	high	2240.24	2217.5	7.568	1864.057	2104.749	54.96	57.8	0.861
C	2	high	2222.76	2140.5	7.752	1174.156	2023.414	80.695	36.4	1.264
C		high	2225.63	2203.1	7.567	1856.037	2091.064	54.518	57.5	0.854
C	4	high	2227.53	2199.2	7.587	1766.307	2087.52	56.722	55	0.888
A	1	low	2206.04	1848.2	8.341	237.902	1594.494	246.314	7.39	3.856
A	2	low	2249.17	1828.7	8.427	187.648	1533.909	288.96	5.83	4.524
A	3	low	2244.17	1894.5	8.322	256.457	1643.812	242.718	7.97	3.8
A	4	low	2218.07	1840	8.37	219.405	1573.253	259.929	6.82	4.069
B	1	mid	2192.1	2039.5	7.946	703.497	1899.638	117.979	21.9	1.847
B	2	mid	2211.92	2021.9	8.03	569.006	1863.538	140.672	17.7	2.201
B	3	mid	2148.76	1914.1	8.133	417.489	1735.139	165.976	13	2.598
B	4	mid	2199.48	1979.2	8.096	473.115	1805.726	158.761	14.7	2.484
C	1	high	2243.33	2132.4	7.833	962.769	2006.37	96.063	30	1.504
C	2	high	2228.86	2095.7	7.893	820.972	1962.263	107.889	25.5	1.688
C	3	high	2226.77	2126.4	7.804	1027.529	2004.451	89.973	32	1.408
C	4	high	2231.28	2117.7	7.842	936.742	1991.375	97.141	29.2	1.52
A	1	low	2213.79	1911.4	8.246	314.536	1690.647	211.021	9.73	2.976
A	2	low	2222.33	1922.3	8.239	321.592	1702.591	209.768	9.94	2.98
A	3	low	2233.54	1925.4	8.251	312.253	1700.332	215.416	9.65	2.935
A	4	low	2234.53	1937.6	8.232	330.171	1719.095	208.301	10.2	2.95
B	1	mid	2169.84	1854.4	8.275	283.073	1628.032	217.616	8.75	2.911
B	2	mid	2200.67	1874.3	8.288	276.854	1639.874	225.87	8.56	2.958
B	3	mid	2183.42	1833.4	8.328	244.085	1585.752	240.108	7.54	3.037
B	4	mid	2195.65	1836.6	8.342	236.116	1582.676	246.625	7.3	2.967
C	1	high	2216.43	1912.2	8.249	311.854	1690.239	212.302	9.66	3.011
C	2	high	2215.87	1889.4	8.286	280.308	1654.23	226.492	8.68	3.11
C	3	high	2238.51	1905	8.293	277.388	1664.474	231.942	8.58	2.978
C	4	high	2240.77	1908	8.292	278.817	1667.74	231.635	8.63	3.061

Table A8

The information of culture experiment (Hard coral, night-time)

Group	ID	class	End							
			TA (μmol/kg)	TA (μmol/kg)	pH (Total)	pCO2 (μatm)	HCO3 (μmol/kg)	CO3 (μmol/kg)	CO2 (μmol/kg)	Ωa
A	1	low	2245.1	1997.6	8.143	424.72	1807.141	177.258	13.201	2.77
A	2	low	2254.5	2009.7	8.137	434.26	1820.319	175.882	13.499	2.75
A	3	low	2255.63	2002.8	8.153	416.21	1808.841	181.017	12.942	2.83
A	4	low	2252.34	1999	8.154	414.08	1804.911	181.214	12.875	2.84
B	1	mid	2248.88	2198.8	7.654	1511.2	2085.534	66.277	46.99	1.04
B	2	mid	2246.12	2196.3	7.654	1510.9	2083.223	66.088	46.989	1.04
B	3	mid	2255.42	2207.6	7.648	1541.8	2094.191	65.461	47.948	1.03
B	4	mid	2255.58	2203.5	7.661	1492.6	2089.751	67.327	46.422	1.05
C	1	high	2284.03	2303.9	7.437	2607	2180.806	42.022	81.072	0.66
C	2	high	2279.69	2280.9	7.494	2266.1	2162.898	47.527	70.475	0.74
C	3	high	2283.61	2303.4	7.437	2605.7	2180.343	42.038	81.019	0.66
C	4	high	2271.84	2301.6	7.406	2790.4	2175.757	39.082	86.761	0.61
A	1	low	2243.17	1987.6	8.154	412.17	1792.684	182.196	12.72	2.85
A	2	low	2230.3	1987.9	8.132	435.49	1800.857	173.596	13.447	2.72
A	3	low	2227.31	1995.9	8.11	461.95	1815.083	166.559	14.257	2.61
A	4	low	2230.92	1990.6	8.128	440.68	1804.683	172.309	13.607	2.7
B	1	mid	2251.96	1986.5	8.172	393.27	1785.603	188.733	12.164	2.95
B	2	mid	2241.25	1991.8	8.144	422.98	1800.38	178.342	13.078	2.79
B	3	mid	2249.87	1995.7	8.152	416.1	1801.225	181.616	12.859	2.84
B	4	mid	2240.26	1995.9	8.133	435.25	1807.381	175.076	13.442	2.74
C	1	high	2237.45	1989.2	8.141	426	1798.649	177.403	13.148	2.78
C	2	high	2238.17	1995.4	8.13	438.72	1807.85	174.011	13.54	2.72
C	3	high	2238.37	1995.1	8.132	437.04	1807.248	174.359	13.494	2.73
C	4	high	2239.42	1996.1	8.132	436.76	1808.133	174.479	13.488	2.73

Group	ID	class	Total number of zooxanthella (×10^9)	measurement deviation	Start							
					TA (μmol/kg)	TA (μmol/kg)	pH (Total)	pCO_2 (μatm)	HCO_3 (μmol/kg)	CO_3 (μmol/kg)	CO_2 (μmol/kg)	Ωa
A	1	low	85.04	10.56	2261.61	1990.7	8.187	379.643	1786.19	192.68	11.84	3.02
A	2	low	92.17	8.69	2265.42	1991.4	8.191	375.719	1784.949	194.75	11.71	3.05
A	3	low	85.68	8.74	2265.22	1990.2	8.193	373.485	1783.091	195.48	11.63	3.06
A	4	low	74.69	6.04	2267.18	1989.5	8.196	370.417	1780.77	197.22	11.52	3.09
B	1	mid	143.39	19.36	2268.36	2201	7.706	1343.53	2084.622	74.666	41.71	1.17
B	2	mid	119.41	14.19	2267.26	2194.7	7.721	1291.59	2077.483	77.112	40.11	1.21
B	3	mid	223.97	25.59	2265.7	2196.3	7.712	1320.4	2079.734	75.557	41.01	1.18
B	4	mid	161.74	12.08	2260.53	2193.6	7.705	1340.02	2077.719	74.252	41.63	1.16
C	1	high	60.13	6.92	2263.25	2282.2	7.439	2570.33	2160.447	41.783	79.97	0.65
C	2	high	163.40	10.35	2265.97	2270.2	7.484	2308.28	2152.2	46.243	71.76	0.72
C	3	high	100.01	17.84	2263.18	2287.9	7.421	2683.77	2164.267	40.208	83.43	0.63
C	4	high	174.69	11.35	2261.98	2282.4	7.434	2598.84	2160.256	41.347	80.8	0.65
A	1	low	85.04	10.56	2267.9	1981	8.208	358.68	1766.935	202.98	11.08	3.18
A	2	low	92.17	8.69	2269.42	1986.6	8.201	365.855	1774.767	200.53	11.31	3.14
A	3	low	85.68	8.74	2269.29	1985.7	8.201	365.383	1773.499	200.92	11.28	3.14
A	4	low	74.69	6.04	2266.81	1985.7	8.198	368.577	1774.996	199.32	11.38	3.12
B	1	low	143.39	19.36	2270.59	1985.2	8.205	362.502	1771.775	202.24	11.18	3.17
B	2	low	119.41	14.19	2265.22	1982.3	8.202	364.4	1770.513	200.54	11.25	3.14
B	3	low	223.97	25.59	2265.88	1980.6	8.206	360.52	1767.433	202.04	11.13	3.16
B	4	low	161.74	12.08	2270.43	1982.6	8.21	357.305	1767.747	203.82	11.03	3.19
C	1	low	60.13	6.92	2263.88	1983	8.2	366.63	1772.529	199.13	11.34	3.12
C	2	low	163.40	10.35	2268.09	1981.4	8.209	357.869	1767.349	202.99	11.07	3.18
C	3	low	100.01	17.84	2272.4	1984.6	8.209	357.913	1769.724	203.82	11.06	3.19
C	4	low	174.69	11.35	2227.64	1984.2	8.134	431.993	1796.726	174.13	13.35	2.73