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Effects of Acidified Seawater on the Skeletal Structure of a Scleractinian Coral from Evidence Identified by SEM

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Isani Chan, Shao-Hung Peng, Ching-Fong Chang, Jia-Jang Hung, and Jiang-Shiou Hwang (2012) Effects of acidified seawater on the skeletal structure of a scleractinian coral from evidence identified by SEM. *Zoological Studies* **51**(8): 1319-1331. This study investigated the response of the scleractinian coral *Acropora valida* to acidified seawater from the Kueishan I. hydrothermal fields off northeastern Taiwan. *Acropora valida* was exposed to seawater with various pH values, and modification of the skeletal structure was examined and recorded through scanning electron microscopy (SEM). The SEM images displayed various degrees of erosion of the vertical rods and radial bars, and considerable and significant acidified seawater effects on the fusiform crystals and small spherulitic tuft clusters of aragonite as the pH decreased in the experimental treatments. The results demonstrated that a low pH may inhibit coral calcium carbonate formation, implying that the pH may be the limiting factor in the coral's distribution around the Kueishan I. hydrothermal fields. Morphological changes in scleractinian coral skeletal structures with erosion of $CaCO_3$ crystals could also be explained by the saturation state (Ω) of the experimental seawater with respect to aragonite under various pH conditions. Because ocean acidification is an urgent global environmental issue, the implication is that Kueishan I. hydrothermal vents have a considerable influence on the spatial distribution of corals around Kueishan I. This was demonstrated by the use of coral, *A. valida*, as a model species in the present study.
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Key words: Scleractinian coral, SEM, pH, Ocean acidification, Kueishantao hydrothermal vents.

Finding hydrothermal plumes on the seafloor along the Galapagos Ridge in 1976 considerably changed our understanding of hydrothermal vent biology and ecology; in particular, vent ecosystems are recognized as extreme environments that can support various life forms in highly unstable physicochemical conditions in the surrounding ecosystems (Lyon et al. 1977, Karl 1995, Dando et al. 2000, Kelly et al. 2001, Luther et al. 2001, Hwang et al. 2008, Ka and Hwang 2011). Hydrothermal systems are characterized by unexpectedly high temperatures, and wide ranges of compositions of fluids, gases, and substantially low pH values that constitute a previously unknown ecosystem and support high biomass with low species diversity (Dando et al. 1995, Cowen et al. 2002, Hwang and Lee 2003, Hwang et al. 2008, Ki et al. 2009). Research on shallow hydrothermal vents has evaluated various aspects of hydrothermal fluids, water parameters, and the abundances, distributions, and adaptations of marine organisms in vent ecosystems (Dando and Leahy 1993, Pichler and Veizer 1999 2004, Selkoe et al. 2008, Ka and Hwang 2011, Peng

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et al. 2011). The shallow-water hydrothermal vents off Kueishan I. in northeastern Taiwan are recognized as a part of the Okinawa Arc; the island is located on a tectonic conjunction of the extension of the fault system of Taiwan and the southern rifting end of the Okinawa Trough (Lee et al. 1980, Sibuet et al. 1998, Hwang and Lee 2003, Liu et al. 2008). The hydrothermal vents discharge highly acidic and sulfur-rich fluids and gases composed of carbon dioxide, sulfur dioxide, and hydrogen sulfide. The highest temperature of the Kueishan I. hydrothermal fields is approximately 116°C; however, it can reach up to 126°C after an earthquake (Hwang and Lee 2003, Chen et al. 2005). In addition, shallow hydrothermal vents also exhibit extreme environmental characteristics; however, relatively few studies of the ecology and biology of vent organisms have been conducted in the Kueishan I. hydrothermal vent ecosystem (Jeng et al. 2004, Hwang et al. 2008, Ki et al. 2009, Ka and Hwang 2011, Peng et al. 2011). The discovery of a coral reef community on the opposite side of Kueishan I., 4 km from the vent side, has intrigued researchers for a long time, particularly the manner in which the coral community flourishes near the vent plumes (Hwang and Lee 2003). Scleractinian coral is formed from calcium carbonate ($CaCO_3$), a unique underwater structure. However, coral reefs are fragile in extreme vent ecosystems, and are especially sensitive to high water temperatures and low pH values from the vent plumes. Therefore, researching the effects of acidified seawater on the skeletal structure of scleractinian corals was the primary objective of the present study.

Recently, the world's coral reef ecosystems have encountered increasing threats from anthropogenic pressures (Marubini and Atkinson 1999, Jokiel et al. 2008, Holcomb et al. 2009, Suwa et al. 2010, Tseng et al. 2011, Chan et al. 2012) and loss of biodiversity (Dai et al. 2002, Wilkinson 2004, Anthony et al. 2007). Hence, corals experience considerable threats from polluted seawaters (Tseng et al. 2011, Chan et al. 2012). The increase in ocean acidification and climate change caused by rising atmospheric carbon dioxide (CO_2) concentrations threaten marine organisms (Caldiera and Wickett 2003, Hoegh-Guldberg et al. 2007), including coral reef ecosystems. Consequently, a decrease in ocean pH contributes to decalcification caused by lowered pH levels in several marine calcifying organisms (Caldiera and Wickett 2003, Orr et al. 2005, Marubini et al. 2008), such as corals (Suwa et al. 2010). However, morphological and physiological responses to lowered pH by scleractinian coral vertical rods, radial bars, fusiform crystals, and small spherulitic tuft clusters of aragonite remain relatively unknown. The range of pH tolerance and effects on the calcium carbonate crystal structure of corals must be examined because of ocean acidification. Kueishan I. hydrothermal fields consist of a yellow vent and a white vents, which discharge highly acidic fluid (low pH), may contribute to the abundance and distribution of corals around Kueishan I. This study focused on understanding the following: (i) the effect of acidified seawater on the skeletal structure of the coral after exposure to the hydrothermal vent water; and (ii) the possible effects of hydrothermal vents on the distribution of corals around Kueishan I. We used the scleractinian coral, *Acropora valida* (Dana, 1846), as a model species to examine the effect of acidified seawater on the skeletal structure of the coral. The detailed structure of the coral skeleton after exposure to acidified vent waters was examined by scanning electron microscopy (SEM).

MATERIALS AND METHODS

Acropora valida is classified into the Animalia, phylum Cnidaria, class Anthozoa, order Scleractinia, family Acroporidae, and genus *Acropora*. Colonies have a wide range of forms from compact bushes to tables, and axial corallites are small. Radial corallites are a mixture of sizes, and are strongly appressed and swollen with small openings. The color of *A. valida* is usually cream, brown, or yellow, and occasionally brown with purple branch tips and cream radial corallites. Habitats cover a wide range of reef environments and rocky foreshores (Almany et al. 2009, Brusca and Brusca 2009).

Coral and water collection

Colonies of *A. valida* were collected from a fringing reef off Kueishan I. (Fig. 1). Specimens were placed in a temperature-insulated box containing seawater obtained from the Aquaculture Department of National Taiwan Ocean Univ., and immediately transported to the laboratory. Experimental seawater was collected from surface water of the hydrothermal fields off Kueishan I. (121°57'E, 24°50'N). Seawater samples collected from the hydrothermal vents (121°57'E, 24°50'N) were also analyzed *in situ* for various chemical parameters, such as temperature, pH, and total dissolved sulfide (Fig. 2). The temperature was measured with a thermocouple device (European Union banned mercury-in-glass thermometer), pH was measured with a portable pH meter (www.up-aqua.com), and total dissolved sulfide was measured according to a method described by Cline (1969).

Experiments

Thirty-three colonies of *A. valida* were equally distributed in 11 aquarium tanks, which contained control and experimental treatments. The coral colonies were exposed to various pH values as experimental conditions (pH 8.0, 7.0, 6.0, 5.0, 4.0, 3.0, and 2.0) and a control condition (pH 8.2) for 7 d at 25°C. The experimental treatments were not renewed during the experimentation. Hydrochloric acid (HCl) was added to the water to reduce the pH in the initial stage of the experiment. The pH and temperature were measured daily, and water samples were also periodically collected and preserved with $HgCl_2$ to measure the pH and alkalinity. During the experiment, survivorship of *A. valida* was classified as either healthy (intact tissue and polyps) or recently dead (white freshly exposed skeleton) (Williams et al. 2008). Alkalinity was determined with Gran's (1952) titration method according to a method by Cai et al. (2010). The saturation state of aragonite (Ω) was calculated according to a method of Lewis and Wallace (1998). The saturation state was defined as the $[Ca^{2+}][CO_3^{2-}]/kp'$, where $[Ca^{2+}]$ and $[CO_3^{2-}]$ are respective concentrations of calcium and carbonate ions in seawater, and kp' is the conditional solubility product of aragonite in seawater.

SEM

Materials for SEM (Chan et al. 2012) were obtained from skeletons of *A. valida*. Samples were cleaned with sodium hydroxide to remove tissue from within the skeletal structure. The *A. valida* skeleton was then attached to SEM stubs using a liquid colloidal silver paste. Samples were subsequently sputter-coated with gold (Jeol JFC 1100 E ion-sputtering system, Tokyo, Japan) and observed with an SEM (Hitachi S-4700, Tokyo, Japan) (Humphreys et al. 1974, Clode and Marshall 2003b, Chan et al. 2012).

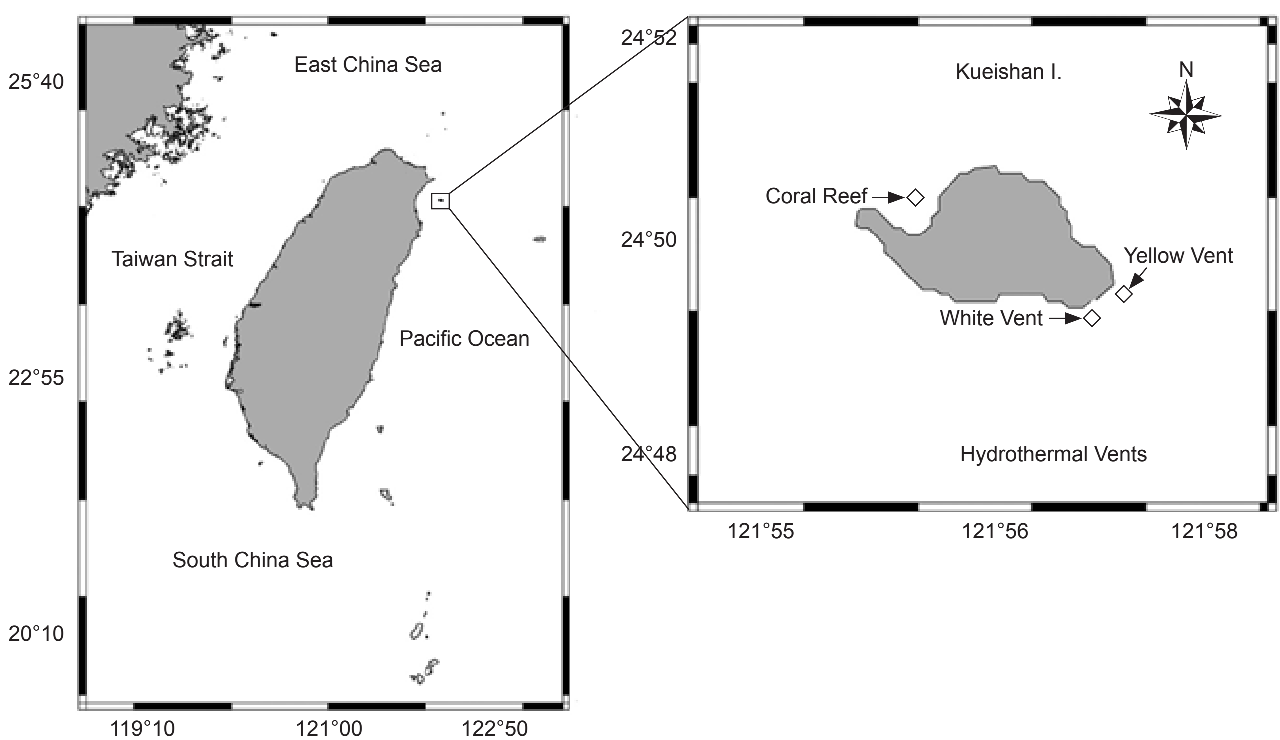

Fig. 1. Location of Kueishan I. hydrothermal vents off northeastern Taiwan.

RESULTS

Environmental conditions at the Kueishan I. hydrothermal vents

Values of pH, temperature, and total dissolved sulfide are shown in table 1 for the sampling expedition on 13 Mar. 2011. The concentration of sulfide was high at 8.95 mg/L HS^- in yellow vents and 15.2 mg/L HS^- in white vents. The temperature measured in the yellow vent was as high as 116°C and 55°C in the white vent. The pH in the yellow vent was as low as 2.81, and it was 5.06 in the white vent.

Effects of acidified seawater on *Acropora valida*

During the experimental period, the highest survivorship of *A. valida* was observed in the control treatment at pH 8.2; no changes in polyp extensions or tissue health in the coral were observed in the control tank (Fig. 3A, B). Survivorship of *A. valida* in the experimental treatment of pH 8.0 and 7.0 was significantly

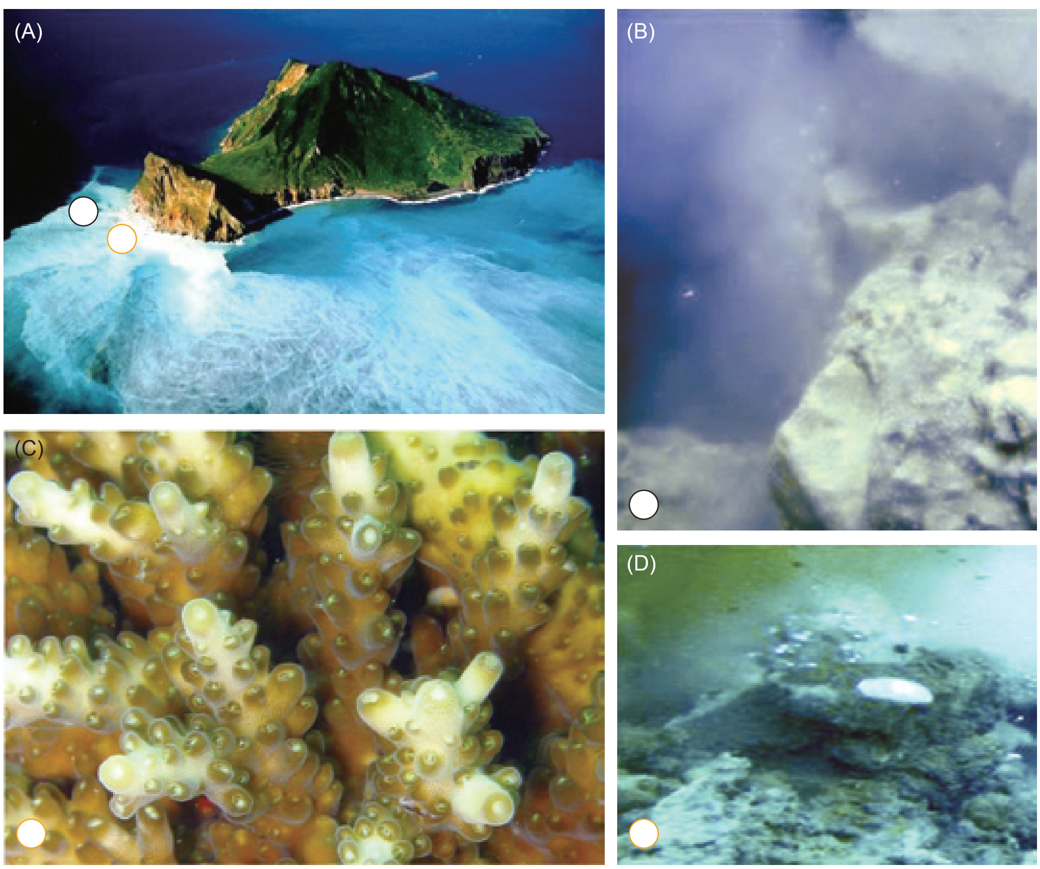

Fig. 2. Photo of Kueishan I. in northeastern Taiwan taken from a helicopter (A), white hydrothermal vents (B), scleractinian coral identified at the northwestern end of Kueishan I. (C), and yellow hydrothermal vents (D).

Table 1. Distributions of sulfide concentration (mg/L), temperature, and pH values measured in the Kueishantao hydrothermal fields

Water	Yellow vent (YV) (HS^-, mg/L)	White vent (WV) (HS^-, mg/L)	YV Temp. (°C)	WV Temp. (°C)	YV pH	WV pH
Surface water	1.04	0.93	24.2	24.1	6.82	6.50
Vent fluid	8.95	15.2	116	55	2.81	5.06
Bottom of vent	0.18	0.83	23.4	22.2	7.84	7.28

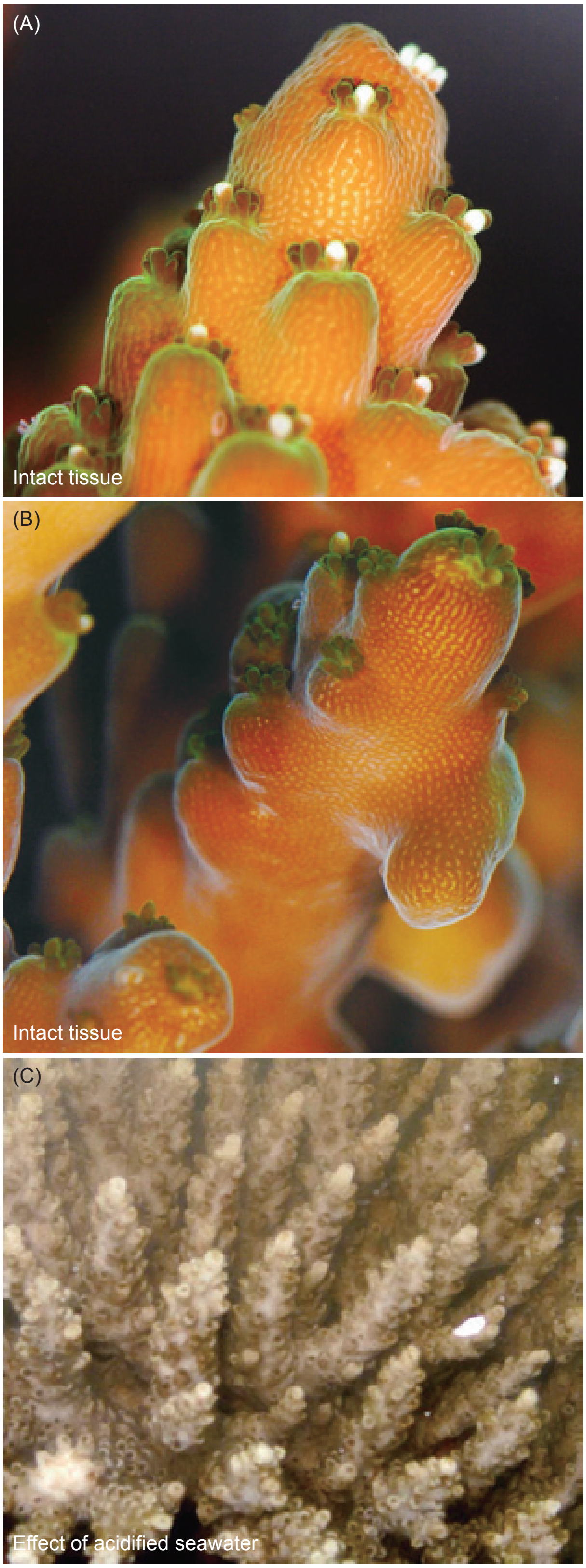

Fig. 3. Intact tissue and polyp extension identified on *Acropora valida* in the control tank environment (A, B). Polyps partially disappeared, and coral was partially bleached (C).

high. Intact tissues and polyp extensions were also observed during the experimental period. However, seawater acidification considerably affected *A. valida* during the experiment at pH 6.0-2.0. Corals exhibited mucus secretions, tissue necrosis, polyp loss, bleaching, and eventually partial mortality of the colony in all treatment groups, even during a short period (e.g., 1 wk) (Fig. 3C). Mortality of polyps in the colony increased after day 2 of the experimental period.

Effects of acidification on the calcium carbonate crystal structure of *A. valida*

Linear extensions of axial corallites of *A. valida* were erected by the distal growth of vertical rods arranged in a concentric ring (Fig. 4A-C). Rods were supported by 2 sets of radial and tangential horizontal bars. Radial bars formed the sclerosepta along the vertical units; the tangential bars were synapticulae that connected adjacent sclerosepta, and were intact in the control tank environment (Fig. 4A-C). In experimental treatments at pH 8.0 and 7.0, the vertical rods and radial/tangential bars connecting the rods appeared to be intact after 1 wk of the experimental period (Figs. 4D-F, 5A-C). However, acidification at pH 6.0 had a substantial erosive effect on the calcium carbonate crystal structure of *A. valida* by the extension of rods, beginning with deposition of fusiform crystals and small spherulitic tufts of aragonite (Fig. 5D-F). The pH 5.0 displayed erosive effects on the initial depositional surface of the calcium carbonate crystals on the bars, rods, fusiform crystals, and spherulitic tuft clusters of aragonite structures that were identified at 20x, 200x, and 4000x (Fig. 5A-C). At magnifications of 20x, 200x, and 4000x, the erosive effects of pH 4.0, 3.0, and 2.0 were respectively identified at branch tips of *A. valida*, thus revealing detrimental effects to the aragonite calcium carbonate crystal structure of the corals (Figs. 6D-F, 7A-F).

Variability of water parameters during the experiment

During the experimental period, the control tank (pH 8.2) and one of the experimental treatments (pH 8.0) exhibited no significant change in pH (Fig. 8). However, in the experimental treatments of pH 7.0 and 6.0, the pH value exhibited a slight change; furthermore, in the experimental treatments of pH 5.0, the tank exhibited increasing pH values during the experimental period. The experimental treatments of pH 2.0, 3.0, and 4.0 exhibited increasing pH values from acidic to neutral conditions. In the control tank of pH 8.2 and experimental treatment at pH 8.0, alkalinity exhibited a stable trend in both groups. In the experimental treatments of pH 7.0 and 6.0, alkalinity changed during the experimental period. In the experimental treatment of pH 5.0, the tank exhibited a slight fluctuating trend of alkalinity (Fig. 9). In the control tank of pH 8.2 and the experimental treatment at pH 8.0, saturation states (Ω) of aragonite were much higher than those in the experimental treatments of pH 7.0, 6.0, and 5.0 (Fig. 10).

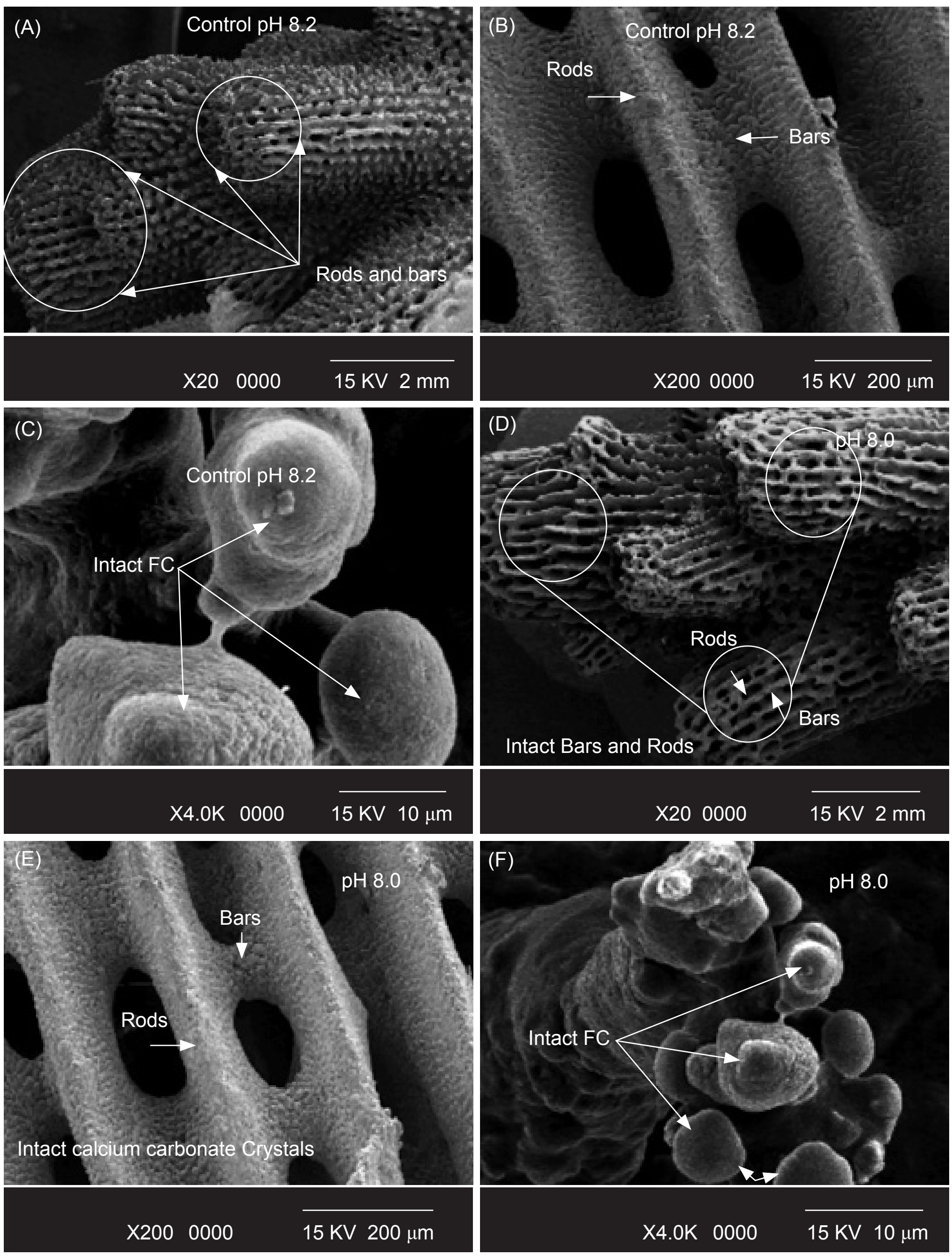

Fig. 4. Branch tips of *Acropora valida*, in the control tank (A-C). Axial corallites of *A. valida* with a series of vertical rods arranged in a concentric ring and radial bars that form the sclerosepta along with vertical rods and fusiform crystals (FC) which were intact in the control tank. *Acropora valida* with a series of vertical rods and radial bars that were intact in the pH 8.0 condition during the experimental period (D-F). Photos were taken by SEM.

DISCUSSION

Coral colonies represent a complex ecosystem, comprising a network of corals and a community of symbiotic dinoflagellates, which is also associated with a bacterial biota that thrives in remarkable harmony. Hence, several environmental factors that influence this delicate ecosystem have been identified. One of the main environmental factors that continue to

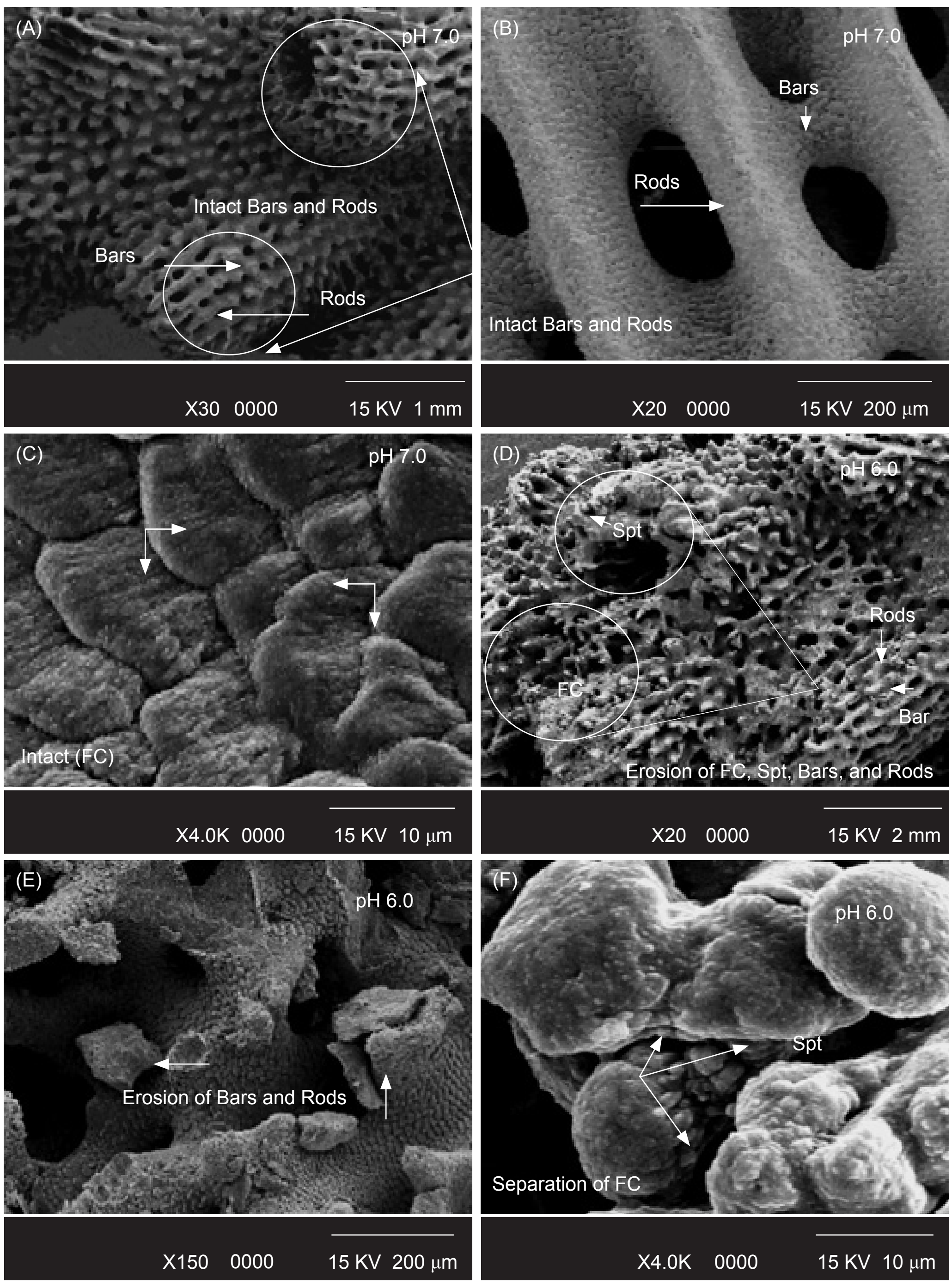

Fig. 5. In the experimental treatment at pH 7.0, vertical rods, radial bars, and the calcium carbonate structure were intact (A-C). Under the experimental treatment at pH 6.0, *Acropora valida* displayed erosive effects to the rods and bars which contributed significant negative effects on the fusiform crystals (FC) and small spherulitic tuft (Spt) clusters of aragonite (D-F). Photos were taken by SEM.

directly affect coral reefs is an increase in the CO_2 concentration, which affects ocean acidification (Gattuso and Buddemeier 2000, Langdon and Atkinson 2005, Veron 2008a b). Ocean acidification is the reduction of pH in the world's oceans; and therefore, was ranked 36th of 40 potential threats to future coral reef communities (Kleypas and Eakin 2007). Subsequently, substantial evidence has emerged which demonstrated that ocean acidification may have more considerable implications for coral reefs than previously assumed (Orr et al. 2005, Raven et al. 2005, Hoegh-Guldberg et al. 2007). CO_2 emissions continue to considerably increase because of the burning of fossil fuels and land clearing (Harley et al. 2006, Solomon et al. 2007).

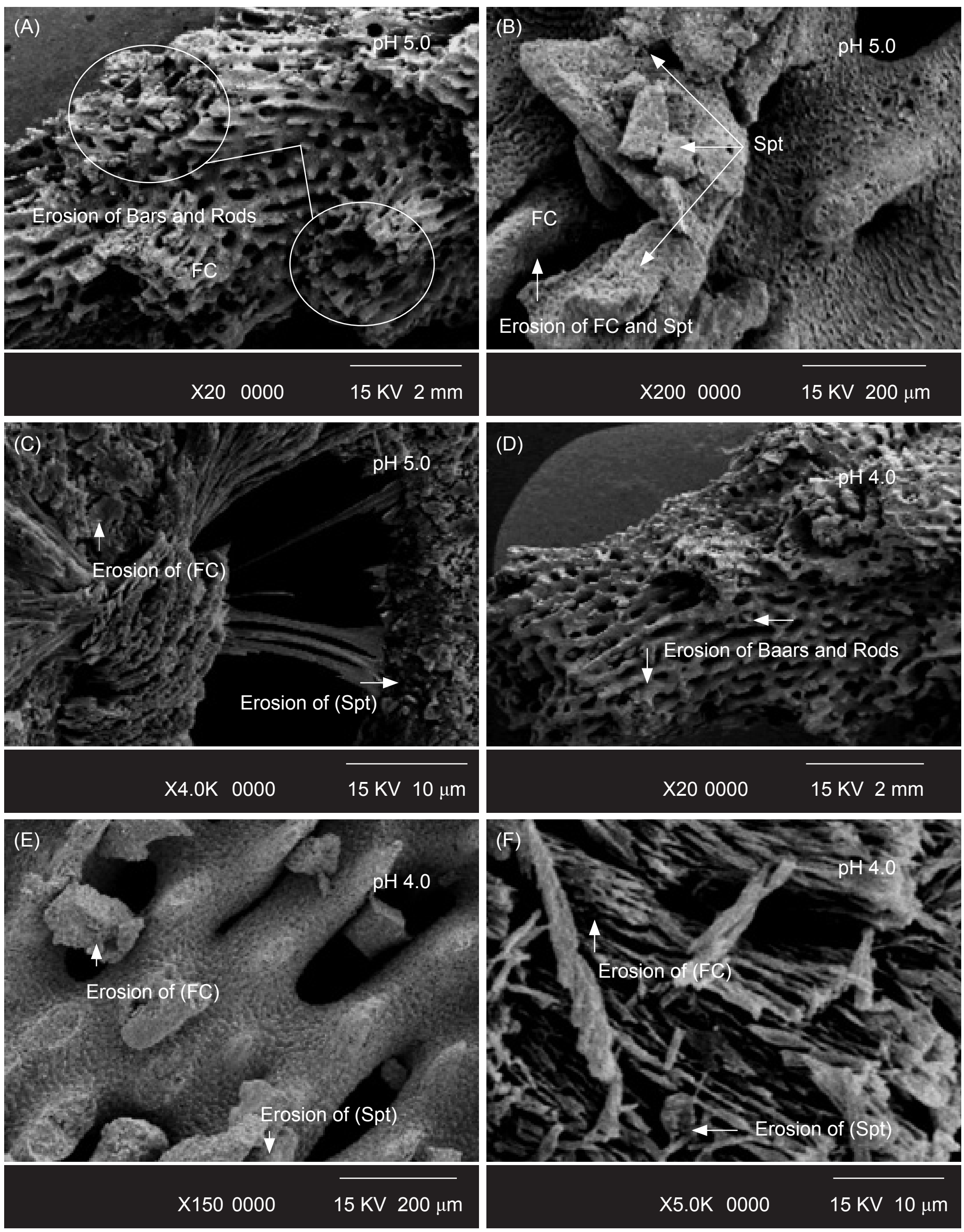

Fig. 6. Under the experimental treatment at pH 5.0 (A-C) and 4.0 (D-F), erosive effects were identified on the fusiform crystals (FC) and small spherulitic tuft (Spt) clusters of aragonite. Photos were taken by SEM.

The current rate of CO_2 increase is approximately 1 ppm/yr. Depending on future CO_2 emission rates, this figure could rise to 2-6 ppm/yr, leading to levels of 540-970 ppm CO_2 by 2100 (Raven et al. 2005). Because the ocean readily exchanges CO_2 with the atmosphere, the increased atmospheric CO_2 leads to a higher partial pressure of CO_2 in the upper oceans, resulting in a increased seawater concentration of H_2CO_3 and a decreased concentration of CO_3^{2-}. The direct effect of these CO_2 emissions is estimated to reduce seawater pH by 0.1 unit, CO_3^{2-} concentration by 30 μmol/kg, and aragonite super saturation from 4.6 to 4.0 (Kleypas et al. 1999, Hoegh-Guldberg et al. 2007).

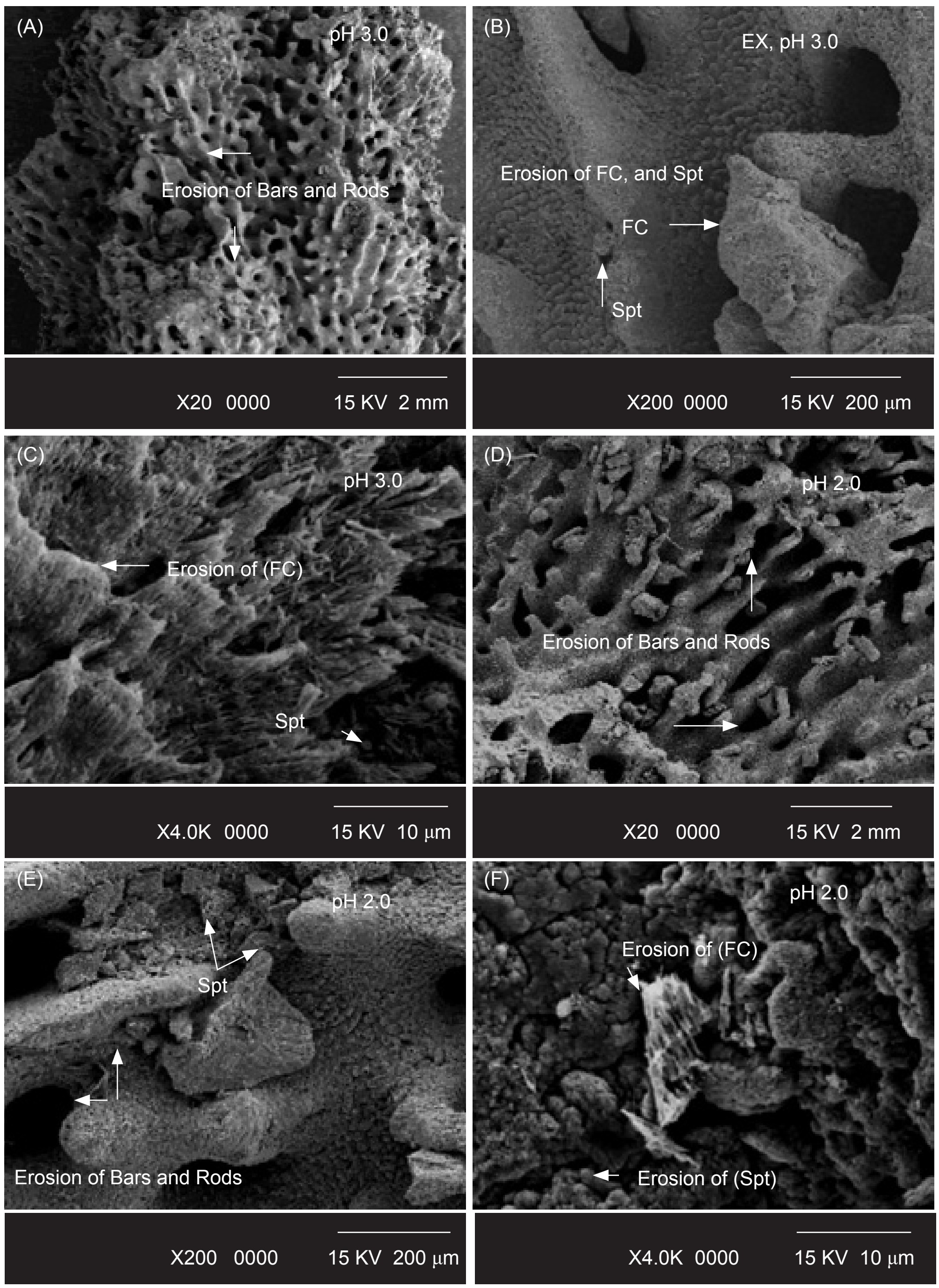

Fig. 7. Effects of the experimental treatments at pH 3.0 (A-C) and 2.0 (D-F) on *Acropora valida*. Erosive effects were identified on bars, rods, fusiform crystals (FC), and spherulitic tuft (Spt) clusters of the aragonite structure of the corals. Photos were taken by SEM.

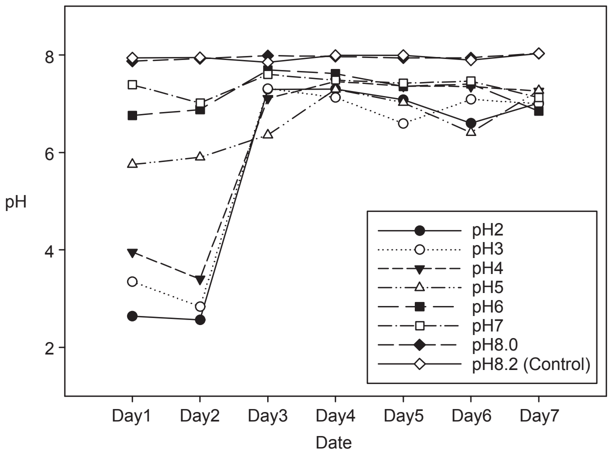

Fig. 8. Summary of pH changes in the control and experimental treatments during the experimental period.

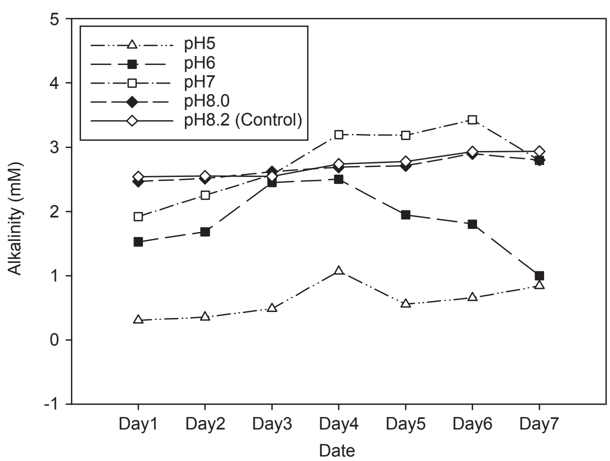

Fig. 9. Summary of changes in total alkalinity (mM) in the control and experimental treatments during the experimental period.

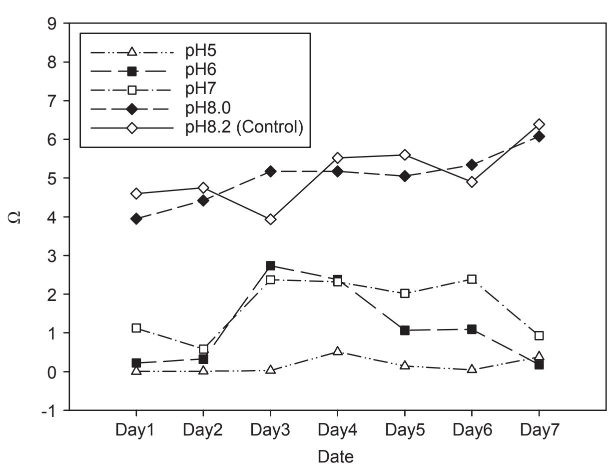

Fig. 10. Summary of the saturation state (Ω) of aragonite in the control and experimental treatments during the experimental period.

Little is known about the effects of changes in pH on the morphology and physiology of corals; in particular, knowledge about the effects of reduced pH on coral-associated microbial communities is limited. In the Kueishan I. hydrothermal fields, the pH value was detected at as low as pH 2, and vent temperatures can reach 116°C (Hwang and Lee 2003, Chen et al. 2005). Therefore, pH values and vent temperatures considerably fluctuate following tides and currents (Hwang and Lee 2003, Chen et al. 2005). Low pH values and high seawater temperatures are considered to be an extreme environment for coral reef ecosystems. The response of the scleractinian coral *A. valida* to acidified seawater from Kueishan Island hydrothermal fields off northeastern Taiwan was investigated. *Acropora valida* was exposed to various pH values, and modifications of the skeletal structure were examined and recorded using SEM (Figs. 4-7). The effects of reduced seawater pH on the skeletal structure of *A. valida* resulted in high mortality of polyps in all treatment groups (Fig. 3C). However, during the experimental period, the highest survival rate of *A. valida* was observed in the control treatment of pH 8.2; no significant changes were identified in polyp extensions or the tissue structure (Fig. 3A, B). Survival rates of *A. valida* in the experimental treatments of pH 8.0 and 7.0 were significantly higher than all other lower-pH treatments. Intact tissues and polyp extensions were also identified during the experimental period. However, seawater acidification at pH 6.0-2.0 affected survival rates of *A. valida* during the experiment, which induced mucus secretions, tissue necrosis, and polyp loss, and resulted in mortality in all treatment groups. The calcium carbonate crystal morphological structure of the corals in the control treatment exhibited a consistent healthy pattern, which was identified by SEM. The decreased pH in each experimental treatment caused substantial changes in the calcium carbonate crystal structure of *A. valida*, as demonstrated by SEM (Figs. 5-7); thus, our results are consistent with those of work by Gladfelter (2007) and Holcomb et al. (2009).

In accordance with this finding, linear extensions of axial corallites of *A. valida* were affected by the acidic environment. Hence, the calcium carbonate crystal structure that forms the rods and supports the radial and tangential sets of horizontal bars was also affected by the acidic environment of the Kueishan hydrothermal vents. The morphology of rods, bars, and calcium carbonate crystals structure of *A. valida* systematically changed at low pH values in the experimental treatments. As described in this study, extensions of rods and bars by *A. valida* under the experimental treatment of pH 6.0 exhibited erosive effects of the fusiform crystals and small spherulitic tuft clusters of aragonite as the densely packed calcium carbonate continued to separate based on the systematic change in pH treatments. This observation is consistent with systematic variations in rods, bars, fusiform crystals, and spherulitic tuft clusters of aragonite morphologies, which were observed in the experimental treatments at pH 5.0-2.0; thus, formation of calcium carbonate crystals in each treatment reflected a change because it was related to the acidic environment with a rough interface associated with spherulitic dislocation on the fusiform crystals. Detrimental effects were clearly identifiable on branch tips of *A. valida* at various magnifications by SEM.

The information presented in this paper is consistent with earlier observations, in that as the pH in each experimental treatment decreased, the morphological changes to the calcium carbonate crystal structure increased in each treatment group. Therefore, results demonstrated that the chemical composition of the vent fluid at the Kueishan I. hydrothermal vents is detrimental to scleractinian corals that form their structure from calcium carbonate. Recently, with an increasing concentration of atmospheric carbon dioxide that is acidifying the world's oceans, sea surface pH continues to drop below preindustrial values, and is predicted to significantly decrease globally by the end of this century (Harley et al. 2006, Solomon et al. 2007). Consequently, the change in pH may result in changes in the morphology and physiology of scleractinian corals; in particular, organisms that build their skeleton from calcium carbonate. Thus, these morphological and physiological changes may also affect other members of the coral holobiont; for example, microbial communities associated with corals, which may affect coral physiology and health (Orr et al. 2005, Raven et al. 2005, Hoegh-Guldberg et al. 2007).

Acidification processes of low pH during the experiments changed the saturation state of aragonite to a lower condition in the control tank of pH 8.2 and the experimental treatment at pH 8.0 and the saturation state of aragonite was maintained at relatively high values causing coral calcium carbonate erosion. Thus, the decrease in pH caused a shift in carbonate equilibrium; therefore, low-pH conditions had substantial negative effects on *A. valida*, causing $CaCO_3$ dissolution in the experimental treatments, thereby contributing to changes in alkalinity and pH as a result of $CaCO_3$ dissolution. Prior studies indicated that vent fluid affected the distribution of marine organisms around hydrothermal vents because of the acidic environment (Grieshaber and Volkel 1998, Von Damm et al. 1985a b, Yang and Scott 1996, Hwang and Lee 2003, Liu et al. 2008).

In conclusion, acidified seawater has considerable negative effects on the skeletal structure of scleractinian corals. This indicates that scleractinian corals might not be able to survive in acidic vent environments. This evidence supports the distribution pattern of scleractinian coral observed at Kueishan I., where scleractinian corals are located on the farthest end away from the vent sites of the island (Fig. 2). Thus, the results of this study provide new insights into the manner in which scleractinian corals are distributed around Kueishan I. The SEM results demonstrated that the low pH may inhibit coral calcium carbonate formation, implying that pH may be the limiting factor in the coral's distribution. Kueishan I. hydrothermal vents have a considerable influence on the spatial distribution of corals around Kueishan I. This was demonstrated by the use of the coral, *A. valida*, as a model species in this study.

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