Studies on Stress Responses of Corals in Japan

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Abstract

Coral reefs in Japan are threatened by multiple environmental stresses at both the global and the local scales. Declining water quality in coastal reefs has been reported in the Okinawa region due to red soil runoff, agricultural fertilizers, and antifouling chemicals. Many studies on the stress responses of corals have been conducted by Japanese researchers. For example, metabolic changes due to stresses such as high temperature and chemical discharge have been reported in quantitative terms. Antioxidant enzyme activities and mycosporine-like amino acids have been studied as possible defense mechanisms against environmental stress. Moreover, coral bleaching has been frequently reported and actively studied since the 1980s in Japan. The synergistic effects of multiple stressors have also been studied, with several studies reporting accelerated bleaching under conditions of high seawater temperature and low water quality (high nitrate concentration and high bacterial abundance). The importance of water flow has been extensively studied, and it has been suggested to allow more rapid recovery from bleaching and a higher survival rate. To mitigate environmental stresses on coral reefs, it is important to evaluate risks due not only to global warming but also to local stresses.

Keywords

Global warming • Eutrophication • Chemical discharge • Coral bleaching • Oxidative stress • Antioxidant • Cold stress

3.1 Environmental Stresses on Coral Reefs in Japan

Coral reefs are threatened by several serious environmental problems, including global warming, coral bleaching, coastal development, increased sedimentation, tourism, overfishing, hurricanes, ocean acidification, and coral diseases (Brown 1997; Hughes et al. 2003; Fabricius 2005; Orr et al. 2005; Hoegh-Guldberg et al. 2007). Many studies have reported that elevated seawater temperature is the most notable environmental change affecting the symbiotic system between the host coral and its symbiotic algae, zooxanthellae (e.g., Brown 1997). In Okinawa, Japan, extensive bleaching due to high seawater temperature was observed in 1998 (Yamazato 1999). Seawater temperature was nearly 30 °C from the surface to a depth of 50 m in July 1998, compared with around 27 °C from the surface to a depth of 30 m in July 1997 (Yamazato 1999). The period of elevated temperature, during which the sea surface temperature (SST) exceeded 30 °C, corresponded with the period of bleaching, which confirmed that the major driver of coral bleaching in the Ryukyu Islands was high SST (Kayanne et al. 2002). On the shallow reef flat, seawater was 2–3 °C warmer than the measured SST in the outer ocean during the daytime and reached 35 °C in the middle of August 1998 (Kayanne et al. 1999). On the other hand, especially in northerly parts of Japan, low temperature

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Fig. 3.1 Monthly averages of
sea surface temperature
around Japan in 2013. ○, at
Sesoko (26ºN); △, at
Shirahama (33ºN); □, at
Tateyama (34ºN).
Temperature data was
extracted from Higuchi et al.
(2015a)
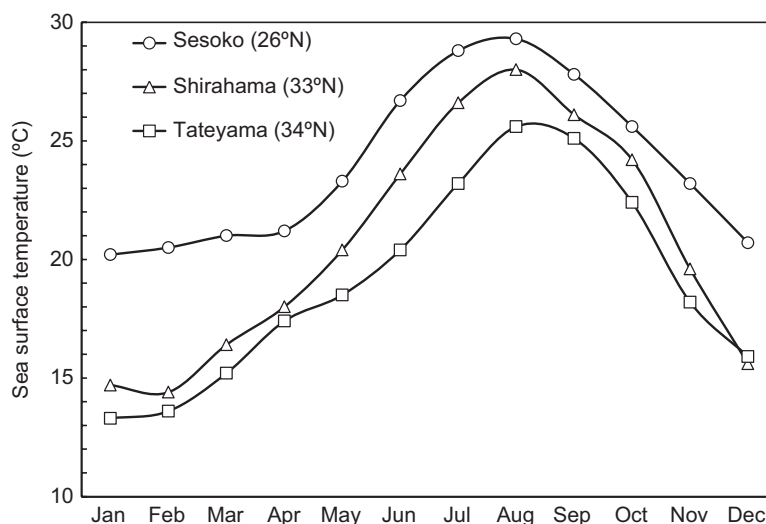
is a serious stress determining coral survival (Higuchi et al. 2015a). SST close to the northern limit of the coral distribution in the temperate zone of Japan can fall as low as 13 °C in winter (e.g., Tateyama: 34°N, Higuchi et al. 2015a). Notably, corals survive winter SST below 13 °C around Iki Island (33.5°N) (Yamano et al. 2001). Thus, the coral distribution in Japan includes a wide range of temperatures across different geographic zones (Fig. 3.1).

Declining water quality in the coastal environment due to coastal development is considered to be one of the primary causes of the global decline in coral reefs (Hoegh-Guldberg 1999; Pandolfi et al. 2003). In Japan, red soil runoff from developed land has been a major environmental issue since the 1980s (Omija 2004). High turbidity after rainfall can often be observed in coral reef areas (Fig. 3.2). Terrestrial runoff of sediments, nutrients, and pollutants can adversely impact corals due to burial and smothering and can also reduce recruitment, decrease calcification, and decrease photosynthetically active radiation (PAR) (Fabricius 2005). The effects of short-term sedimentation with red soil on the corals *Coelastrea aspera*, *Porites lobata*, and *Pavona frondifera* were investigated in laboratory experiments (Ismail and Tsuchiya 2005). The results showed that massive corals, such as *C. aspera* and *P. lobata*, were more resistant to sediment stress than the platelike coral *P. frondifera*. In addition to the direct effects of red soil particles covering the corals, photochemical studies of red soil-polluted seawater have shown that photoformation of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), was higher in red soil-polluted seawater than in unpolluted seawater (Nakajima et al. 2004). This increase in H_2O_2 photoformation was correlated with the total dissolved iron (Fe) concentration, which was higher in red soil-polluted seawater than in clean seawater (Nakajima et al. 2004; Okada et al. 2005). Furthermore, Nakajima et al. (2004) reported that H_2O_2 photochemical formation was further enhanced by strong UV

radiation, suggesting that the decrease in the stratospheric ozone layer may increase concentrations of H_2O_2 in seawater in the future. In the Red Sea, H_2O_2 concentrations are 0.10–0.25 μM during the daytime in the lagoon at low tide (Shaked and Armoza-Zvuloni 2013). The concentration of seawater H_2O_2 in coral reef areas of Okinawa Island can reach 0.16 μM on sunny days (Arakaki et al. 2005), and higher concentrations (ca. 0.5 μM) of H_2O_2 were also observed when seawater was kept in a quartz test tube in the sunlight (Arakaki et al. 2007). This suggests that seawater that is not well mixed, such as in tidal pools at low tide, could have much higher concentrations of H_2O_2, reaching levels toxic to corals (Higuchi et al. 2009a).

Chemical discharge to coral reefs through rivers is also important to consider when assessing coral reef stressors. Pollution from domestic agricultural, industrial, and shipping activities has not been investigated as a problem affecting coral reefs around the Ryukyu Archipelago (Kitada et al. 2008; Sheikh et al. 2007; Tanabe et al. 2008). Terrestrial discharge onto coral reefs can be linked to changes in coral population and community dynamics in Japan (West and van Woesik 2001; Dikou and van Woesik 2006). Based on 15 years of data, it appeared that high turbidity influenced by recent terrestrial runoff reduced the resilience of *Acropora* species to severe thermal stress events because the corals could not adapt to the decline in water quality (Hongo and Yamano 2013). In coastal areas, pollution with nutrients, especially nitrate, via inflow from rivers and groundwater has increased over the last few decades (Umezawa et al. 2002; Higuchi et al. 2014). Nutrient pollution due to human activity has increased due to several factors but most notably the increased use of chemical fertilizers in agriculture (Umezawa et al. 2002). Reefs that receive groundwater discharge, such as the Sesoko reef in Okinawa, exhibit increased nitrate concentrations of about 10 μM (Meekaew et al. 2014). This increase is most notable at low tide due to the concen-

Fig. 3.2 Effect of red soil pollution on coastal reef. (**a**) Sunny day and (**b**) after rainfall

trated effect of groundwater at that time (Higuchi et al. 2014). Inoue et al. (2014) indicated that coral skeletons of *Porites* sp. recorded clear seasonal variations in manganese (Mn), which is a proxy for river discharge. Tanahara et al. (2013) studied temporal changes in heavy metal concentrations in sediment cores from tidal land downstream of the main rivers in Naha, Okinawa, a densely populated area. Analysis of the soil revealed contamination by heavy metals from development upstream that had flowed into the tidal area. Diuron, [*N′*-(3,4-dichlorophenyl)-*N*,*N*-dimethyl-urea] (DCMU), is a photosystem II herbicide and is considered a priority hazardous substance by the European Commission (Malato et al. 2002). In 2004, around 11 tons of diuron was used for agricultural purposes in Okinawa Prefecture, which had the highest usage of this toxic substance in Japan outside of the Tokyo metropolitan region (Sheikh et al. 2009a). Diuron concentrations were periodically measured between 2007 and 2009 using 93 transects of the Shiraho coral reef waters of the Ryukyu Archipelago and the Todoroki River, which receives runoff from sugarcane production and discharges to the reefs (Sheikh et al. 2009a). Average DCMU concentrations in the river and reefs were 60.2 ± 157 and 2.3 ± 7 ng L^{-1}, respectively, with concentrations on the Shiraho reef ranging from below detection to 90 ng L^{-1}. The maxi-

mum concentration of DCMU was detected in winter after a period of intense rainfall. The DCMU contamination of Ryukyu Archipelago coral reefs is higher than that of other tropical reefs, as measured in Hong Kong (1.9–6.3 ng L^{-1}; Shaw et al. 2008) and in the Great Barrier Reef, Australia (0.2–1.6 ng L^{-1}; Shaw and Mueller 2005). In contrast, the maximum DCMU concentration in Okinawa sediment (0.22 μg kg^{-1}) was low relative to that in the Great Barrier Reef World Heritage Area, Australia (<0.1–10.1 μg kg^{-1}) (Haynes et al. 2000; Kitada et al. 2008; McMahon et al. 2005). Although DCMU is frequently found at high concentrations close to farmland and not cities, high concentrations were detected in both rural and moderately urbanized areas (Kitada et al. 2008). DCMU concentrations in urban areas were sometimes higher than in rural areas, which might be caused by greater usage in the gardens of urban homes (Kitada et al. 2008). Irgarol 1051 is used in antifouling paint, and high concentrations were observed in ports with shipping activity (Sheikh et al. 2009b). Kitada et al. (2008) reported that Irgarol 1051 can be detected in river areas far downstream of the source pier or fishery harbor (0.6–3.2 km). These results suggested that Irgarol 1051 could be transported from river mouths to the coastal ocean (Kitada et al. 2008). The average concentration of Irgarol detected in com-

mercial and fisheries ports was 24.70 ± 9.88 ng l⁻¹, and in Naha Bay, its average concentration was 10.00 ± 12.98 ng l⁻¹, approaching the environmental risk limit for marine organisms (Sheikh et al. 2009b). Irgarol 1051 has been used since around 2003 instead of tributyltin (TBT), which is on the list of compounds legally restricted by the International Maritime Organization (IMO) (Gatidou et al. 2007). Sheikh et al. (2007) reported the presence of organotin compounds [TBT and triphenyltin (TPhT)] in water and sediments of the Manko and Okukubi estuaries with high boating and agricultural activities. Although high TBT concentrations were not detected in reef areas, the concentrations of TBT reported in upper streams may pose a risk to marine life (>1 ng L⁻¹; Tong et al. 1996). Bisphenol A (BPA) is classified as an endocrine disruptor and is released into the environment as a result of its use in polycarbonate plastic and epoxy resin (Krishnan et al. 1993). Concentrations of BPA in river water were low in reef areas in Okinawa (Kawahata et al. 2004; Kitada et al. 2006). Although BPA was detected in river sediment, its concentration remained too low for effects such as estrogenic stimulation of corals and coral reef organisms (Kitada et al. 2008). Therefore, the BPA concentrations presently observed in Okinawa are not likely to damage coral reefs. Benzophenone-3 (BP-3; oxybenzone) is used in sunscreen lotions as it protects against the damaging effects of ultraviolet radiation (Downs et al. 2016). Downs et al. (2016) suggested that oxybenzone may be a hazard to coral reef conservation by threatening the resiliency of coral reefs to climate change. In Okinawa, BP-3 levels on coral reefs 300–600 m away from public swimming beaches ranged from 0.4 to 3.8 ng L⁻¹. BP-3 was detected up to 600 m from the public beach, suggesting that this distance represents its maximum dispersion from the source (Tashiro and Kameda 2013). Although the concentration of BP-3 in Okinawa is not higher than those in other areas such as Hawaii (Downs et al. 2016), the threat of BP-3 from swimmers to corals and coral reefs remains important, as a concentration of 6.5 μg L⁻¹ has been shown to affect coral planulae. Coral reefs in Japan are affected by multiple environmental stresses, many of which can be linked to anthropogenic activities. For other effects of anthropogenic activities such as the impact of ocean acidification on coral reefs, please see Chap. 5. Predation of corals by organisms such as crown-of-thorns starfish is discussed in Chap. 9.

3.2   Stress Responses of Corals in Japan

Stress responses of hermatypic corals in Japan have been the subject of numerous studies (Table 3.1). Many indicators have been used to evaluate coral stress levels. Coral metabolism, an indicator of the physiological condition of coral

colonies, has been studied using an apparatus called the continuous-flow, complete-mixing (CFCM) experimental system (Fujimura et al. 2008), which can quantitatively measure changes in coral metabolism under increasing stress levels. This system can simultaneously control temperature, light intensity, and chemical composition of the seawater. The CFCM system can acquire quantitative information during long-term experiments while continuously supplying fresh seawater (Fujimura et al. 2008). Using this experimental system, Fujimura et al. (2008) reported that high temperature increased respiration rate and decreased both photosynthesis and calcification rates in corals. Agostini et al. (2016) proposed that mitochondrial electron transport system activity (ETSA) could be used as an indicator of coral health, especially for the health of the host animal. ETSA showed positive correlations with metabolic rates and physiological variables such as zooxanthella density, biomass, photosynthesis, respiration, and calcification. Thus, measurement of all of the different metabolic rates during incubation could be avoided by measuring ETSA as a global indicator of coral health.

Contamination by the new antifouling biocide Irgarol is prevalent in the coastal areas around the coral reefs of Okinawa Island. However, current concentrations in the environment are <1 to 35 ng L⁻¹ and thus do not seriously impact the survival of reef-building corals in Japan (Sheikh et al. 2009b). Regarding other chemical contaminants, Watanabe et al. (2006) reported tissue detachment and decreases in symbiont density in *Acropora tenuis* juvenile polyps at a concentration of 1 μg L⁻¹ TBT or 10 μg L⁻¹ DCMU. A long-term experiment (about 50 days) showed adverse effects on coral growth at lower concentrations (0.4 μg L⁻¹ TBT and 1 μg L⁻¹ DCMU; Watanabe et al. 2007). Sheikh et al. (2012) indicated that DCMU inhibited photosynthesis and calcification of corals at a concentration of 10 μg L⁻¹. Yuyama et al. (2012b) examined the effects of thermal and chemical (DCMU and TBT) stresses on *A. tenuis* juvenile polyps. Oxidative stress-responsive protein was commonly expressed by stressed corals, regardless of the stressor.

ROS can lead to significant oxidative damage to the coral-algae symbiotic system (Lesser 2006; Weis 2008). Smith et al. (2005) hypothesized that H_2O_2 is the most important ROS associated with coral bleaching because it can act as a signaling molecule between zooxanthellae and their symbiotic hosts. Considering the high concentration of H_2O_2 in red soil-polluted areas, the physiological effects of H_2O_2 on reef-building corals were tested. Higuchi et al. (2009b) reported the effects of various concentrations of H_2O_2 on carbon metabolism by the coral *Coelastrea aspera* (formerly *Goniastrea aspera*). Higher concentrations of H_2O_2 decreased photosynthesis and calcification but did not affect respiration

Table 3.1 List of studies in Japan on responses of corals against environmental changes

Parameter	Response	References
High temperature	1. Photosynthesis, respiration, and calcification of *Coelastrea aspera* were maximum in 29 °C. Photosynthesis and calcification were decreased from 31–33 °C, and respiration was increased with 31–33 °C	Fujimura et al. (2008)
	2. Under 32 °C a large number of *Symbiodinium* were expelled, and loss of *Symbiodinium* from coral tissues led to coral bleaching. Six species of scleractinian corals, *Acropora selago*, *Acropora muricata*, *Heliofungia actiniformis*, *Ctenactis echinata*, *Oxypora lacera*, and *Pocillopora eydouxi*, were used	Fujise et al. (2013)
UV radiation	3. UV-reduced condition (UV cut 78%) decreased 40% photoformation of OH radical in tissue extract of *Galaxea fascicularis*	Higuchi et al. (2010)
Hydrogen peroxide (H_2O_2)	4. 0.3 μM does not affect on coral metabolisms. >3 μM H_2O_2 decreased photosynthesis (−18% with 3 μM) and calcification (−23% with 3 μM) of *Coelastrea aspera*	Higuchi et al. (2009b)
	5. 0.3 and 3 μM H_2O_2 increased activities of antioxidant enzyme catalase	Higuchi et al. (2008)
H_2O_2 plus high temperature	6. 3 μM H_2O_2 decreased photosynthesis (−21%) and calcification (−7%) of *Galaxea fascicularis*. The synergistic effect of 3 μM H2O2 combined with high seawater temperature (31 °C) increased respiration rate (+134%).	Higuchi et al. (2009a)
Red soil	7. The massive corals, *Coelastrea aspera* and *Porites lobata*, are more resistant to sediment stress than the platelike coral *Pavona frondifera*	Ismail and Tsuchiya (2005)
Nitrate and phosphate plus high temperature	8. The corals *Acropora tenuis* in high nutrient (4.8 μM NO_3, 0.28 μM PO_4) lost more zooxanthellae per unit surface area than those in low nutrient (0.2 μM NO_3, 0.06 μM PO_4), but the lost percentages were not significantly different between low and high nutrient under thermal stress of 31 °C	Tanaka et al. (2014)
Nitrate plus high temperature or high light	9. 10 μM nitrate alone does not induce stress with ambient temperature (27 °C) and low light (200 μmol m^{-2} s^{-1}). However, high nitrate increased bleaching severity (about 70% of zooxanthellae lost) under increased light (600 μmol m^{-2} s^{-1}) or high temperature (32 °C) within 6 days	Higuchi et al. (2015b)
High light	10. Corals containing clade C showed a greater decline in Fv/Fm (−74%), compared to decline in corals associated with clade D (−50%) with 500 μmol m^{-2} s^{-1} after 3 h	Yuyama et al. (2016)
Low temperature	11. Measuring photosynthesis and respiration of *Acropora pruinosa* under 3 h exposure	Nakamura et al. (2004)
	12. 13 °C stress led to death on *Acropora solitaryensis* and *Acropora hyacinthus* before 5 days. *A. pruinosa* survived with bleaching for 10 days under 13 °C. After water temperature increased, *A. pruinosa* recover from cold bleaching	Higuchi et al. (2015a)
Bacteria plus high temperature	13. Increased abundance of bacteria such as *Vibrio coralliilyticus* and *Sulfitobacter* sp. did not affect coral metabolisms and zooxanthellae density under ambient temperature (27 °C). However, *Sulfitobacter* sp. accelerated coral bleaching and severely decreased photosynthesis and calcification under high temperature (32 °C)	Higuchi et al. (2013)
DCMU	14. Tissue detachment and decreased in the symbiont density of *Acropora tenuis* at the concentrations of 10 μg L^{-1} within 10 days exposure	Watanabe et al. (2006)
	15. Adverse effects on coral growth at 1 μg L^{-1} with 50 days exposure	Watanabe et al. (2007)
	16. Decreased photosynthesis and calcification of corals with 10 μg L^{-1} for 4 days	Sheikh et al. (2012)
Water velocity	17. Reduced water flow amplified photodamage of zooxanthellae under strong irradiance. Higher water-flow rates strongly reduced the degree of photoinhibition and the severity of bleaching in *Acropora digitifera*	Nakamura et al. (2005)

(Higuchi et al. 2009b). The synergistic effect of high H_2O_2 with high temperature significantly increased respiration rates, which surpassed the effect of either H_2O_2 or high temperature alone (Higuchi et al. 2009a). High temperature alone decreased photosynthesis and calcification and increased respiration. These results suggest that the current level of H_2O_2 in Okinawa seawater is not likely to cause sig-

nificant acute effects on the metabolic activities of corals. However, if the ozone layer in the stratosphere becomes thinner in the future, the amount of UV radiation reaching the surface of the ocean may increase, which in turn will increase the photochemical formation of H_2O_2. It is therefore important to continue monitoring the concentrations of seawater H_2O_2 in coral reef areas. Incidentally, high concentrations of

H_2O_2 (5 mM) induced spawning in Okinawan staghorn corals (Hayashibara et al. 2004). This spawning technique is used for many studies of larval and juvenile corals (Suzuki et al. 2011; Yamashita et al. 2013).

Environmental stresses such as high temperature can also induce increased production of ROS, including H_2O_2, by the coral host and its symbiont. The production of H_2O_2 was induced by inhibition of the chlorophyll a–chlorophyll c_2–peridinin protein complex (acpPC) in *Symbiodinium* (Takahashi et al. 2008). One defense mechanism against increased ROS is an increase in the activity of free radical scavenger enzymes such as superoxide dismutase (SOD) and catalase (CAT). SOD and CAT act in concert to inactivate superoxide radicals and H_2O_2. Under high-temperature conditions, both SOD and CAT activities increased in coral tissue and zooxanthellae (Yakovleva et al. 2004; Higuchi et al. 2008). Agostini et al. (2016) reported that high temperature increased Mn SOD, which may correlate with the production of ROS in the mitochondria. Yakovleva et al. (2004) suggested that coral bleaching triggered by high temperature was caused by the production of ROS, including H_2O_2, within the zooxanthellae. In H_2O_2 exposure experiments, CAT activities in both coral tissue and zooxanthellae increased (Higuchi et al. 2008). However, CAT activities in corals were not significantly affected by even tenfold increased concentrations of H_2O_2. It was thus suggested that corals have a limit to their ability to increase CAT activity (Higuchi et al. 2008, 2012). Thus, both high temperature and high H_2O_2 affect the metabolic and antioxidant activities of corals.

Strong UV radiation is a direct stress on the coral symbiotic system (Gleason and Wellington 1993). For many coral reefs, the overlying water column allows UV radiation to penetrate to depths of 20 m or deeper because oligotrophic waters are dominated by the optical properties of the water itself and not by dissolved or particulate constituents in the water column (Banaszak and Lesser 2009). Higuchi et al. (2010) reported photochemical production of hydroxyl radical (•OH) in extracts of both coral hosts and endosymbiotic zooxanthellae. Their results indicated that, whether or not coral bleaching occurred, coral tissues and symbiotic zooxanthellae had the potential to produce •OH photochemically under exposure to sunlight. Moreover, reducing the amount of UV radiation reduced •OH formation rates by 40%, indicating that UV radiation strongly affects •OH formation in coral tissue and zooxanthellae in addition to its formation through photochemical processes. Gleason and Wellington (1993) showed that reef-building corals neutralize the damaging effects of UV light through the synthesis or accumulation of UV-absorbing compounds, known as mycosporine-like amino acids (MAAs). Shick et al. (1999) indicated that MAAs originate from the algae in algal-invertebrate symbioses during a bleaching event. As more zooxanthellae are lost from coral tissue, more UV radiation can penetrate into the corals, enhancing the formation of •OH in the coral tissues and causing more damage. On the other hand, the whole genome sequence of *Acropora digitifera* indicated the presence of homologues encoding all four shinorines, a group of MAAs. Thus, MAA synthesis in corals is not symbiont dependent (Shinzato et al. 2011). Yakovleva and Hidaka (2009) reported that MAA concentration and composition varied among color morphotypes of the coral *Galaxea fascicularis*. Yakovleva et al. (2004) proposed that mycosporine-glycine, an MAA, also plays a role in providing rapid protection against oxidative stress, functioning as a biological antioxidant in the host coral and zooxanthellae under thermal stress.

Responses of host corals to stressors can vary depending on the types of zooxanthellae associated with them. Using monoclonal cultures of *Symbiodinium* clades, Yuyama et al. (2012a) observed changes in the expression of stress marker genes in the coral depending on the symbiont type. The expression levels of four genes, encoding green and red fluorescent proteins, an oxidative stress-responsive protein, and an ascorbic acid transporter, decreased at high temperatures when juvenile corals were associated with clade A zooxanthellae but increased when the zooxanthellae were in clade D. Yuyama et al. (2016) reported that juvenile corals hosting *Symbiodinium* clade C showed a lower survival rate than those with clade D symbionts during thermal stress, although photosynthetic efficiency (F_v/F_m) was lower at 30 °C than at 25 °C in both clades. In addition, after 3 h of strong light exposure (500 μmol m^{-2} s^{-1}), corals containing clade C1 showed a greater decline in F_v/F_m (-74%) than corals associated with clade D (-50%), indicating that an association with clade D leads to a greater tolerance to strong light than with clade C (Yuyama et al. 2016).

Under natural conditions, a single source of stress, such as elevated water temperature, rarely occurs in isolation, and instead multiple stressors often occur simultaneously. While most studies have focused on responses to individual stressors, the synergistic interactions between increased temperature and other factors have also been examined, including UV radiation (Lesser et al. 1990), salinity (Porter et al. 1999), copper (Nyström et al. 2001), and nitrate (Nordemar et al. 2003). Researchers in Japan have investigated the combined effects of multiple stresses on corals for better elucidation of the impacts of environmental changes on Japanese coral reefs (Higuchi et al. 2009a, 2013; Tanaka et al. 2014). Higuchi et al. (2013) reported the effects of high seawater temperature and bacterial inoculation (a mixture of five potential coral pathogens naturally found in coastal regions of Okinawa, Japan) on coral bleaching, metabolism, and antioxidant defenses in *Montipora digitata*. At ambient temperature (27 °C), the added bacteria did not cause coral bleaching and did not affect metabolism or antioxidant

enzyme activities. However, bacterial inoculations in addition to high-temperature stress (32 °C) resulted in severe coral bleaching, decreased photosynthetic efficiency (F_v/F_m), and decreased photosynthesis and calcification rates. Corals under thermal stress alone showed signs of bleaching, but the changes in zooxanthella density and metabolic rates were less severe than for those under combined thermal stress and bacterial challenge. Among the bacteria examined, the sulfite-oxidizing bacteria *Sulfitobacter* sp. had the greatest capacity to enhance and accelerate the bleaching process under thermal stress. *Sulfitobacter* sp. was isolated from the water surrounding bleached *M. digitata*. These results suggest that bacterial challenge and thermal stress can have synergistic negative effects on living corals (Higuchi et al. 2013). As shown above, high concentrations of nitrate caused by agricultural fertilizer runoff (>10 μM) have been measured in coastal areas (Umezawa et al. 2002; Higuchi et al. 2014). Higuchi et al. (2015b) reported the combined effects of elevated nitrate concentrations with physical stresses (temperature and light intensity) on coral bleaching and antioxidant enzyme activity. Two nitrate concentration levels (<1, 10 μM) were tested along with two temperature conditions (27, 32 °C) and two levels of photon intensity (200, 600 μmol m^{-2} s^{-1}). The density of zooxanthellae did not significantly change when subjected to only high nitrate or only high light. On the other hand, the combined stresses of high light or high temperature with high nitrate significantly decreased zooxanthella density. Moreover, there was a negative correlation between zooxanthella density and activities of the antioxidant enzymes SOD and CAT, leading to the conclusion that the observed coral bleaching occurred due to high ROS levels (Higuchi et al. 2015b). High nitrate concentration also affects the resilience of the zooxanthella-coral relationship at high temperatures (Chumun et al. 2013). Taken together, these results suggested that both high temperature and high light intensity affected coral bleaching when nitrate concentrations were high, but their combined effect cannot be estimated by simply summing the effects of the individual stressors.

3.3 Studies on Coral Bleaching in Japan

Massive coral bleaching incidents have recently increased around the world due to changes in the environment with both natural and anthropogenic causes. The main causes of coral bleaching are believed to be high seawater temperatures, high irradiance, and strong UV radiation (Fitt et al. 2001). High seawater temperature disrupts the symbiotic association between the animal host and the symbiotic algae, leading to the bleaching of the coral. During the 1997–1998 El Niño, sea surface temperatures rose well above normal, and many tropical coral reefs were bleached or experienced high mortality (Glynn et al. 2001). In the past, several bleaching events have been reported on Japanese coral reefs (e.g., 1980, 1983, 1987, 1991, 1993, 1994, 1998, 2001, 2003, 2005, 2007, 2010, 2013; Yamazato 1999; Yara et al. 2009; Muko et al. 2013; Harii et al. 2014). Uniquely, Japanese divers and researchers used the Sango (coral) Map Project (Namizaki et al. 2013), a citizen science project, to collect bleaching information in 2013. The most severe bleaching event in Japan occurred in 1998 (Fig. 3.3), when many corals were bleached and died in Okinawa (Yamazato 1999). Bleaching was first observed in early July, peaked in August and September, and ceased in early October (Hasegawa et al. 1999, Yamazato 1999). Seawater temperature in 1998 was 1–2 °C higher than in normal years (Kayanne et al. 2002). Harii et al. (2014) showed that a shift in dominant coral taxa, from branching *Montipora* and *Acropora* to *Porites* and *Heliopora*, resulted from multiple disturbances, such as the bleaching events in 1998 and 2007, physical damage from strong typhoons, and inputs of sediment after heavy rain.

As observed in mesocosm experiments, coral bleaching severely affects carbon metabolism including photosynthesis and calcification (Fujimura et al. 2001). While Takahashi et al. (2004) showed that photosynthesis in zooxanthellae is very sensitive to water temperature and is impaired when temperature is high, the exact mechanism of the loss of zooxanthellae during bleaching remains unclear. In one thermal stress experiment, a large number of *Symbiodinium* were expelled, and this loss from coral tissues led to coral bleaching (Fujise et al. 2013). However, in another investigation of corals during a bleaching event, degraded zooxanthellae were found in high numbers in the coral tissue (Mise and Hidaka 2003). Fujise et al. (2014) proposed that the expulsion mechanisms differed depending on temperature. Under ambient temperature conditions, the expulsion of *Symbiodinium* was undertaken as part of a regulatory mechanism to maintain constant zooxanthella density. However, at 30 °C (moderate thermal stress), *Symbiodinium* were damaged, and corals selectively digested the damaged cells or expelled them without digestion. Under high-temperature (32 °C) stress, the abundance of healthy zooxanthellae in coral tissues decreased, the proportion of abnormal cells increased, and the rate of zooxanthella expulsion was reduced (Suzuki et al. 2015). Within the shrunken cells, a chlorophyll-like pigment, 13^2, 17^3-cyclopheophorbide a enol (cPPB-aE), which is not ordinarily found in healthy zooxanthellae, was detected. The presence of cPPB-aE in zooxanthellae suggests that chlorophyll was degraded to cPPB-aE, a compound that is not fluorescent. The lack of fluorescence reduced the rate of ROS formation in zooxanthellae. Therefore, it has been suggested that the formation of cPPB-aE in zooxanthellae is a defense mechanism by the host coral to avoid oxidative stress (Suzuki et al. 2015).
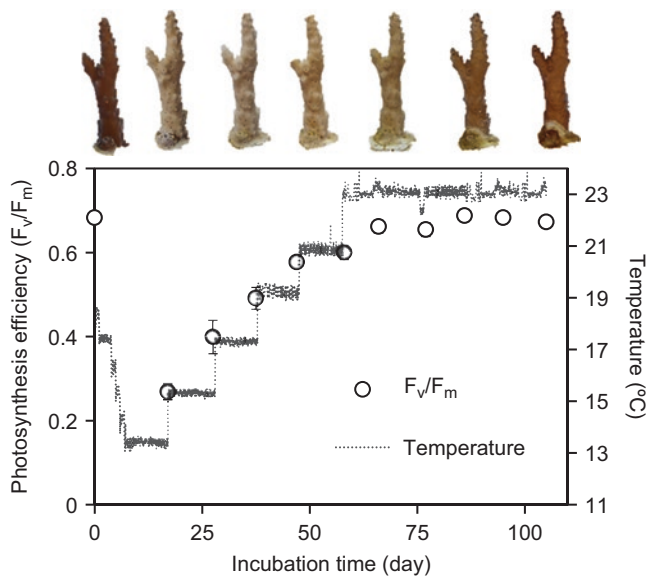
Fig. 3.4 Variation in the photosynthetic quantum yield F_v/F_m and photograph of *Acropora pruinosa* under cold stress and recovery process (Modified from Higuchi et al. 2015a)

On the other end of the temperature range experienced by corals in Japan, Nakamura et al. (2004) investigated the effect of cold stress on the coral *Acropora pruinosa* found in the warm temperate zone of Japan and found that photosynthesis and respiration were reduced under low-temperature conditions. Higuchi et al. (2015a) also reported that acroporid corals incubated at low temperature (13 °C) showed serious bleaching symptoms (Fig. 3.4). Photosynthetic efficiency, respiration, calcification, and activities of the antioxidant enzymes SOD and CAT all decreased at low temperature. Decreases in photosynthesis and calcification were also observed with high-temperature bleaching stress, but the decreases in respiration and antioxidant enzyme activities were the opposite of the responses to heat stress. *A. pruinosa*,

which is distributed up to the northernmost limit for hermatypic corals in the world, did not die after 10 days under cold stress and was able to recover when temperature was increased (Fig. 3.4). Cold stress bleaching might be a result of the disposal of zooxanthellae that lost their photosynthetic function due to low temperature. Cold stress bleaching could also serve to limit the energy consumption of zooxanthellae with low photosynthetic efficiency. We suggested that cold bleaching is an important mechanism for *A. pruinosa* to survive low winter temperatures (Higuchi et al. 2015a).

The effect of water flow on the response of corals to high-temperature stress and the bleaching mechanism has been extensively studied in Japan (Nakamura 2010). Limited water flow lowers the temperature threshold for inhibition (Nakamura and Yamasaki 2013), leading to enhanced bleaching and increased damage to zooxanthella photosystems. On the other hand, high water flow allows faster recovery from bleaching in terms of chlorophyll concentration and zooxanthella density and a higher survival rate for colonies exposed to high temperature, both experimentally and in the field (Nakamura and van Woesik 2001; Nakamura et al. 2005).

In a global study, Downs et al. (2002) suggested that bleaching is the final defense of corals against oxidative stress. Several studies in Japan have investigated this bleaching mechanism under high-temperature conditions (Yakovleva et al. 2004; Higuchi et al. 2008; etc.). If antioxidant activity is insufficient, the final defense mechanism (i.e., bleaching) might be activated. However, bleaching in cold conditions might not be due to oxidative stress (Higuchi et al. 2015a) and was not triggered by the addition of a high concentration of H_2O_2 (Higuchi et al. 2008). It may be that because the oxidative stress was not caused by zooxanthellae, corals did not expel them (Fig. 3.5). Furthermore, coral bleaching was inhibited by simultaneous H_2O_2 and high-temperature stresses (Higuchi et al. 2012). It is likely that the bleaching mechanism was not triggered because the high

Fig. 3.5 Reactive oxygen species in zooxanthellae (zoox) and host tissue (**a**) under high-temperature stress and (**b**) under H_2O_2 stress. Both conditions cause oxidative stresses in coral cells and damage to metabolic system. However, under H_2O_2 stress only, coral does not expel zoox
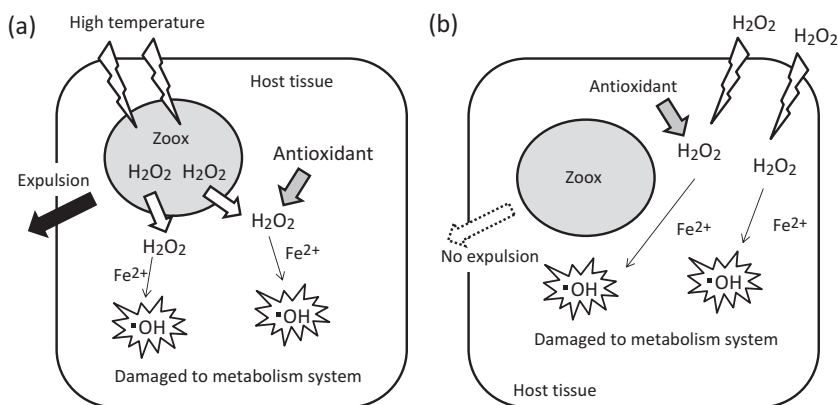
H_2O_2 concentration was in the coral cytosol during H_2O_2 exposure, rather than by the zooxanthellae.

3.4   Future Directions

As discussed above, many studies on the effects of environmental stresses on corals have been conducted in Japan. The response to each stressor has been tested quantitatively, and this information will help to determine conservation standards for coral reefs in Japan. However, stress response studies were mainly conducted under manipulated conditions in the laboratory, and more field-based quantitative research is still required. Coral bleaching was studied under both hot- and cold-temperature stresses. In Japan, Yara et al. (2009) suggested that both the frequency and the areal extent of coral bleaching around the Ryukyu Islands are expected to increase in the second half of the twenty-first century. Moreover, as multiple stressors enhance the bleaching of corals in a synergistic manner (Higuchi et al. 2013, 2015b), we need to account not only for global warming but also for local stresses such as nutrient discharge in reef assessments. These stresses should be avoided to mitigate environmental impacts on coral reefs.

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