

Influence of salinity and sedimentation on *Vibrio* infection of the Hawaiian coral *Montipora capitata*

A. Shore-Maggio^{1,*}, G. S. Aeby², S. M. Callahan³

¹Institute of Marine and Environmental Technology, Baltimore, Maryland 21202, USA

²Hawaii Institute of Marine Biology, Kaneohe, Hawaii 96744, USA

³University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA

ABSTRACT: Environmental cofactors alter host–pathogen interactions and influence disease dynamics by impairing host resistance and/or increasing pathogen virulence. Terrestrial runoff is recognized as a major threat to coral reef health. However, the direct links between runoff and coral disease are not clear. *Montipora* white syndrome (MWS) is a coral disease that occurs in the Hawaiian archipelago, can be caused by various bacterial pathogens, including *Vibrio* species, and is linked to conditions associated with heavy rainfall and runoff. The objective of this study was to determine whether a short-term hyposalinity stress (20 ppt for 24 h) or sedimentation stress (1000 g m⁻² d⁻¹) would influence bacterial infection of the coral *Montipora capitata*. Hyposalinity increased *M. capitata* susceptibility to infection by 2 MWS pathogens, *Vibrio coralliilyticus* strain OCN008 and *Vibrio owensii* strain OCN002. Specifically, hyposalinity allowed OCN008 to infect at lower doses (10⁶ CFU ml⁻¹ compared with 10⁸ CFU ml⁻¹) and reduced the amount of time before onset of OCN002 infection at high doses (10⁸ CFU ml⁻¹). In contrast, short-term sedimentation stress did not affect *M. capitata* infection by either of these 2 pathogens. Although several studies have found a correlation between runoff and increased coral disease prevalence in field studies, this is the first study to show that one aspect of runoff (reduced salinity) enhances bacterial infection of coral using manipulative experiments.

KEY WORDS: Coral disease · *Vibrio* · Environmental stress · Runoff · Salinity · Kaneohe Bay

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Disease is a global threat to marine ecosystems and is a main contributor to declines in coral populations worldwide (Peters 2015), prompting a need to better understand disease dynamics in coral reef ecosystems. Disease in coral, like in humans, results from a complex interaction between host resistance and pathogen virulence, which can be altered by environmental cofactors. Environmental cofactors alter host–pathogen interactions and influence disease dynamics in 2 fundamental ways. Environmental cofactors affect basic physiological properties of hosts, altering their ability to fight infection, and pathogens may experience changes in abundance or virulence in response

to environmental cues (Keane & Kerr 1997). Investigating changes in host–pathogen interactions due to environmental cofactors is particularly challenging in coral disease systems; the coral holobiont is complex (symbiosis with photosynthetic *Symbiodinium* and a diverse community of microorganisms), several environmental cofactors may act synergistically, and coral diseases have varying etiologies and ecologies (Harvell et al. 2007). Despite these challenges, understanding the effects of environmental cofactors on coral disease dynamics is particularly important given the ability of human activities to cause environmental change on local and global scales.

Many environmental parameters affect coral physiology such as temperature, irradiance, eutrophica-

*Corresponding author: asmaggio@umbc.edu

tion, sedimentation, and freshwater input (Rogers 1990, Bruno et al. 2003, 2007, Fabricius 2005, Harvell et al. 2007). Increased sea surface temperature is well studied in connection with coral disease processes. Many coral disease outbreaks are linked to thermal stress (Bruno et al. 2007, Miller & Richardson 2014), warm temperatures dampen certain coral immune responses, such as antimicrobial activities (Ritchie 2006, Mydlarz et al. 2009), and some coral pathogens have enhanced virulence during higher temperatures (Banin et al. 2003, Ushijima et al. 2016). Terrestrial runoff is also recognized as a major threat to coral reef health, and the effects of eutrophication, decreased salinity, and sedimentation from terrestrial runoff on coral ecology and coral reef distribution are well characterized (reviewed in Fabricius 2005). In relation to coral disease, terrestrial runoff has been correlated with higher disease prevalence (Haapkylä et al. 2011), eutrophication increases coral disease severity (Bruno et al. 2003, Voss & Richardson 2006a), and terrestrial runoff is hypothesized to be a source of some coral pathogens, such as *Aspergillus sydowii* (etiologic agent of Aspergillosis in sea fans) (Smith et al. 1996).

Although many environmental cofactors are associated with terrestrial runoff, both salinity and sedimentation are known to have large impacts both on the distribution and structure of coral reef ecosystems and directly impact the physiology of the coral animal. There are fewer coral reefs near outlets of major rivers (Birkeland 1997), growth and reproduction are reduced in corals near sources of freshwater (Lirman et al. 2003), and severe episodes of reduced salinity can cause mass mortality on coral reefs (Bahr et al. 2015a). Changes in salinity may impact disease dynamics by disrupting cellular processes in the coral host (Muthiga & Szmant 1987, Downs & Kramarsky-Winter 2009), disrupting symbiosis with *Symbiodinium* (Manzello & Lirman 2003), or by altering associated microbial communities (Röthig et al. 2016). Sedimentation has similar influences on coral distribution, cover, and growth (Rogers 1990, Fabricius 2005). Sedimentation has also been correlated with coral disease prevalence (Voss & Richardson 2006a,b, Pollock et al. 2014, Sheridan et al. 2014a) and may influence disease processes by inducing direct damage, altering associated microbial communities (Hodgson 1990, Weber et al. 2012), decreasing energy resources (hypothesized to decrease resistance to infection) (Abdel-Salam & Porter 1988), or serving as an abiotic reservoir or vector of coral pathogens (Voss & Richardson 2006b, Sheridan et al. 2014a).

In recent years, *Montipora* white syndrome (MWS) has become a coral disease of concern for Hawaiian coral reefs (Friedlander et al. 2008). MWS is a progressive tissue loss disease that primarily affects *Montipora capitata*, an abundant reef-building coral in Kaneohe Bay, Oahu, USA. MWS is transmissible by direct contact and can present as a chronic, sub-acute lesion, causing tissue loss at a rate of $\sim 3\% \text{ mo}^{-1}$, or as an acute lesion, causing tissue loss at a rate of $\sim 24\% \text{ mo}^{-1}$ (Aeby et al. 2010, 2016). The southern region of Kaneohe Bay, where water circulation is more restricted and has the longest residence time (Bathen 1968, Drupp et al. 2011), has the highest disease levels during outbreak (Aeby et al. 2016) and non-outbreak (Aeby et al. 2010) time periods. Therefore, it was hypothesized that a cofactor associated with terrestrial runoff, such as sedimentation or decreased salinity, may influence MWS disease dynamics. Under laboratory conditions, 2 species of *Vibrio* have been identified as MWS pathogens, causing progressive tissue loss in *M. capitata*. *Vibrio owensii* strain OCN002 causes diffuse tissue loss (Ushijima et al. 2012) and *Vibrio coralliilyticus* strain OCN008 causes acute tissue loss (Ushijima et al. 2014). The objective of this study was to test whether a short-term hyposalinity or sedimentation stress could increase the susceptibility of *M. capitata* to bacterial infection. Specifically, we measured whether hyposalinity or sediment stress could decrease the time or the dosage needed for a MWS pathogen to infect *M. capitata*.

MATERIALS AND METHODS

Salinity stress

The average salinity of Kaneohe Bay during non-rainfall conditions is approximately 35 parts per thousand (ppt) (Bathen 1968). Kaneohe Bay has runoff originating from nearby streams (Cox et al. 2006, Ostrander et al. 2008) that discharge at low flow rates with sporadic intense runoff associated with rainstorms during the winter (Ostrander et al. 2008, Drupp et al. 2011). During rainfall events, salinity can decrease to 20 ppt (or even as low as 15 ppt) over a range of 12 h to 5 d (Ostrander et al. 2008). Based on these patterns of salinity changes in Kaneohe Bay, a reduction to 20 ppt for 24 h was chosen as the salinity stress to conduct infection trials with MWS pathogens. Preliminary experiments determined that this exposure did not induce any gross signs of bleaching or tissue loss in *Montipora capitata* fragments.

Salinity in each aquarium was changed by removing a set volume of seawater and replacing with an equal volume of 0.2 μm filtered, distilled freshwater to lower salinity or by replacing with an equal volume with 0.2 μm filtered 35 ppt seawater to raise salinity. Salinity was reduced or raised at a rate of 3 ppt h^{-1} until the desired salinity was reached, and was monitored using a Sper Scientific salinity/conductivity pen. Salinity changes due to evaporation were prevented by lids on each aquarium, and aeration was provided constantly to prevent haloclines.

Sedimentation stress

The sedimentation rate in Kaneohe Bay during non-rainfall conditions is less than 10 $\text{g m}^{-2} \text{d}^{-1}$ (Kinzie et al. 2001, Te 2001). Sedimentation rates during heavy rain events can range from 40 $\text{g m}^{-2} \text{d}^{-1}$ (Kinzie et al. 2001) to 35 000 $\text{g m}^{-2} \text{d}^{-1}$ (Maragos 1972), and the majority of the sediment settles in less than 1 d (Te 2001). In the southern region of Kaneohe Bay, the yearly average sedimentation rate is approximately 1000 $\text{g m}^{-2} \text{d}^{-1}$ (Maragos 1972). Therefore, a loading rate of 1000 $\text{g m}^{-2} \text{d}^{-1}$ was chosen as the sediment stress to conduct infection trials with MWS pathogens. *M. capitata* can tolerate a 100 $\text{g m}^{-2} \text{d}^{-1}$ sediment stress without signs of tissue loss, even when applied repetitively over multiple days (Te 2001), and preliminary experiments determined that exposure at 1000 $\text{g m}^{-2} \text{d}^{-1}$ did not induce any gross signs of bleaching or tissue loss in *M. capitata* fragments.

The amount of sediment to be placed on each fragment was scaled for the surface area of each coral fragment, which was estimated by photometric analysis (Image J software, v1.46 NIH). The perimeter of each coral fragment was digitally outlined and the encircled area calculated in cm^2 . Digital measurements were conducted in triplicate for each photograph and the averages were used for subsequent calculations. Sediments used in experiments were collected from the top 5 cm of sediment from the same fringing reef where coral fragments were collected. Sediments were drained of excess seawater, dried for 48 h at 60°C, and then aliquoted into glass vials (with the appropriate mass of sediment for each coral fragment) before sterilization via autoclaving. Sterilization was conducted to remove any potential pathogens that may have been present in reef sediments. This allowed for accurate dosage of MWS pathogens and prevented the introduction of other unknown potential coral pathogens. Sediments were applied directly onto the coral surface, using sterile serological pipettes.

Infection trial experimental design

Set up for infection trials was conducted similarly as previously described (Ushijima et al. 2014). Each infection trial utilized a block design in which replicate fragments from the same colony were exposed to the environmental stress (stressed group) or kept at ambient conditions (non-stressed group) and were subsequently exposed to 0.2 μm filtered seawater (FSW) only or to an MWS pathogen (OCN008 or OCN002) at one of 3 different dosages (expressed in colony forming units per ml of FSW, or CFU ml^{-1}). Non-pathogen (FSW only) exposed fragments were included in the block design to show that tissue loss was due to bacterial infection rather than possible stress created by laboratory conditions (light, temperature, water motion, water quality). The 3 dosages chosen for inoculation were high (10^8 CFU ml^{-1}), intermediate (10^6 CFU ml^{-1}), and low (10^4 CFU ml^{-1}). The highest dose (10^8 CFU ml^{-1}) was previously used to confirm infectivity of OCN008 (Ushijima et al. 2014). For each infection trial, 8 fragments from 12 *M. capitata* colonies were collected from fringing reefs off Moku O Loe, allowed to acclimate for 2 d in an outdoor, flow-through seawater table, and then placed in individual 4 l aquaria. Each block of individual aquaria was maintained in a larger, freshwater-filled secondary container that was fitted with aquarium heaters to maintain temperature at 27°C, the temperature used to determine infectivity of OCN008 and near the mean summer seawater temperature in Kaneohe Bay (27.4°C, 2003–2012; <http://tidesandcurrents.noaa.gov/>). Water pumps and temperature loggers were placed at both ends of both control and treatment water tables to ensure even heat distribution and monitor seawater temperature. For salinity stress infection trials, salinity was reduced to 20 ppt for 24 h (as described above), and then all coral fragments were inoculated with a MWS pathogen within 1 h of the stressed group returning back to ambient salinity (35 ppt). For sediment stress infection trials, coral fragments from the stressed group were moved from individual aquaria to 6 l glass aquaria (1 aquarium per block) to apply sediments (as described above). Sediments were applied in 3 dosages every 3 h to reach a total dose of 1000 g m^{-2} . After 24 h, coral fragments were cleared of remaining sediments and then moved back to individual aquaria. All coral fragments were inoculated with a MWS pathogen within 1 h of the stressed group returning back to individual aquaria. Infection trials with salinity stress or with sediment stress were conducted individually for both OCN008 and OCN002.

To prepare inocula for the infection trials, an overnight culture of the MWS pathogen (OCN008 or OCN002) was inoculated into fresh glycerol artificial seawater (GASW) medium and incubated at 27°C. Cultures were grown to an optical density of 0.8 (at 600 nm) and were then centrifuged at $3220 \times g$ for 15 min. The supernatant was removed and then the bacterial pellet was resuspended in FSW. High dose aquaria received this standard inocula, and serial 10-fold dilutions of the standard inocula were used to inoculate the intermediate and low doses. To confirm the dosage, inocula were diluted in FSW and plated onto GASW media. The final concentrations of inocula were determined to be 1.3×10^8 CFU ml⁻¹, 1.1×10^6 and 2.4×10^4 CFU ml⁻¹ for high, intermediate, and low doses, respectively. Coral fragments were monitored daily and water quality maintained with partial seawater changes every 3 d. Infection trials using OCN008 were run for a maximum of 25 d, whereas infection trials using OCN002 were run for a maximum of 35 d, as previously described (Ushijima et al. 2012, 2014).

Infection trial data were analyzed using the Mantel-Cox log-rank test, a non-parametric method used for nominal data. The number of days to infection

between stressed and non-stressed groups is presented as mean (\pm SEM) and was compared using a non-parametric Mann-Whitney *U* test.

RESULTS

Salinity stress

Hyposalinity stress did not reduce time to infection but allowed *Vibrio coralliilyticus* strain OCN008 to infect at a lower dose. OCN008 is capable of infecting *Montipora capitata* in less than 5 d at a concentration of 10^8 CFU ml⁻¹ (Ushijima et al. 2014). The high dose (10^8 CFU ml⁻¹) infected 9 out of 12 (75%) fragments in both non-stressed and stressed groups after an average of 2.7 ± 0.4 d and 2.5 ± 0.4 d, respectively (Mann-Whitney: $W = 97.5$, $p = 0.309$; Fig. 1A). The intermediate dose (10^6 CFU ml⁻¹) infected 7 out of 12 (58%) stressed fragments after an average of 16.1 ± 0.7 d but did not infect any fragments in the non-stressed group (Mantel-Cox log-rank test: $\chi^2 = 9.604$, $df = 1$, $p = 0.001$; Fig. 1A). The low dose (10^4 CFU ml⁻¹) failed to infect coral fragments in either the stressed or non-stressed groups and no

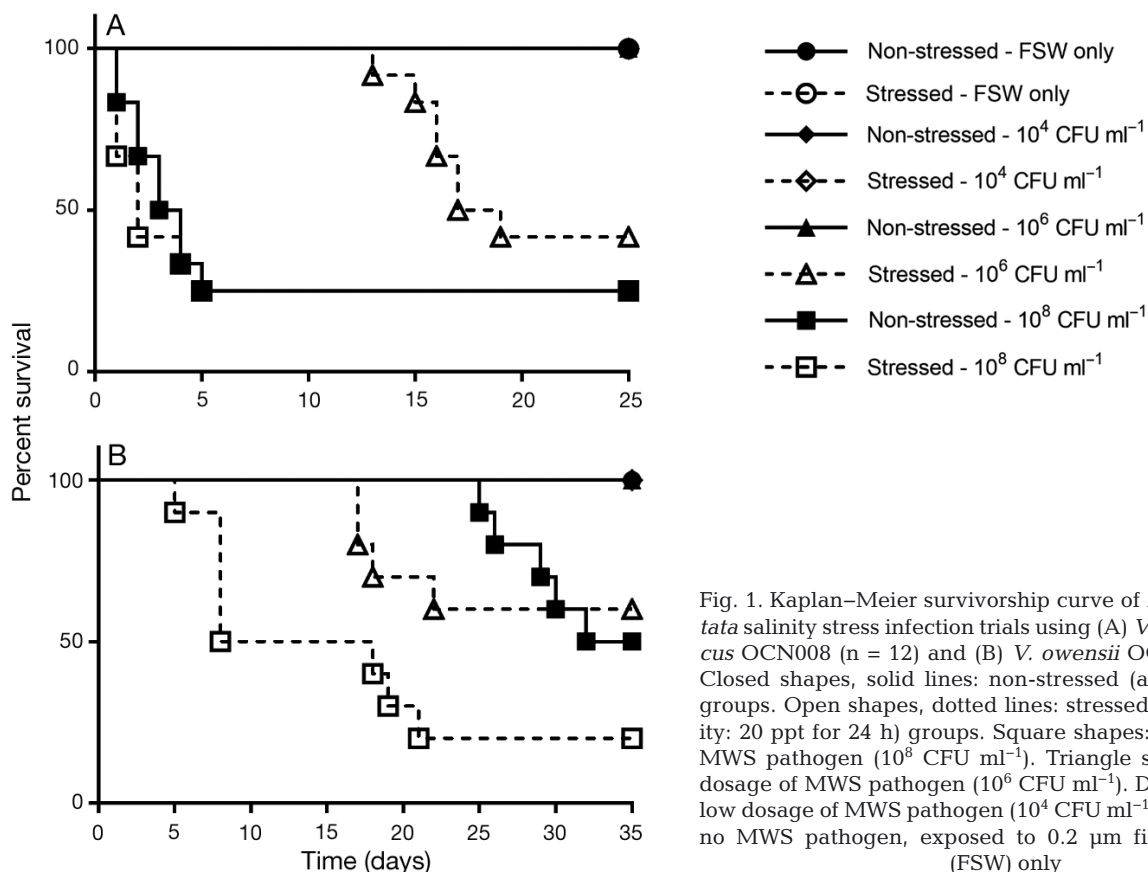


Fig. 1. Kaplan–Meier survivorship curve of *Montipora capitata* salinity stress infection trials using (A) *Vibrio coralliilyticus* OCN008 ($n = 12$) and (B) *V. owensii* OCN002 ($n = 10$). Closed shapes, solid lines: non-stressed (ambient salinity) groups. Open shapes, dotted lines: stressed (reduced salinity: 20 ppt for 24 h) groups. Square shapes: high dosage of MWS pathogen (10^8 CFU ml⁻¹). Triangle shapes: medium dosage of MWS pathogen (10^6 CFU ml⁻¹). Diamond shapes: low dosage of MWS pathogen (10^4 CFU ml⁻¹). Circle shapes: no MWS pathogen, exposed to 0.2 μ m filtered seawater (FSW) only

fragments exposed to FSW only developed tissue loss over the course of the infection trial (Fig. 1A).

Hyposalinity stress decreased the time to infection at the high dose but did not allow *Vibrio owensii* strain OCN002 to infect at a lower dose. OCN002 is capable of infecting *M. capitata* after an average of 28 d at a concentration of 6×10^6 CFU ml⁻¹ (Ushijima et al. 2012). The high dose infected 8 out of 10 (80%) stressed fragments at an average of 11.9 ± 2.2 d and 5 out of 10 (50%) non-stressed fragments at an average of 28.4 ± 1.3 d (Mantel-Cox log rank test; $\chi^2 = 5.595$, df = 1, p = 0.018; Fig. 1B), demonstrating a significant reduction in the average number of days to infection (Mann-Whitney: $W = 95.0$, p = 0.004). The intermediate dose infected 4 out of 10 (40%) stressed fragments after an average of 17.5 ± 0.8 d but did not infect any fragments in the non-stressed group (Mantel-Cox log rank test; $\chi^2 = 4.773$, df = 1, p = 0.028; Fig. 1B). The low dose failed to infect any fragments in either the stressed or non-stressed groups, and no fragments exposed to FSW only developed tissue loss over the course of the infection trial (Fig. 1B). Two blocks in this infection trial were removed from analysis, hence n = 10, because electricity was lost to the water heaters and pumps 4 d post-inoculation, causing the temperature to drop to 24°C.

Sedimentation stress

Sediment stress did not reduce time to infection or allow OCN008 to infect at a lower dose. The high dose infected 8 out of 12 (66%) stressed fragments after an average of 2.3 ± 0.6 d and 10 out of 12 (83%) of non-stressed fragments after an average of 1.8 ± 0.3 d (Mann-Whitney: $W = 87.5$, p = 0.534; Fig. 2A). The intermediate dose and the lowest dose failed to infect any coral fragments in either the stressed or non-stressed groups (Fig. 2A). None of the fragments in either the stressed or non-stressed group exposed to FSW only developed tissue loss over the course of the infection trial (Fig. 2A).

Sediment stress did not allow OCN002 to infect at a lower dose nor did it lower the time to infection. The highest dose infected 7 out of 12 (58%) stressed fragments after an average of 23.8 ± 1.4 d and 6 out of 12 (50%) of non-stressed fragments after an average of 27.2 ± 1.6 d (Mantel-Cox log-rank test; $\chi^2 = 1.450$, p = 0.228; Fig. 2B). The intermediate dose and the lowest dose failed to infect any coral fragments in either the stressed or non-stressed groups (Fig. 2B). None of the fragments in either the stressed or non-stressed group exposed

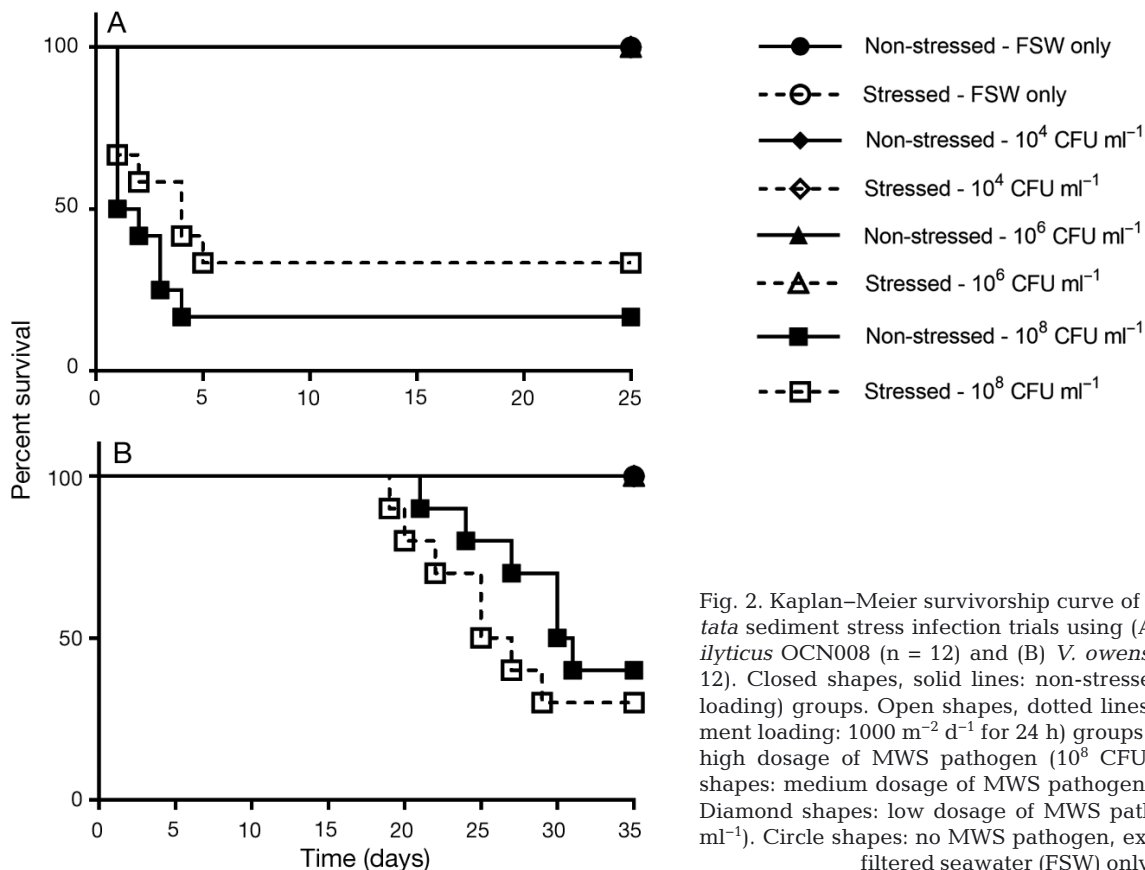


Fig. 2. Kaplan–Meier survivorship curve of *Montipora capitata* sediment stress infection trials using (A) *Vibrio corallilyticus* OCN008 (n = 12) and (B) *V. owensii* OCN002 (n = 12). Closed shapes, solid lines: non-stressed (no sediment loading) groups. Open shapes, dotted lines: stressed (sediment loading: $1000 \text{ m}^{-2} \text{ d}^{-1}$ for 24 h) groups. Square shapes: high dosage of MWS pathogen (10^8 CFU ml⁻¹). Triangle shapes: medium dosage of MWS pathogen (10^6 CFU ml⁻¹). Diamond shapes: low dosage of MWS pathogen (10^4 CFU ml⁻¹). Circle shapes: no MWS pathogen, exposed to $0.2 \mu\text{m}$ filtered seawater (FSW) only

to seawater only developed tissue loss over the course of the experiment (Fig. 2B).

All tissue loss observed in infection trials using OCN008 inocula had a distinct lesion front that progressed rapidly, consistent with the pattern of tissue loss originally established for OCN008 (Ushijima et al. 2014). All tissue loss observed in infection trials using OCN002 inocula presented as diffuse tissue loss, some of which developed a progressive lesion front, consistent with the pattern of tissue loss originally established for OCN002 (Ushijima et al. 2012).

DISCUSSION

Short-term hyposalinity stress increased the susceptibility of *Montipora capitata* to infection by 2 coral pathogens, *Vibrio coralliilyticus* strain OCN008 and *V. owensii* strain OCN002. We show that reduced salinity decreased the infectious dose of OCN008 by 2 orders of magnitude (10^6 CFU ml⁻¹ compared with 10^8 CFU ml⁻¹). Although we found a significant difference in OCN002 infection rates for stressed and non-stressed corals at the intermediate dose (1.1×10^6 CFU ml⁻¹), OCN002 is capable of infecting *M. capitata* at a concentration of 6.0×10^6 CFU ml⁻¹ under normal salinity conditions (Ushijima et al. 2012). Therefore, hyposalinity stress did not reduce the minimum infectious dose for this pathogen. Reduced salinity did significantly reduce the amount of time before onset of OCN002 infection at the high dose. In contrast, short-term sediment stress did not affect the susceptibility of *M. capitata* to infection by either of these 2 pathogens. Although several studies have found a correlation between runoff or rain events and increased coral disease prevalence or disease severity (Voss & Richardson 2006a, Haapkylä et al. 2011, Pollock et al. 2014, Sheridan et al. 2014a), this is the first study to test 2 aspects of runoff (reduced salinity and sediment loading) on a coral's susceptibility to bacterial infection using manipulative experiments.

Environmental stress can increase the risk of disease either through the reduction of host defenses or the creation of a favorable environment for pathogen proliferation and invasion, or both. Because MWS pathogens were not exposed to the stress conditions, virulence is assumed to be similar between stressed and non-stressed groups. Therefore, it is probable that the effects of hyposalinity on host resistance were responsible for the change in disease susceptibility we found in *M. capitata*. Salinity stress could lower coral resistance to bacterial infection in a number of ways. First, cellular damage can occur during salinity

stress in corals. Corals are osmoconformers, rapidly absorbing water to become iso-osmotic with their surroundings (Rankin & Davenport 1981), which can cause tissue swelling, tissue necrosis, and the induction of an oxidative-stress response that is visible with histology in some species (Downs & Kramarsky-Winter 2009). Hyposalinity can also disrupt symbiosis with *Symbiodinium* (Muthiga & Szmant 1987, Coles & Jokiel 1992, Kerswell & Jones 2003), leading to the production of damaging reactive oxygen species that begin apoptotic and/or necrotic pathways (Tchernov et al. 2011). Damage at the cellular level from these processes could have aided bacterial invasion of tissues. Second, hyposalinity could impair immune responses. Hemocyte function, phagocytosis, and/or prophenyloxidase activity is reduced in other marine invertebrates when exposed to reduced salinity (Cheng et al. 2004, Wang et al. 2008, Li et al. 2010). To date, no studies have investigated what aspects of coral immunity are affected by hyposalinity. Finally, hyposalinity could disrupt symbiosis with associated microbial communities, considered the first line of defense for corals (Shnit-Orland & Kushmaro 2009). Dysbiosis of coral-associated microbial communities occurs in response to many environmental stressors, including hypersalinity (Vega Thurber et al. 2009, Röthig et al. 2016), and microbial dysbiosis is generally thought to increase disease susceptibility in many marine animals, including corals (Egan & Gardiner 2016). Therefore, changes in microbial communities due to hyposalinity stress may have aided bacterial infection of *M. capitata*. Even though the corals exposed to salinity stress appeared healthy macroscopically, we did not measure heat-shock proteins or photoefficiency, indicators of stress to the coral host and *Symbiodinium*, respectively (Abrego et al. 2008, Seveso et al. 2013). Nor did we conduct any measurements of immune response or microbial dysbiosis. Future studies will be needed to address the mechanism by which *M. capitata* susceptibility to MWS pathogens increased with hyposalinity stress.

Increased disease susceptibility in response to hyposalinity stress may help explain patterns of MWS prevalence across Kaneohe Bay, which is roughly divided into 3 sections (northern, central, and southern). MWS is found within all 3 regions, but the southern region displays a significantly higher MWS prevalence before and during outbreak conditions (Aeby et al. 2010, 2016). Even though the greatest volume of runoff enters the northern region, the southern region experiences the greatest fluctuation in salinity during rain events mostly due to decreased water circulation (Bathen 1968). The freshwater layer formed during

rain events can vary in salinity and depth depending on several meteorological variables, including the amount of rainfall, tidal flow, and wind direction (Ringuet & Mackenzie 2005, Ostrander et al. 2008, Drupp et al. 2011), and reductions in salinity at or below 20 ppt are constrained to a depth of 1 m (Ringuet & Mackenzie 2005, Ostrander et al. 2008). However, the flats and reef crests of many patch and fringing reefs in Kaneohe Bay are less than 1 m depth and can be exposed to air during extreme spring tides (Bathen 1968). Therefore, shallow *M. capitata* colonies in Kaneohe Bay likely experience the level of hyposalinity stress conducted in manipulative experiments.

Sedimentation can also interfere with coral health in a variety of ways. Long-term, chronic sediment loading is known to depress growth rates and reproductive output in corals, highlighting the high energy costs incurred for sediment removal (Rogers 1990). Kaneohe Bay is a shallow coastal embayment with a history of sedimentation issues (Bahr et al. 2015b), but *M. capitata* is capable of quickly removing sediment (Te 2001), maintains high levels of energy reserves compared with other abundant coral species in Kaneohe Bay (Grottoli et al. 2006, Rodrigues & Grottoli 2007), and thus may be well adapted to chronic sedimentation. We aimed to replicate conditions that occur during heavy rain events and address potential contributions of short-term stress to disease development, as opposed to long-term stress. Increases in mucus production and respiration rates (concurrent with decreases in photosynthesis) during sediment removal (Abdel-Salam & Porter 1988, Riegl & Branch 1995) and reductions in lipid stores 24 h after sediment loading (Sheridan et al. 2014b) suggest that corals also undergo high energy expenditures in response to short-term sedimentation stress. Sediments can physically abrade coral tissues or create anoxic environments that induce microbial dysbiosis (Woolfe & Larcombe 1999, Weber et al. 2012). Therefore, we hypothesized that energy reallocation for sediment removal, tissue damage through physical processes, or changes in microbial community structure may weaken coral defenses. However, sediment stress did not influence infection by OCN008 or OCN002. Therefore, the amount of sediment applied or the short duration of exposure in this study may not have constituted enough of a stress to reduce coral defenses.

We did not see a decrease in host resistance in response to short-term sedimentation stress, suggesting that sediment loading during rain events may contribute to disease dynamics in other ways rather than through impairing coral health, depending on

the severity of sediment loading. In fact, sediment loading can result in a short-term increase in some aspects of coral immunity, including melanin production and phenoloxidase activity (Sheridan et al. 2014b). However, reef sediments harbor a high abundance of bacteria, which can include *Vibrio* species (Rusch et al. 2009, Sheridan et al. 2014a). Thus, sediments could be delivering high doses of potential pathogens to already stressed corals (via hyposalinity or other conditions). Sediments used in this study were sterilized prior to use; therefore, sediment did not function as an abiotic vector of MWS pathogens in this study. Future studies should investigate whether sediments directly from watersheds already harbor potential coral pathogens and address the potential for sediment to transport coral pathogens across reefs or transfer pathogens to coral surfaces.

Reduction in salinity and sedimentation occur simultaneously during heavy rain events, and it is reasonable that there could be synergistic effects. For example, the reef coral *Siderastrea radians* can effectively deal with sediment burial unless also exposed to reduced salinity, which slowed sediment removal rates (Lirman & Manzello 2009). We did not test the combined effect of reduced salinity and sedimentation on *Vibrio* infection in *M. capitata*. However, investigating the effects of single stressors systematically is an important first step to assess the relative impact of various stressors and to help subsequent studies put into context (additive, antagonistic, or synergistic) the effect of combined stressors.

Runoff includes other factors that were not investigated here, including organic nutrients, pollutants, and subsequent phyto- and zooplankton blooms. In addition, temperature is an important driver in many coral disease systems (Miller & Richardson 2014). Past prevalence of MWS (2006–2007) was not affected by season (Aeby et al. 2010); however, recent studies suggest that increased temperature or that a mild winter condition is a good predictor of MWS prevalence (Williams et al. 2010, Caldwell et al. 2016). All of these factors, individually or in combination, could dampen coral defenses or may have different roles in the disease process and will need to be considered in future investigations.

Surface ocean salinity has been decreasing in many nearshore areas (Antonov 2002) and the incidence of intense tropical storms is expected to increase due to anthropogenic climate change (Knutson et al. 2010). In addition, urban development, which increases the volume and intensity of runoff, continues to expand in coastal regions (McGranahan & Balk 2007, Smith 2011). The combination of these anthropogenic effects

will further increase the likelihood of abrupt salinity decreases due to heavy rain events, potentially increasing susceptibility to coral diseases. Fortunately, management of runoff is a feasible option to increase coral reef health and resilience to future climate change impacts (Bellwood et al. 2004).

Acknowledgements. All coral fragments used in this study were collected under special activity permits SAP2015-17 and SAP2016-69 from the Hawaii Department of Aquatic Resources. This paper was funded by a grant/cooperative agreement from the National Oceanic and Atmospheric Administration, Project No. R/TR-15, which is sponsored by the University of Hawaii Sea Grant College Program, SOEST, under Institutional Grant No. NA14OAR4170071 from NOAA Office of Sea Grant, Department of Commerce. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. UNIHI-SEAGRANT-JC-15-07

LITERATURE CITED

- Abdel-Salam HA, Porter JW (1988) Physiological effects of sediment rejection on photosynthesis and respiration in three Caribbean reef corals. *Proc 6th Int Coral Reef Symp, Townsville* 2:285–292
- Abrego D, Ulstrup K, Willis B, van Oppen M (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proc R Soc B* 275:2273–2282
- Aeby GS, Ross M, Williams GJ, Lewis TD, Work TM (2010) Disease dynamics of *Montipora* white syndrome within Kaneohe Bay, Oahu, Hawaii: distribution, seasonality, virulence, and transmissibility. *Dis Aquat Org* 91:1–8
- Aeby GS, Callahan S, Cox EF, Runyon C and others (2016) Emerging coral diseases in Kāneʻohe Bay, Oʻahu, Hawaiʻi (USA): two major disease outbreaks of acute *Montipora* white syndrome. *Dis Aquat Org* 119:189–198
- Antonov J (2002) Steric sea level variations during 1957–1994: importance of salinity. *J Geophys Res* 107:SRF 14-1–SRF 14-8
- Bahr KD, Jokiel PL, Rodgers KA (2015a) The 2014 coral bleaching and freshwater flood events in Kāneʻohe Bay, Hawaiʻi. *PeerJ* 3:e1136
- Bahr KD, Jokiel PL, Toonen RJ (2015b) The unnatural history of Kaneohe Bay: coral reef resilience in the face of centuries of anthropogenic impacts. *PeerJ* 3:e950
- Banin E, Vassilakos D, Orr E, Martinez R, Rosenberg E (2003) Superoxide dismutase is a virulence factor produced by the coral bleaching pathogen *Vibrio shiloi*. *Curr Microbiol* 46:418–422
- Bathen KH (1968) A descriptive study of the physical oceanography of Kaneohe Bay, Oahu, Hawaii. Tech Rep 14. Hawaii Institute of Marine Biology, Kaneohe, HI
- Bellwood DR, Hughes TP, Folke C, Nyström M (2004) Confronting the coral reef crisis. *Nature* 429:827–833
- Birkeland C (1997) Introduction. In: Birkeland C (ed) *Life and death of coral reefs*. Chapman & Hall, New York, NY, p 536
- Bruno JF, Petes LE, Harvell CD, Hettinger A (2003) Nutrient enrichment can increase the severity of coral diseases. *Ecol Lett* 6:1056–1061
- Bruno JF, Selig ER, Casey KS, Page CA and others (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. *PLOS Biol* 5:e124
- Caldwell JM, Heron SF, Eakin CM, Donahue MJ (2016) Satellite SST-based coral disease outbreak predictions for the Hawaiian Archipelago. *Remote Sens* 8:93
- Cheng W, Juang F, Chen J (2004) The immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus* at different salinity levels. *Fish Shellfish Immunol* 16:295–306
- Coles S, Jokiel P (1992) Effects of salinity on coral reefs. In: Connell D, Hawker D (eds) *Pollution in tropical aquatic systems*. CRC Press, Boca Raton, FL, p 147–166
- Cox EF, Ribes M, Kinzie RA III (2006) Temporal and spatial scaling of planktonic responses to nutrient inputs into a subtropical embayment. *Mar Ecol Prog Ser* 324:19–35
- Downs CA, Kramarsky-Winter E, Woodley CM, Downs A, Winters G, Loya Y, Ostrander GK (2009) Cellular pathology and histopathology of hypo-salinity exposure on the coral *Stylophora pistillata*. *Sci Total Environ* 407:4838–4851
- Drupp P, DeCarlo E, Mackenzie F, Bienfang P, Sabine C (2011) Nutrient inputs, phytoplankton response, and CO₂ variations in a semi-enclosed subtropical embayment, Kaneohe Bay, Hawaii. *Aquat Geochem* 17:473–498
- Egan S, Gardiner M (2016) Microbial dysbiosis: rethinking disease in marine ecosystems. *Front Microbiol* 7:991
- Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Mar Pollut Bull* 50:125–146
- Friedlander AM, Aeby G, Brainard R, Brown E and others (2008) The state of coral reef ecosystems of the Main Hawaiian Islands. In: Waddell J (ed) *The state of coral reef ecosystems of the United States and Pacific Freely Associated States: 2008*. NOAA Tech Memo NOS NC-COS 11. NOAA/NCCOS Center for Coastal Monitoring and Assessment, Biogeography Team, Silver Spring, MD
- Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. *Nature* 440:1186–1189
- Haapkylä J, Unsworth RK, Flavell M, Bourne DG, Schafelke B, Willis BL (2011) Seasonal rainfall and runoff promote coral disease on an inshore reef. *PLOS ONE* 6:e16893
- Harvell D, Jordán-Dahlgren E, Merkel S, Rosenberg E and others (2007) Coral disease, environmental drivers, and the balance between coral and microbial associates. *Oceanography (Wash DC)* 20:172–195
- Hodgson G (1990) Tetracycline reduces sedimentation damage to corals. *Mar Biol* 104:493–496
- Keane P, Kerr A (1997) Factors affecting disease development. In: Brown J, Ogle H (eds) *Plant pathogens and plant diseases*. Rockvale Publications for the Division of Botany, University of New England, Armidale, NSW, p 287–297
- Kerswell AP, Jones RJ (2003) Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of 'low-salinity bleaching'. *Mar Ecol Prog Ser* 253:145–154
- Kinzie R, Mackenzie F, Smith S, Stimson J (2001) CISNet: linkages between a tropical watershed and reef ecosystem. Final Report to NOAA. University of Hawaii, Honolulu, HI
- Knutson T, McBride J, Chan J, Emanuel K and others (2010) Tropical cyclones and climate change. *Nat Geosci* 3:157–163

- Li CC, Yeh S, Chen J (2010) Innate immunity of the white shrimp *Litopenaeus vannamei* weakened by the combination of a *Vibrio alginolyticus* injection and low-salinity stress. *Fish Shellfish Immunol* 28:121–127
- ✦ Lirman D, Manzello D (2009) Patterns of resistance and resilience of the stress-tolerant coral *Siderastrea radians* (Pallas) to sub-optimal salinity and sediment burial. *J Exp Mar Biol Ecol* 369:72–77
- ✦ Lirman D, Orlando B, Macia S, Manzello D, Kaufman L, Biber P, Jones T (2003) Coral communities of Biscayne Bay, Florida and adjacent offshore areas: diversity, abundance, distribution, and environmental correlates. *Aquat Conserv* 13:121–135
- ✦ Manzello D, Lirman D (2003) The photosynthetic resilience of *Porites furcata* to salinity disturbance. *Coral Reefs* 22: 537–540
- Maragos J (1972) A study of the ecology of Hawaiian reef corals. PhD dissertation, University of Hawaii at Manoa, Honolulu, HI
- ✦ McGranahan G, Balk D (2007) The rising tide: assessing the risks of climate change and human settlements in low elevation coastal zones. *Environ Urban* 19:17–37
- Miller A, Richardson L (2014) Emerging coral diseases: a temperature-driven process? *Mar Ecol* 36:1–14
- ✦ Muthiga NA, Szmant AM (1987) The effects of salinity stress on the rates of aerobic respiration and photosynthesis in the hermatypic coral *Siderastrea siderea*. *Biol Bull* 173: 539–551
- ✦ Mydlarz LD, Couch CS, Weil E, Smith G, Harvell CD (2009) Immune defenses of healthy, bleached and diseased *Montastraea faveolata* during a natural bleaching event. *Dis Aquat Org* 87:67–78
- ✦ Ostrander C, McManus M, DeCarlo E, Mackenzie F (2008) Temporal and spatial variability of freshwater plumes in a semienclosed estuarine–bay system. *Estuaries Coasts* 31:192–203
- Peters E (2015) Diseases of coral reef organisms. In: Birke-land C (ed) *Coral reefs in the Anthropocene*. Springer, Dordrecht, p 147–178
- ✦ Pollock FJ, Lamb JB, Field SN, Heron SF (2014) Sediment and turbidity associated with offshore dredging increase coral disease. *PLOS ONE* 9:e102498
- Rankin J, Davenport J (1981) *Animal osmoregulation*. Halsted Press, New York, NY
- ✦ Riegl B, Branch G (1995) Effects of sediment on the energy budgets of four scleractinian (Bourne 1900) and five alcyonacean (Lamouroux 1816) corals. *J Exp Mar Biol Ecol* 186:259–275
- ✦ Ringuelet S, Mackenzie FT (2005) Controls on nutrient and phytoplankton dynamics during normal flow and storm runoff conditions, southern Kaneohe Bay, Hawaii. *Estuaries* 28:327–337
- ✦ Ritchie K (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar Ecol Prog Ser* 322:1–14
- ✦ Rodrigues L, Grotoli A (2007) Energy reserves and metabolism as indicators of coral recovery from bleaching. *Limnol Oceanogr* 52:1874–1882
- ✦ Rogers CS (1990) Responses of coral reefs and reef organisms to sedimentation. *Mar Ecol Prog Ser* 62:185–202
- ✦ Röthig T, Ochsenkuhn MA, Roik A, van der Merwe R, Voolstra CR (2016) Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. *Mol Ecol* 25:1308–1323
- ✦ Rusch A, Hannides A, Gaidos E (2009) Diverse communities of active Bacteria and Archaea along oxygen gradients in coral reef sediments. *Coral Reefs* 28:15–26
- ✦ Seveso D, Montano S, Strona G, Orlandi I, Galli P, Vai M (2013) Exploring the effect of salinity changes on the levels of Hsp60 in the tropical coral *Seriatopora caliendrum*. *Mar Environ Res* 90:96–103
- ✦ Sheridan C, Baele J, Kushmaro A, Frejaville Y, Eeckhaut I (2014a) Terrestrial runoff influences white syndrome prevalence in SW Madagascar. *Mar Environ Res* 101:44–51
- ✦ Sheridan C, Grosjean P, Leblud J, Palmer C, Kushmaro A, Eeckhaut I (2014b) Sedimentation rapidly induces an immune response and depletes energy stores in a hard coral. *Coral Reefs* 33:1067–1076
- ✦ Shnit-Orland M, Kushmaro A (2009) Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiol Ecol* 67:371–380
- ✦ Smith K (2011) We are seven billion. *Nat Clim Chang* 1: 331–335
- ✦ Smith G, Ives L, Nagelkerken I, Ritchie K (1996) Caribbean sea-fan mortalities. *Nature* 383:487
- ✦ Tchernov D, Kvitt H, Haramaty L, Bibby T, Gorbunov M, Rosenfeld H, Falkowski P (2011) Apoptosis and the selective survival of host animals following thermal bleaching in zooxanthellate corals. *Proc Natl Acad Sci USA* 108:9905–9909
- Te F (2001) Responses of Hawaiian scleractinian corals to different levels of terrestrial and carbonate sediment. PhD dissertation, University of Hawaii at Manoa, Honolulu, HI
- ✦ Ushijima B, Smith A, Aeby GS, Callahan SM (2012) *Vibrio owensii* induces the tissue loss disease *Montipora* white syndrome in the Hawaiian reef coral *Montipora capitata*. *PLOS ONE* 7:e46717
- ✦ Ushijima B, Videau P, Burger AH, Shore-Maggio A and others (2014) *Vibrio coralliilyticus* strain OCN008 is an etiological agent of acute *Montipora* white syndrome. *Appl Environ Microbiol* 80:2102–2109
- ✦ Ushijima B, Videau P, Poscablo D, Stengel JW and others (2016) Mutation of the *toxR* or *mshA* genes from *Vibrio coralliilyticus* strain OCN014 reduces infection of the coral *Acropora cytherea*. *Environ Microbiol* 18: 4055–4067
- ✦ Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C and others (2009) Metagenomic analysis of stressed coral holobionts. *Environ Microbiol* 11:2148–2163
- ✦ Voss J, Richardson L (2006a) Nutrient enrichment enhances black band disease progression in corals. *Coral Reefs* 25: 569–576
- ✦ Voss JD, Richardson LL (2006b) Coral diseases near Lee Stocking Island, Bahamas: patterns and potential drivers. *Dis Aquat Org* 69:33–40
- ✦ Wang F, Yang H, Gao F, Liu G (2008) Effects of acute temperature or salinity stress on the immune response in sea cucumber, *Apostichopus japonicus*. *Comp Biochem Physiol A Mol Integr Physiol* 151:491–498
- ✦ Weber M, de Beer D, Lott C, Polerecky L and others (2012) Mechanisms of damage to corals exposed to sedimentation. *Proc Natl Acad Sci USA* 109:E1558–E1567
- ✦ Williams GJ, Aeby GS, Cowie ROM, Davy SK (2010) Predictive modeling of coral disease distribution within a reef system. *PLOS ONE* 5:e9264
- ✦ Woolfe KJ, Larcombe P (1999) Terrigenous sedimentation and coral reef growth: a conceptual framework. *Mar Geol* 155:331–345