

Title:

The interactive effects of nutrient and salinity stress on corals from distinct environments on the Belize Barrier Reef System

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Abstract

Global climate change has induced shifts in local weather patterns near many coral reef ecosystems, resulting in changes in precipitation, which, combined with land use changes, have resulted in greater nutrient loading and more severe freshwater invasion events on local coral reefs. In order to investigate how different populations of corals may respond to these stressors, we quantified the effects of salinity and nitrate (NO_3^-) concentrations on calcification and bleaching susceptibility for corals from nearshore (stress-frequent) and forereef (stress-infrequent) environments. Colonies of *Siderastrea siderea*, were collected from the southern portion of the Belize Barrier Reef System (BBRS), fragmented, and reared for 30 days in weekly salinity/nutrient treatments that simulate one of four seasonal conditions: 1) control- approximate local average salinity (32) and approximate local average nutrient concentration ($[\text{NO}_3^-] = 2.5 \mu\text{mol}$) held constantly throughout the experiment, 2) low salinity (24) stress events, 3) high nutrient dosing events (peak $[\text{NO}_3^-] = 6.5 \mu\text{mol}$), and 4) double stress- low salinity events and nutrient dosing events. Buoyant weight and colony color were quantified at the beginning of the experiment and 30 days later after a sudden temperature drop of 6 °C. Coloration was also monitored during a three-week recovery process. Results indicate no significant difference in growth between corals from different environments across all conditions, however corals from the fore-reef showed consistently lower variation in change in buoyant weight across all treatments when compared to corals from the nearshore environment. Additionally corals exposed to combined stressor conditions show significantly more bleaching and slower recovery. Understanding these interactive roles of nutrients and salinity, as well as the role that local environments play in coral survivorship and recovery, will be important considerations for the future of coral reefs in the face of climate change.

IntroductionImportance of Coral Reefs

Coral reefs are essential to the life cycle of over 800,000 marine species worldwide, and through indirect association can affect as many as one million species globally (Fisher et al 2015). The essential architects of these reefs are calcifying corals, also known as scleractinian corals (Fisher 2015). Understanding the biological and physiological response of scleractinian

corals to modern shifting environments brought on by climate change is essential in understanding the dynamics of reef ecosystems globally. Recent studies detailing the decline of coral health have been tied to overall reef ecosystem decline, resulting in losses of biodiversity, productivity, and reef accretion, and these reef processes are directly tied to highly valued ecosystem services by the reef system (Haan 2015; Hoegh-Guldberg et al 2005; Zhou et al 2015). The Belize Mesoamerican Barrier Reef System (MBRS), the world's second longest barrier reef system, yielded 15 million US dollars in commercial and recreational fish catch on average (Cooper, Burke, and Bood 2008). Likewise barrier reef systems such as the Belize MBRS have been shown to be an essential buffer from storm surge and wave action, saving as much as 347 million US dollars of coastline in Belize alone (Cooper et al., 2008).

Local Threats to Coral Reefs of Belize

Increasing global ocean temperatures worldwide has been correlated with coral bleaching, or the loss of the coral's symbiotic dinoflagellate *Symbiodinium spp* (Hoegh-Guldberg 2007; Sabine et al 2015). Prolonged bleaching results in mortality, leading to reef degradation (Hoegh-Guldberg 2007; Sabine et al 2015). Additionally, increasing carbon dioxide (pCO₂) in the world's oceans has been tied to a reduction in calcification or reef building ability of corals, therefore warming and acidification are identified as two of the most significant stressors impacting to coral health at present and in the near future (Hoegh-Guldberg 2007).

Additionally several studies have examined the effect of local conditions on reef communities and reef health. Reef salinity, dissolved nutrient concentrations, light levels, and wave action are all localized factors that have been shown to impact coral health as well as limit growth in corals (Alutoin et al 2001; Browne et al 2015; Faxneld et al 2010; Haan 2015; Sabine

et al 2015; Yang et al 2008; Zhou et al 2015; Zhu et al 2004). Indeed, many studies have demonstrated that even small changes in salinity have significant impacts on coral growth and potentially induce bleaching in many coral species (Alutoin et al 2001; Faxneld et al 2010; Ferrier-Pagès et al 1999; Kjerfve 1986; Li et al 2009; Moberg et al 1997; Muthiga et al 1987).

The effects of nutrient influx on reefs appear to have a result in a different kind of response. Increasing nutrients such as nitrate primarily triggers a shift in dominance from a coral dominated system to an algal dominated system, since macroalgal competition is typically limited by bottom up nutrients processes in reef systems (Haan et al 2015; Heyman & Kjerfve 1999; Koop et al 2001; Lapointe et al 1992; Larned 1998; Reefs of Tomorrow 2015). Recent studies have attempted to better understand the effects of the nutrients on corals, and on the relationship between the coral host and its intracellular zooxanthellae symbiont. Several studies have shown that in the presence of higher nutrients, there is breakdown of carbon exchange between the coral host and algal symbiont, leading to a decrease in the amount of fixed carbon provided to the coral resulting in a decline in host calcification despite increased symbiont proliferation (Browne et al 2015, Ezzat et al 2015, Faxneld et al 2010, Marubini & Davies 1996, Rådecker et al 2015, Zhou et al 2015, and Zhu et al 2004).

These findings provide important insight into the nature of the relationship between the coral host and its algal symbiont, however there are additional considerations when understanding the relationship between the coral holobiont (coral and symbiotic algae) to nutrient concentration and salinity. Firstly, sudden increases in nutrients will often take place simultaneously with a drop in salinity during high-runoff events (Burke & Sugg 2006; Butler et al 2013; Fabricius 2005; Paris & Chérubin 2008). During high-runoff events, intense rainfall results in high volumes of fresh water containing agricultural and human waste which ultimately

overwashes the surrounding reef environment (Burke & Sugg 2006; Butler et al 2013; Carillo et al 2009; Fabricius 2005; Heyman & Kjerfve 1999; Paris & Chérubin 2008). *In situ* water sampling in southern Belize has found nutrient spikes as high as 8.5 μmol for $[\text{NO}_3^-]$, and salinity drops as low as 18 as the result of these runoff events during the rainy season (San & Road 2015). However, despite the drastic shift in conditions as a result of these events, stressful conditions are soon washed away by ocean mixing and dissolution, returning conditions to a pre-runoff state usually within 3 days after the initial flooding event (Burke & Sugg 2006; Paris & Chérubin 2008). Corals on a reef therefore, experience sharp declines in salinity and sharp increases in nutrients over a very short period, but only experience these conditions very briefly before returning to pre-flood conditions. In order to understand how corals are responding to nutrient and salinity stress within the context of the reef environment, these sharp changes in salinity and nutrients must be replicated in a laboratory setting.

Shifting trends in nutrient loading and storm frequency/intensity

When discussing nutrient and runoff events, it is important to consider how these conditions are altered by anthropogenic activity. The Belize and Honduras water systems are responsible for any freshwater influence on the reef environment, and therefore any alteration to these watersheds will ultimately impact the severity and frequency of runoff events on the Belize MBRS (Burke & Sugg 2006; Paris & Chérubin 2008; San & Road 2015). Recent shifts from forested dominated environments to agricultural lands in both Belize and Honduras have resulted in a two-fold effect on these watersheds and the Belize MBRS. The loss of forested land firstly results in a significant increase in water that is washed downstream instead of retained on land (Paris & Chérubin 2008). Additionally, the increased runoff results in increased erosion of

fertilizer-heavy land, resulting in an increase in the concentrations of nitrates and other fertilizer-based nutrients washed downstream and ultimately onto the reef (San & Road 2015).

In addition to this increased runoff volume, anthropogenic activities have also been correlated with a global shift in storm frequency and intensity (Bender et al 2010; Chan 2006; Emmanuel 2005; Knutson et al 2010). In the Caribbean, the frequency of intense or severe storms has significantly increased, resulting in more frequent rainfall and more frequent severe runoff events that affect the Belize MBRS (Bender et al 2010; Chan 2006; Emmanuel 2005; Knutson et al 2010). Combined with increased nutrient concentration and salinity stress as the result of land use change, corals will likely experience more frequent and more intense runoff events as the climate and local land use in Honduras and Belize continues to shift.

Synergism of high nutrient and low salinity stress

When discussing the combined impact of two or more effectors on a system, ecologists often identify the interaction as an antagonistic effect, an additive effect, or a synergistic effect of their simultaneous presence (Figure 1) (Darling et al 2010). In an antagonistic interaction, the combined impact of the effectors is reduced by the contrasting influence of each independent effector. As a result, the interaction of two factors can sometimes result in a net zero impact on an organism (Figure 1a) (Darling et al 2010). Alternatively, an additive effect refers to the interaction where two factors combine to have an impact equal to the sum of the two separate factors. In this case, although the two factors produce the same effect, they do not influence each other (Figure 1b) (Darling et al 2010). Lastly there is synergistic interaction, where two factors combine to have an impact greater than the calculated sum of the two separate factors. In

synergism, the two factors influence the impact of each other in an additional additive way, created a magnified effect (Figure 1c) (Darling et al 2010).

In the case of nutrient and salinity stress on the Belize MBRS, corals will often experience nutrient stress in the presence of salinity stress, and therefore may respond differently to their simultaneous presence compared to exposure to each stressor individually. Because of the stressful nature of both factors, it is likely that increased nitrate and low salinity stress will have an additive, or even synergistic, impact on coral growth and resilience. In order to investigate this interaction, we exposed colonies of *Siderastrea siderea* from two separate environments of the Belize MBRS to 1 month of periodic nutrient and salinity stress, both in isolation and simultaneously, and measured the change in growth and bleaching resistance of the corals in a controlled laboratory experiment.

Methods

Collection

Colonies of *Siderastrea siderea* were collected at approximately 3-5 meters depth from the BBRS in June of 2015. In order to ensure that no clonal colonies were selected, corals were collected on reefs at least 2 meters from each other. Corals were selected to be of similar size (<0.5 m) and had no observable areas of bleaching or necrosis. Nine corals were collected from a lagoonal patch reef in the nearshore reef environment and nine corals were collected from a lagoonal patch reef near the barrier reef structure. Colonies were then transported to Northeastern University's Marine Science Center in Nahant, Massachusetts for recovery and sectioning.

Sectioning and acclimation

After 7 days of recovery in the Northeastern Aquarium Research Center flow-through system, all corals were sectioned into approximately 4 cm² nubbins using a 10 inch wet tile saw and attached to plastic petri dishes using cyanoacrylate with an etched colony and nubbin identifying label. Following 48 days of recovery, nubbins were transported to the Castillo laboratory's Aquarium Research Center at the University of North Carolina at Chapel Hill. Corals were then allowed to acclimate to new tank conditions of 28.0 °C and 32 salinity for an additional 48 day interval. The experiment began on September 29, 2016.

Design

Coral nubbins were placed into one of twelve experimental tank aquaria, 38L in volume. Three coral nubbins from each reef environment (fore-reef and nearshore) were placed in each tank, such that each tank contained six coral nubbins. Water in the tanks was circulated constantly with two power heads (*Hydor USA*, Sacramento, CA) rated at 908.5 Lh⁻¹, in order to sustain flow within each tank. Three tanks were then connected to one 190L sump system to create a uniform system of filtration, temperature, and salinity for each tank, resulting in 4 treatment systems (3 tanks per treatment system): control, double stress, low salinity, and high nutrient.

Treatment sumps contained a filtration system with a filter sock to remove particulates and a protein skimmer to remove organic materials. All systems were maintained at 28 °C using an in-sump 90W heater (*Eheim*, Deizisau, Germany) and connection to a chiller (*AquaEuroUSA*, Los Angeles, CA). Each aquarium was covered with a plexiglass sheet to reduce evaporative

water loss, and all treatment systems were illuminated by a high output T5 lighting system (*Current-USA*, Vista, CA) containing two 460 nm actinic bulbs and two 10,000 K daylight bulbs (156 watt fixture). All treatment systems were illuminated on a twelve hour light cycle with *ca* 250 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ during the daylight hours and *ca* 100 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ during the hours of only actinic light at the beginning and end of the light cycle. Coral nubbins were scrubbed free of visible algae once a week in order to prevent effects of macroalgal competition on nubbin response.

All seawater used was created using deionized water and *Instant Ocean Sea Salt*, which has been shown to be the most similar to natural seawater in trace metal and micronutrient composition (Atkinson & Bingman 1998). *Instant Ocean Sea Salt* was mixed into a 454L mixing tank using both a 250GPH recirculating pump and split phase motorized propeller (*Dayton Electric Motoring Company*, Lake Forest, IL). Water for each salinity treatment (32 and 12 for low salinity) was mixed independently. In order to create necessary concentrations of nitrate in the systems, water from each salinity mix was transported to isolated mixing tanks, where 0.33g of potassium nitrate (KNO_3) was added to 227 L of each salinity mixed and dissolved using a 2400 GPH recirculating pump (*Danner Manufacturing*, Islandia, NY) for at least four hours to ensure uniform dissolution.

Target nutrient concentrations and target salinities were reestablished during weekly 50% water changes of the systems. In order to prevent contamination, water was delivered from the mixing tanks to the treatment systems through a plumbing system that was rinsed with deionized water between each water change. Treatment conditions in each of the respective systems were as follows: 1) No change to the system, the system was maintained at a salinity 32 and $[\text{NO}_3^-] = 2.5 \text{ M}$ (background level of nitrate in the system); 2) a lowered salinity to 24 with $[\text{NO}_3^-] = 2.5$

M; 3) a maintained salinity of 32 and increased nitrate concentration such that $[\text{NO}_3^-] = 8.0 \text{ M}$; 4) a lowered salinity to 24 and increased nitrate concentration such that $[\text{NO}_3^-] = 8.0 \text{ M}$. Following the water change, 32 and nitrate reduced ($[\text{NO}_3^-] = 2.5 \text{ M}$) seawater was added each day to all systems, such that after three days all systems were maintained at 32 and $[\text{NO}_3^-] = 2.5 \text{ M}$.

Dosing Monitoring and Analysis

Temperature, salinity, and pH were monitored three times a week. Temperature was measured using NIST calibrated partial-immersion organic filled glass thermometer, and pH was measured using an Orion Star A211 pH meter with a ROSS Sure-Flow Combination pH probe calibrated with certified NBS pH 4.01, 7.00, and 10.01 buffers. Salinity was measured using both a YSI 3200 conductivity meter and an automatic temperature compensating (ATC) refractometer (*Genesis Reef Systems*, Plainfield, IL). Temperature and salinity were adjusted as needed using deionized water and heater adjustments in the sumps.

In order to ensure accuracy of nitrate dosing, water samples were collected twice a week: approximately 2 hours following a water change and 72 hours following a water change. During each collection point, three samples of approximately 75mL were taken from each tank, to ensure sampling was representative of conditions in each tank system. These samples were then preserved in a $-80 \text{ }^\circ\text{C}$ freezer until they could be processed.

Water samples were carefully thawed from $-80 \text{ }^\circ\text{C}$ over a four-hour period before analysis using the miniSEAS© spectrophotometer, developed for total inorganic nitrogen (TIN) analysis by the Martens Laboratory. The miniSEAS© was selected for analysis because its application of Beers law of light absorbance as a proxy for nutrient measurement, allowing it to detect extremely precise changes in concentration. Each sample was drawn over a cadmium reducing column in order to first convert all nitrate to nitrite and then mixed with a light reactive

color reagent (composed of 5 parts sulfanilamide and 1 part N-(1-naphthyl)ethylenediamine dihydrochloride). The color reagent and nitrite combine to absorb light at the 742 nm wavelength, and is then drawn through a 1 cm-long fiber optic tube in order to measure precise absorbance of light. During each analysis time point, a calibration curve was created using 100 nM, 200 nM, 500 nM, 1000 nM, 5000 nM, and 10000 nM nitrate standards, along with a 500 nM nitrite standard as a reference for conversion of nitrate to nitrite. Concentrations of each sample were analyzed using Matlab® and compared against samples taken from the tanks in the absence of coral, in order to account for biological effects on concentration.

Quantification of growth

Coral growth was estimated via buoyant weight analysis. Corals were weighed with a bottom-loading balance (precision=0.0001 g) (*Mettler-Toledo*, Columbus, OH) at the beginning of the experiment (day 0) and following treatment (day 30). The difference in weight was then used as a proxy for coral growth. Corals were each weighed three times and averaged at each time point to ensure weights were precise, and weights were normalized using a 17 g washer and 5 g nickel for standardization across temperature and salinity. Salinity did not vary from 32 by more than 1 and temperature did not vary from 28 °C by more than 0.5 °C.

Acute stressor treatment and recovery monitoring

Following 25 days of nutrient and salinity dosing of each system, all systems were exposed to a cold-shock event, where all systems were changed from 28 °C to 21 °C over the course of 4 hours. This temperature was maintained for 63 hours, after which temperature was slowly returned to 28 °C over 14 days, to ensure that rapid heating would not impact response of the coral. No salinity stress or nutrient stress was conducted during the recovery period.

Each nubbin was photographed at day 30, day 37, and day 51 (directly following the cold shock, 1 week following the cold shock, and 3 weeks following the cold shock, respectively) and compared to photos taken at the beginning of the experiment (day 0). These photos were then categorized as bleached, somewhat bleached, or completely bleached in comparison to day 0 at each time point.

Statistical Analysis of Data

All statistical analyses were implemented using R software, version 3.1.1 (R Development Core Team, 2015). Differences in average change in buoyant weight for nubbins of each habitat in each treatment were analyzed for significance using the analysis of variance (ANOVA, function *aov()*). Averages and standard error were determined and plotted using the package *ggplot2* (version 1.0.0, Wickham & Chang 2014).

Results

Change in buoyant weight

We found no significant trends in change in buoyant weight across treatment or habitat of origin (Figure 1). Average change in buoyant weight for nubbins from the nearshore was not significantly different from zero change in any of our four treatments, including control. Average change in buoyant weight in nubbins from the fore-reef showed significant positive change in control, high nutrient, and low salinity treatments, but not significantly more than zero change in the combination, or double stress treatment. Standard deviation was greater in the nearshore nubbin growth across all four treatments compared to the fore-reef.

Effect on coloration

When compared to photos from day 0 of the experiment, all nubbins in both the control treatment and high nitrate treatment showed no signs of visible bleaching directly following the

cold shock (Figure 3). These nubbins remained unbleached for the entirety of the recovery process. This was true for all nubbins in these two treatments, regardless of habitat (nearshore vs. fore-reef).

Nubbins in the low salinity treatment and combination treatment all showed significant bleaching directly following the cold shock, as photographed in day 30 (figure 3). All nubbins in the combination treatment remained completely bleached at experiment day 37 and day 51. All nubbins in the low salinity treatment recovered to only partially bleached by day 37 and by day 51 had returned to pre-cold shock coloration. There was no distinction in visible bleaching between nubbins from the fore-reef and nubbins from the nearshore.

Discussion and Conclusions

Effect of nutrient and salinity stress on coral growth

Our results suggest that in the presence of nutrient and salinity stress, changes in coral growth are indistinguishable. Due to the slow growing nature of *Siderastrea siderea*, 30 days is likely too little time to exacerbate changes in growth for this species (Darling et al 2012, Elahi & Edmunds 2007). This is particularly true for corals of the nearshore environment, which all demonstrated highly varied amounts of growth, likely due to the greater variability of the nearshore environment compared to the fore-reef (Kjerfve 1986).

Corals from the fore-reef in only high nutrient and only low salinity appear to have some form of growth (average change in weight significantly greater than 0), however, these changes are still indistinguishable from fore-reef corals in control treatment. This suggests that corals from the fore-reef may independently have a faster growth rate that is overall observable within 30 days of measurement. The variation in growth from fore-reef corals, however, makes it unclear if this higher growth rate is impacted by simultaneous nutrient and salinity stress.

Additionally more time within these experimental parameters may help differentiate growth rates of corals from high nutrient and low salinity environments compared to control conditions.

Effect of nutrient and salinity stress on coral stress tolerance and stress recovery

The demonstrated visible bleaching of all corals in the low salinity and combination treatment compared to the lack of visible bleaching in the control treatment indicates that regular sharp declines in salinity may have a negative impact on coral resistance to cold water bleaching. Because temperature decreases are often associated with runoff events onto a reef, this is a relevant relationship in the reef environment. The rapid recovery of the corals in the low salinity following the cold shock event also indicates that while bleaching susceptibility is impacted, the resilience of the coral does not appear to be affected by a decreased salinity regime.

The lack of recovery from the cold shock event by corals of the combination treatment compared to the rapid recovery of corals in the low salinity treatment additionally suggest that there is another added effect to the recovery of the coral in the presence of periodic nitrate concentration spikes. This effect is only manifested in the presence of low salinity stress as well, since corals in nutrient stress alone were not impacted in any observable way by the cold shock.

Interestingly, since the cold shock event after 25 days of stress treatment, both response and recovery of corals takes place in entirely control, or “non-stressful” conditions. It appears that the stress regimes still exert an effect on coral stress tolerance and recovery even after the stressors themselves are no longer present. It is unclear the mechanism behind this, and will require further investigation as to how exactly the pulses of low salinity and nutrients interact with the coral’s physiology.

Presence of synergism

While it would be expected based on the response of the nubbins in isolated treatment (low salinity and high nutrient) would indicate the response of the combination treatment, this is not the case. Instead, the temperature impacts the corals in a way greater than in the presence of low salinity alone or high nitrate alone. This is the definition of synergism, where two agents produce a combined effect greater than the sum of their separate effects (Darling et al. 2012, Dunne 2010). The additive effect suggests that when exposed to high nutrients in addition to low salinity stress, high nutrients will continue to have no effect and corals in the combination treatment will mimic the corals of the low salinity treatment. Instead, the increased bleaching susceptibility is magnified by a nutrient input regime. Since these stressors often appear concurrently in anthropogenic-affected systems, this observation is directly applicable to the reef environment today. Many studies have also found multiple stressors in reef environment to have a synergistic impact on the coral holobiont, particularly involving temperature stress on corals (Dunne 2010, Nyström et al 2000, Hoegh-Guldberg & Smith 1989).

Effect of habitat

We hypothesized that forereef corals experience these high-runoff events only occasionally compared to nearshore corals during the annual rainy season in Belize, and therefore corals from the forereef will be more sensitive to runoff-based stressors (nutrient spikes, salinity drops, temperature drops). However, our findings indicate that there is no difference in growth rate between corals from the forereef and corals from the nearshore in neither control conditions nor stressful conditions. Since the colonies were sectioned into smaller nubbins (~4 cm²) and only subjected to growth treatment for 30 days, it is possible that more

distinguishable trends may have emerged on a longer timescale, however further testing will be needed to identify these trends with certainty (Darling et al 2012, Elahi & Edmunds 2007).

Acute stress response was entirely invariant by habitat, and driven solely by treatment. The conserved response across habitat suggests that corals of these two environments have similar evolutionary mechanisms and weaknesses in place, meaning that the environments may be less distinctly different than previously thought in terms of nutrient and salinity stress. Lowered salinity and increased nitrate concentration may be experienced in forereef environments in greater amounts than previously suggested, driving similar behaviors and responses comparable to corals of the nearshore or “stress-frequent” environment. This has been indicated by a study conducted by Carilli et al 2009, identifying that because of heavy amounts of rainfall in the tropical system, that large watersheds surrounding the MBRS may affect coral reefs on the nearshore and further offshore alike. Additionally Nunes et al 2011 found that *Siderastrea siderea* has the capacity to maintain population connectivity across reefscapes and even longer distances, meaning that differences in behavior between nubbins from each environment may not necessarily be adaptive to the environment as much as plastic response to distinct environmental conditions.

Results as a part of a changing system

These results have significant impacts for understanding how these reefs will handle local stressors as the result of increasing human activity. As land use in Belize continues to shift from forest-heavy to agricultural-heavy, the resulting increase in runoff will generate more powerful and more far-reaching runoff events (Burke & Sugg 2006; Paris & Chérubin 2008; San & Road 2015). Combined with increased nitrate concentrations as the result of agricultural fertilizer runoff, these future runoff events have the potential be more powerful in both salinity and nitrate

flux (Burke & Sugg 2006; Paris & Chérubin 2008; San & Road 2015). The loss of bleaching resistance and recovery ability as the result of these events potentially places these reefs at much greater risk of decline as the result of other stressors such as increasing ocean temperatures and habitat destruction.

Additionally it has been shown that changes in the atmosphere as the result of anthropogenic climate change has led to more frequent intense large storm events in the tropics (Bender et al 2010; Chan 2006; Emmanuel 2005; Knutson et al 2010). An increase in storm frequency and intensity will result in larger and more frequent runoff events, putting corals in an increasingly fragile state during the rainy season of Belize (Burke & Sugg 2006, Carilli et al 2009, Paris & Chérubin 2008). Fragility of these reef environments may persist into the drier months, as in our study where even after nutrient and salinity dosing had ceased bleaching recovery ability was significantly inhibited.

Areas for future research

Looking forward, it will be important to understand the precise mechanism that is driving the synergism between salinity stress and nitrate stress within corals. This may be answered by an analysis of the change in the energy reserves, symbiont community analysis, and changes in gene expression of the coral. Additionally it will be important to understand how these stressors alter growth on a longer time scale, such as one full rainy season in Belize. With a longer time frame, changes in coral growth rate will potentially become more distinct, allowing differences in growth to manifest. Lastly, it will be important to understand the connectivity of the two habitats (fore-reef and nearshore), particularly in terms of the frequency and intensity of stresses corals experience in both habitats.

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Figures

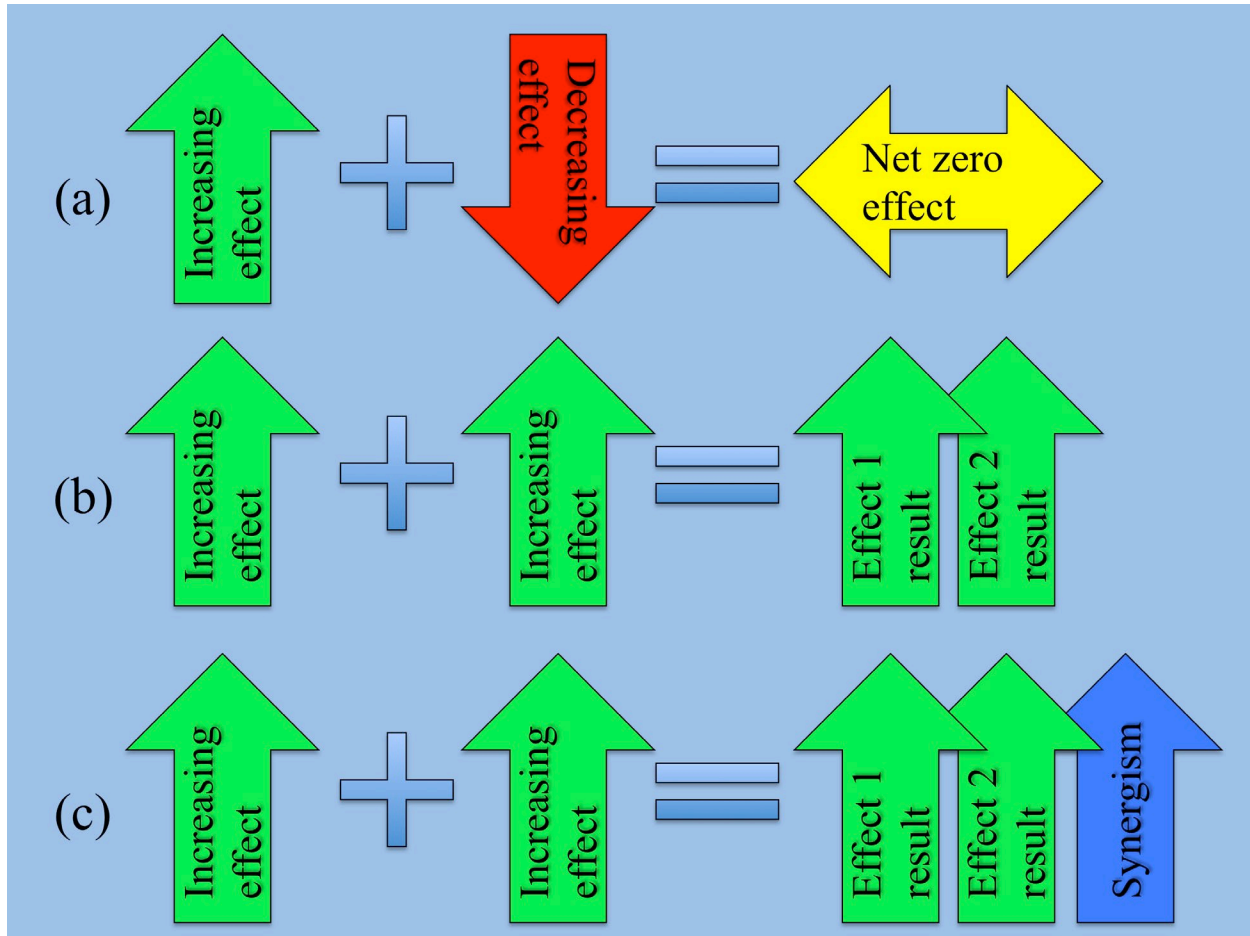


Figure 1) Three possible interactions of two factors on an organism. (a) Antagonistic interaction: When the two factors have a contrasting effect on an organism, the result is a net zero effect. (b) Additive interaction: When the two factors influence an organism in the same way, but do not affect the impact of each other. (c) Synergistic interaction: When two factors influence an organism in the same way, but also have an additional effect that exceeds the summed effect of the two factors.

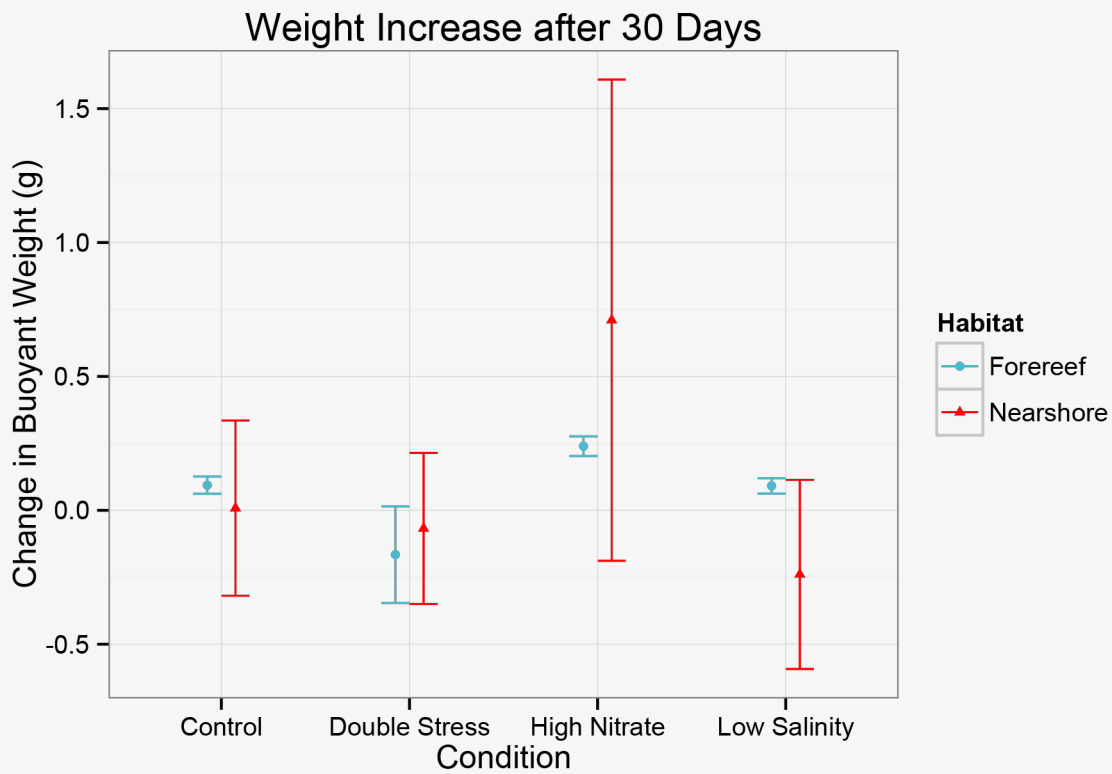


Figure 2) Average change in nubbin buoyant weight (g) from experiment day 0 to day 30 across all four treatments (control, double stress or combination, high nitrate, and low salinity, respectively). Blue points indicate average change in weight for corals harvested from the forereef area of the MBRS, while red points indicate average change in weight for corals harvested from the nearshore area of the MBRS. Bars indicate standard error.

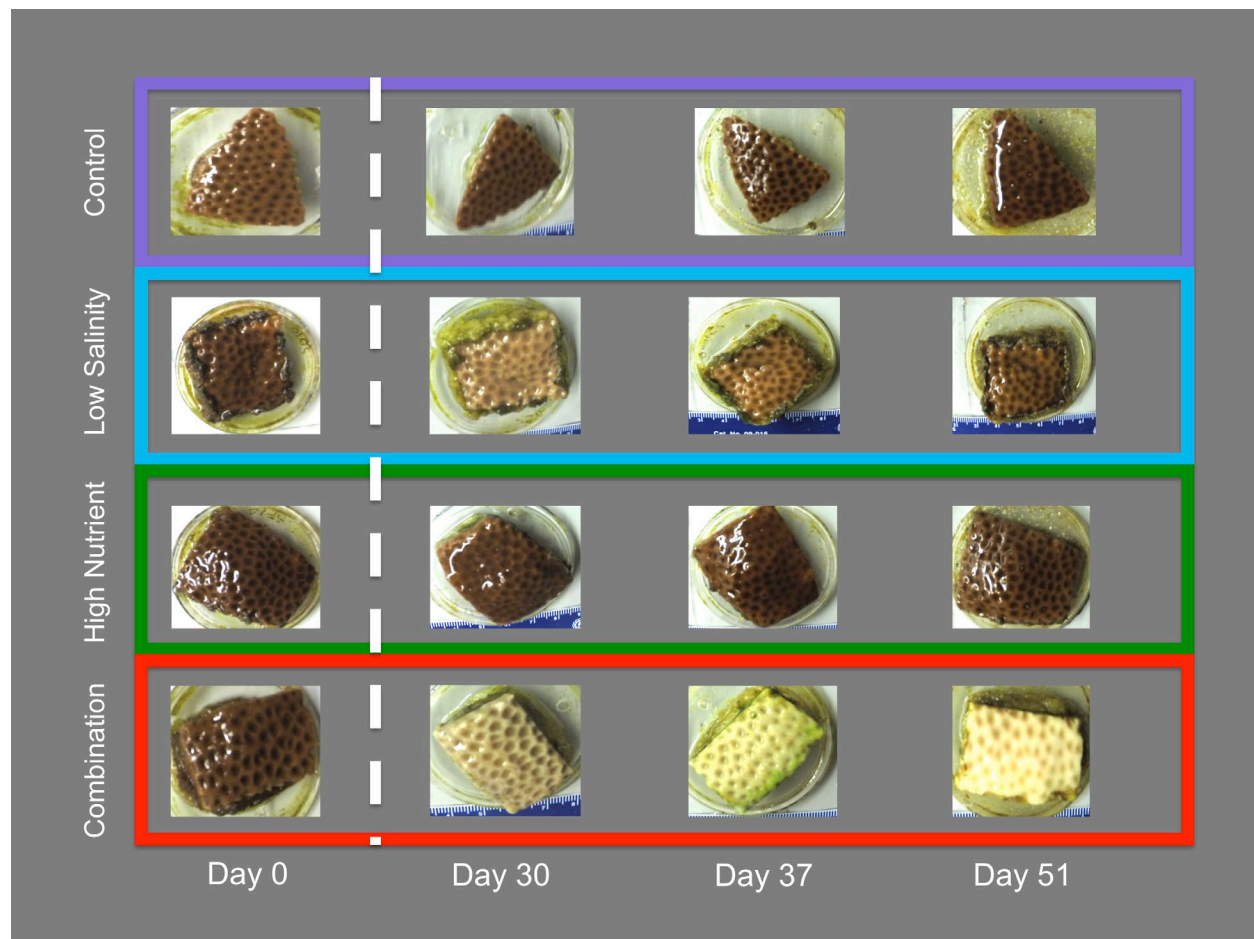


Figure 3) Photographs of representative nubbins from each treatment (control, low salinity, high nutrient, and combination, respectively) at the start of the experiment (day 0), following nutrient and salinity treatments and following the cold shock (day 30), seven days following the cold shock (day 37), and three weeks following the cold shock (day 51).

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