Algal symbionts in the genus *Breviolum* increase the susceptibility of corals to stony coral tissue loss disease (SCTLD)

Algas simbióticas en el género *Breviolum* aumentan la susceptibilidad de los corales a la enfermedad pérdida de tejido en corales escleractinios (SCTLD)

Les algues symbiotiques du genre *Breviolum* augmentent la vulnérabilité des coraux a la maladie de perte de tissu des coraux durs (SCTLD)

CAROLINE DENNISON[1], RICHARD KARP[1], ALEXANDRA WEN[1], SAMANTHA COOK[2], ESTHER PETERS[2], ASHLEY GONCALVES[3], NIKKI TRAYLOR-KNOWLES[1], BRADLEY WEILER[1], JAVIER DEL CAMPO[1,4], STEPHANIE ROSALES[5], ANDREW BAKER[1]

[1]Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Cswy, Miami, FL, United States, [2]George Mason University, 4400 University Drive MS 5F2, David King Hall, Room 3005, Fairfax, VA, United States, [3]University of Miami, 1301 Memorial Dr, 215, Coral Gables, FL, United States, [4]Institute of Evolutionary Biology (UPF-CSIC), Passeig MarÃ-tim de la Barceloneta 37-49, Barcelona, Spain, [5]NOAA Atlantic Oceanographic and Meteorological Laboratory, 4301 Rickenbacker Causeway, Miami, FL, United States,

caroline.dennison@rsmas.miami.edu

EXTENDED ABSTRACT

Globally, the persistence of coral reefs is threatened by a suite of stressors including increasing sea surface temperatures (SSTs) due to climate change, anthropogenic factors, and more recently the emergence of coral related diseases. Such stress often results in extensive coral bleaching, coral mortality, and phase-shifts away from coral dominated ecosystems. Although global climate change remains the greatest threat to coral reefs, reefs in Florida have experienced unparalleled coral mortality associated with the outbreak of stony coral tissue loss disease (SCTLD). Regionally, Florida falls within what is known as a disease "hotspot" due to increased SSTs (van Woesik and Randall 2017). Since the 1970s, there have been over 30 named coral diseases reported in the Caribbean that range in their distribution, affected species, and abundance and severity (Bruckner 2009).

SCTLD was first reported in September 2014 in Southeast Florida off Virginia Key, Miami-Dade County (Precht et al. 2016). Since 2014, SCTLD has spread across all of Florida's Coral Reef including the Dry Tortugas which remained unaffected until late May 2021 (NPS 2021). Endemic to all of Florida, this disease has since been reported in various locations including The Bahamas, US Virgin Islands, British Virgin Islands, Belize, and parts of the Eastern Caribbean and the Greater Antilles (Kramer *et al* 2019). Monitoring efforts in south Florida found that in the first two years of the outbreak, there was a reduction by ~30% of coral diversity in addition to ~60% declines in live coral cover on affected reef habitats (Walton *et al* 2018).

Early observations of this disease highlight distinct differences between other similar coral diseases (namely white-plague I and II) in particular the appearance of lesions, the persistence of this disease through time, and the hierarchy in which susceptible species are affected by SCTLD. SCTLD typically appears on species deemed "highly susceptible" early during an outbreak and progresses across an entire colony, via focal or multi-focal lesions, over the course of two-weeks to a month.

Consequently, "intermediately susceptible" species present with lesions that progress over a colony slower and may even arrest (FKNMS 2018). Interestingly, it is reported that highly susceptible coral species associate exclusively or predominantly with algal symbionts (Family Symbiodiniaceae) in the genus *Breviolum*. On the other hand, intermediately susceptible corals generally associate with multiple algal partners based on environmental conditions, including *Breviolum*. Finally, species assumed to have a "low" susceptibility to SCTLD do not associate with algal symbionts in the genus *Breviolum*.

Using a series of experimental manipulations, we altered native algal associations in five species of Caribbean corals, with varying SCTLD susceptibility, before challenging fragments with different algal associations generated from the same parent colony to a SCTLD assay. Ultimately, we tested whether associated algal symbionts influence a coral's susceptibility to SCTLD, in particular algal associations with symbionts in the genus *Breviolum*.

To test this hypothesis, 10 colonies of five coral species (*Colpophyllia natans*, *Diploria labyrinthiformis*, *Orbicella faveolata*, *Meandrina meandrites*, and *Pseudodiploria strigosa*) affected by SCTLD were collected from the Dry Tortugas, FL ahead of the disease front (FKNMS-2019-178) in January 2020. Unlike corals found on offshore reefs in Miami-Dade County, colonies from the Dry Tortugas had a greater variability in their algal associations and were truly naïve to SCTLD which allowed us to reduce the chances of SCTLD resistance in the experiment.

Following collections, colonies were maintained at the University of Miami's Rosenstiel School of Marine and Atmospheric Science (RSMAS) in 2 replicate, 75-gallon flow through systems on UV sterilized seawater with 4 pumps

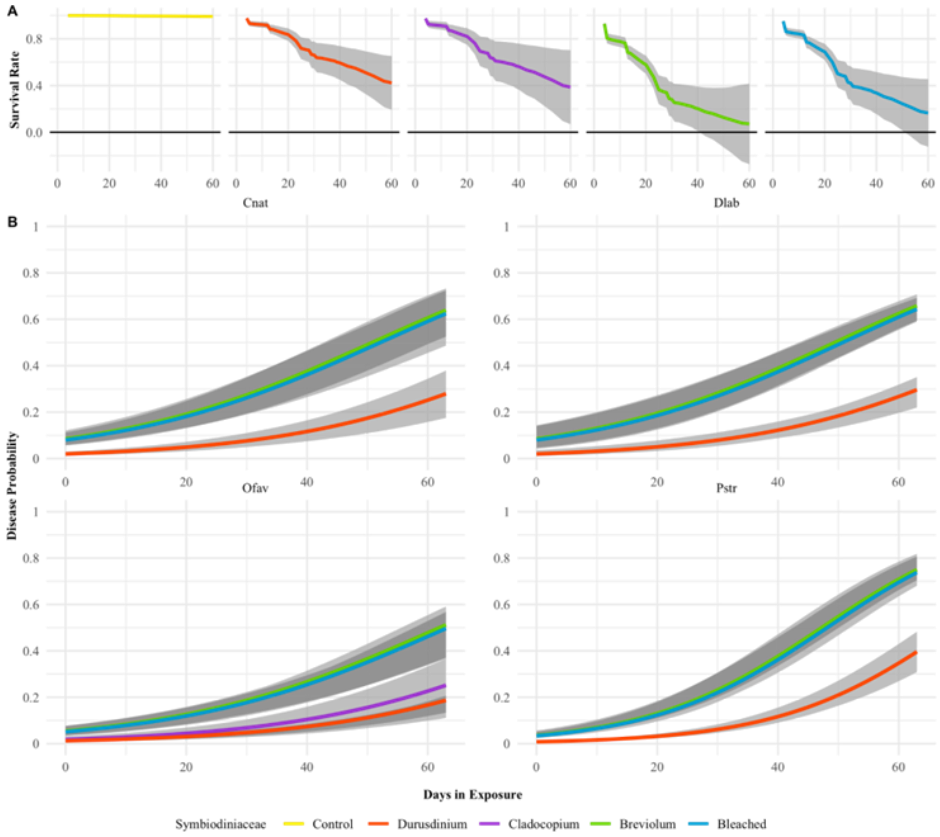

Figure 1. Survival and disease probability of experimental cores throughout a 60-day SCTLD exposure. (A) Cox proportional hazards model (coxph) of experimental cores based on predominant (>95%) associated Symbiodiniaceae. Cores associated with *Durusdinium* (P<0.0001) and *Cladocopium* (P< 0.001) have a significantly higher survival rate during SCTLD exposures than those with *Breviolum* or manipulated to have few symbionts. (B) Generalized linear mixed effects model considering associated Symbiodiniaceae and coral species. Genet was found to not be significant in predicting disease probability.

and 2 heaters to ensure flow and maintain temperature. After a week of acclimating to tank conditions (28°C, 125uEinsteins using AI Hydra 52 on a 12:12 schedule), coral colonies were fragments into 10 replicate, 2.54 cm diameter cores using a diamond bit drill press. Cores were allowed to recover to laboratory conditions for one-month. Throughout the experiment corals were fed 3 times a week for 30 minutes using Reefroids. Temperature and light were measured every 30 minutes using a HOBO data logger (Onset).

In April 2020, a subset of cores was ramped from 28° C to 32.5°C over the course of a 10-day period (0.5°C per day). Photochemical efficiency was measured twice a week using a MAXI Imaging Pulse Amplitude Modulator (IPAM; Walz,

Germany). Corals were removed from heat stress once visually bleached, which ranged from 5- to 11-degree heating weeks (DHWs). Once removed, cores were allowed to recover at 29°C for two months where photochemical efficiency was measured biweekly. An additional subset of cores was aggressively bleached out in August 2020, prior to disease exposures, using high temperature (32.5°C) and high-light (~200uEinsteins).

In late August 2020, coral colonies from five species (*C. natans*, *O. faveolata, O. annularis*, *Montastraea cavernosa*, and *P. strigosa*) showing active disease were collected from the Lower Florida Keys and transported back to RSMAS for disease exposures. Colonies were divided in half so there was an equal area of disease margin and equal live "unaffected" tissue and placed in two replicate experimental tanks with cores manipulated cores, bleached cores, and cores containing native symbiont assemblages. Experimental tanks were outfitted with 4 pumps to ensure water movement. Additionally, cores were moved daily within the tanks to guarantee exposure to all disease colonies (via proximity and shared water). Additionally, a subset of cores was kept as a control in a separate experimental tank. Cores were monitored and photographed daily for signs of SCTLD for 65 days.

During the disease portion of this experiment, tissue samples were taken before the start of disease exposures and every three-weeks while in disease. Samples were flash frozen in cryovials before being stored in the -80°

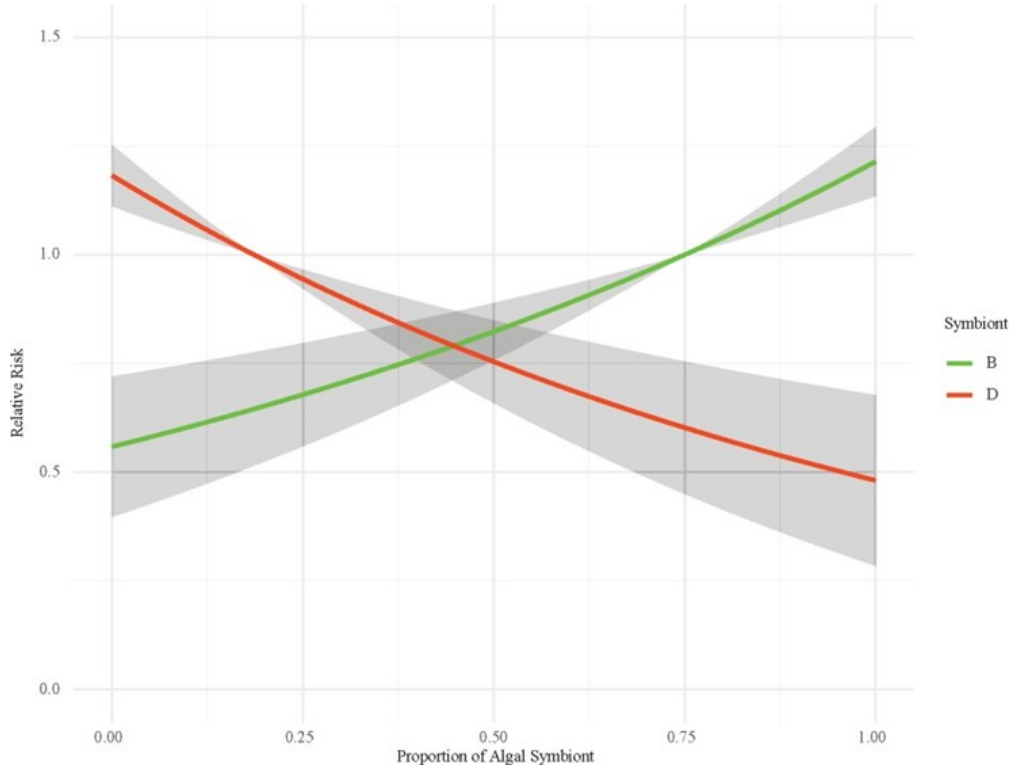

Figure 2. Coxph to calculate relative risk over time based on the proportions of associated Symbiodiniaceae in experimental cores. This model excludes *Cladocopium* as it was only found in *O. faveolata*. Cores containing more than 20% *Durusdinium* (D) are less likely to become infected with SCTLD. Cores containing ~75% *Breviolum* (B) are more likely to present with SCTLD.

C freezer. This approach allowed researchers to identify interesting cores following disease exposures to co-extract DNA and RNA on a KingFisher (ThermoFisher Scientific) using the ZymoBIOMICS™ MagBead DNA/RNA kit (Zymo Research).

SCTLD-like lesions appeared between days 10 and 50 as focal or multi-focal lesions. Survival curves using Kaplan Meier estimates were analyzed in RStudio using survminer. The proportion of surviving cores declined as the number of days in disease increased. Using a Cox Proportional Hazards model (coxph) cores containing predominantly *Cladocopium* or *Durusdinium* were significantly less likely to present with SCTLD ($P<0.001$ and $P<0.0001$, Figure 1A) when compared to cores predominantly associated with *Breviolum* or manipulated to have few symbionts (bleached). However, *Cladocopium* and *Durusdinium* dominated hosts are not immune to SCTLD. The results of a generalized linear mixed effects model suggest that in addition to the species susceptibility hierarchy there also appears to be a Symbiodiniaceae susceptibility hierarchy, where we tentatively rank cores associated with *Breviolum* as the most susceptible to SCTLD followed by *Cladocopium* and *Durusdinium* cores which appear to be about equally susceptible and finally corals associated with *Symbiodinium* given field observations (Figure 1B). Quantification data generated from qPCR was used to calculate the abundance of symbionts in each experimental core. Cores with multiple algal associations were selected and used to develop a coxph to calculate relative risk (RR) over time based on the proportion of *Breviolum* and *Durusdinium* in the experiment cores tissue (Figure 2). This analysis found that cores containing "background" levels, ~20%, of algal symbionts belonging to the genus *Durusdinium* afford the host some level of protection from SCTLD. Furthermore, associating exclusively with *Breviolum* carries a risk 2.5x greater than that of exclusively associating with *Durusdinium*. Ultimately, a 2.5-fold increase in RR in a laboratory experiment may be very significant when translated to the field. The results of this experiment indicate there is a clear effect of algal symbiont type on SCTLD susceptibility but can help explain some of what is observed in the field especially as it related to intermediately SCTLD-susceptible coral species and their spatio-temporal symbiont mosaics.

KEYWORDS: coral mortality, disease incidence, algal symbionts, coral disease

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