

Detecting background levels of coral Symbiodinium clades in Eilat sand, water, and reefs

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Funding Opportunity: The SEAL (Sustainability, Environmental Achievement & Leadership) Award

**Funding Opportunity Guidelines:**

**Target audience:** General reviewers not necessarily scientists

Project Narrative

- a. The narrative should include the following 4 sections which are listed below. You are encouraged to submit relevant images (eg - graphs, representations, diagrams, etc).  
Maximum 1,500 words (excluding references and figures).
- b. Introduction to the project explaining the rationale for the project, specific goals and objectives, and hypothesis for how it has a reasonable chance of producing results that can influence either: environmental policy (eg - corporate policy, governmental policy), and/or the public's understanding of an environmental issue.
- c. If applicable, include your methods/plan of action describing data collection methods, sample size, and detailed timetable including project's completion date and dissemination plan.
- d. Implementation plan detailing how the project results will be used.
- e. Outcome evaluation process explaining how the project's impact(s) on environmental policy and/or the public's understanding of an environmental issue will be assessed.

**Introduction:** Coral reefs are one of the most important world ecosystems. They provide a home to millions of aquatic species and serve as breeding grounds for many commercially important fish (1). Their beauty attracts tourists to many impoverished countries while their buffering capacity protects coastal resources like hotels, cities, and homes from being washed away via erosion or storm surge (2). The services coral reefs provide are estimated at \$375 billion annually and are largely possible because of the algal symbiont that corals host (3).

The relationship between the coral organism and its symbiotic algae, *Symbiodinium* spp, is complex. First, there are many species of *Symbiodinium* that associate with corals. These are separated into 9 different clades (A-I) and an individual coral can host multiple symbiont types (4). Second, corals assimilate the symbiont intracellularly (5). Within the cell, the symbiont receives nutrients that are limiting in reef waters and protection from predators (6). In return, the symbiont provides the coral with both photosynthetically-derived sugars and the brilliant coloration corals exhibit (5,7). However, these colors and sugars are readily lost when ocean temperatures exceed the tolerance of either species, rendering the colony white or bleached (8). During these bleaching events, symbionts cease photosynthesis, become deleterious to coral health, and are therefore consumed or expelled by the coral (9–11).

Bleaching events often demonstrate the importance of the symbiont to overall reef health. Bleached corals cease growth and are more susceptible to disease outbreaks (12–14). Thus, the longer a reef experiences bleaching, the more likely it is to die (8,14). Once dead, reefs are rapidly overgrown by algae making them difficult to recolonize by coral progeny. Furthermore, dead reefs rapidly erode away, thereby transforming into flat reefs (15). These flat reefs serve very limited functions; they don't protect the shoreline from wave energy, are not pleasing to look at, and host much less life (15,16). As such, these reefs provide little economic benefit to the nations they fringe.

Unfortunately, mass coral bleaching events have led to the approximate loss of 20% of the world's coral reefs (17). This die-off is predicted to continue as global ocean temperatures increase (8). This sustained warming is predicted to cause severe annual bleaching of over 50% of the world's coral reefs by the year 2050 (18). However, not all corals will be affected equally. Some corals, such as those in the Persian Gulf, evolved in waters 3-4 °C warmer than that required for bleaching (19). Other corals may acclimate to the warmer oceans by shifting their *Symbiodinium* to thermally tolerant variants, such as those in clade D (20–23).

*Symbiodinium* in clade D are distributed globally but are rarely the dominant symbiont in a coral colony (24). Instead, this symbiont is most prevalent in corals that live in stressful environments, such as backreefs, lagoons, or reefs that have been recently subjected to mass bleaching (20–24). Its presence in these corals has been demonstrated to increase their thermal tolerance and reduce their bleaching propensity and mortality (22,23). This increased likelihood of survival is important because it may provide opportunity for a greater portion of corals to survive future bleaching events.

The ability of corals to survive bleaching events by switching to thermally tolerant symbionts is of great interest to the scientific community and monitoring agencies, such as the National Oceanic and Atmospheric Administration (NOAA). In NOAA's most recent research plan, they highlight the importance of studying *Symbiodinium* clade distributions, specifically as it pertains to management and

reef health forecasting (25). Similarly, in a recent review of *Symbiodinium* (24), the authors highlight the importance of constraining the free-living diversity and biogeography of *Symbiodinium*, with emphasis on clade D. They continue by asserting the importance of documenting clade D presence in corals, even in trace amounts.

Until recently, a hurdle for understanding the distribution of trace *Symbiodinium*, such as clade D, was the sensitivity of the techniques employed (26). Now, however, quantitative polymerase chain reaction (qPCR) methods exist that are 1000x more sensitive than molecular methods previously utilized (26,27). This new methodology has furthered our understanding of symbiont distribution. For example, in the Caribbean, these techniques resulted in the discovery of clade D in corals that previously had not been thought to contain this clade (26). Similar results were obtained for the south China Sea, reefs in the Persian Gulf, and other areas (27–29).

One system of interest for use of these new techniques is the Gulf of Eilat (GoE) in southern Israel. Studies there have been unable to document clade D presence in its reefs (30). This is especially interesting because clade D has been documented further south in the Red Sea (31), which is the source of the GoE's water. Furthermore, clade D is a dominant genus in the nearby Persian Gulf (19,29). It is possible that, like the Caribbean, clade D exists beyond the detection limits of the traditional PCR methods employed in early studies. Therefore, the use of qPCR in the GoE may further our understanding of clade D and other trace symbiont distributions. This information may aid in predicting which corals have the capacity to utilize this clade in future bleaching events.

**Objectives & Importance for Policy/Management:** In furtherance of NOAA's mission and in adherence to the goals set forth in the *Symbiodinium* review (24), this study aims to (a) document the distributions of *Symbiodinium* clades A-D in 7 GoE reefs and (b) determine the ability of Eilat's corals to establish a symbiotic relationship with *Symbiodinium* in clade D (a symbiont they have not yet been shown to associate with). This is the first study in the GoE to investigate symbiont composition with the qPCR method, at a large spatial scale, and for clades A-D. Therefore, its implications are wide ranging. First, this study will offer insight into coral background symbiont concentrations that may have been undetectable in previous studies. Second, it has the potential to discover clade D in the GoE, where it was previously determined to be absent. Third, this study will tell us where symbionts reside in Eilat's reefs when they are not in corals, such as in the water column or sand. Finally, this study will serve as a primary investigation into the ability of Eilat's corals to switch symbionts after bleaching. In summary, this study is a vital step towards understanding the symbiosis of coral and *Symbiodinium*, specifically as it pertains to their ability to adapt to warmer oceans.

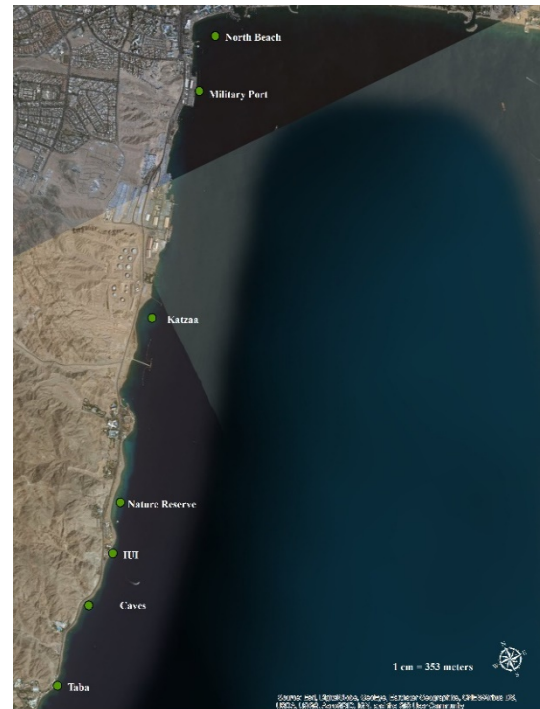


Figure 1. Sample locations along the Eilat, Israel coastline.

**Methods (Field):** This study will utilize qPCR to detect the genetic information of four *Symbiodinium* clade types (A, B, C, and D) at seven sites, within five coral species, reef sediments, and the water column (Figure 1). In total, 195 coral, 13 water, and 13 sand samples will be analyzed for *Symbiodinium* clade presence. Genomic DNA (gDNA) from each sample will then be extracted via an established protocol (32). The resultant gDNA will be amplified in qPCR reactions using an established method (26) to determine the presence or absence of each clade.

**Methods (Lab):** The ability of Eilat’s corals to establish symbiosis with *Symbiodinium* in clade D will be determined by culturing corals in a laboratory. These cultures will be exposed to higher than in situ temperature to induce bleaching. Once bleached, corals will be returned to ambient temperature waters that are free of *Symbiodinium*. Thereafter, these corals will be introduced to cultured clade D *Symbiodinium* on a daily basis. The presence of clade D in coral tissues will be determined visually by pigmentation increases and quantitatively via gDNA extractions and qPCR analysis.

**Broader Impacts:** Public engagement with this project will be achieved through multiple formats. The first is the publishing of weekly blog entries about the project. In these blog posts, I will detail the current plight of coral reefs, focus on their symbiosis, and provide detailed accounts of this study and its progress. The second way that I will engage the public consists of short videos on my project and general progress therein. These will be freely available on multiple online formats such as YouTube and Vimeo. The third way that I will engage the public is through semi-formal talks, such as “Science on Tap” in Santa Cruz, CA, where I am pursuing my doctoral work. Finally, I will participate in the international #SkypeAScientist event. Through these interactions, I hope to increase the public’s understanding of coral symbiosis, their future, and how marine science is conducted.

**Outcome Evaluation:** Surveys will be utilized to determine the impact of my outreach on the public understanding of coral bleaching and symbiosis. These surveys will be specific to each participant and will include a pre-survey to determine the participants prior knowledge and a post-survey to determine the impact of my media/talk content. Long-term understanding of the issue will be tested via 1, 6, and 12-month follow up surveys.

**Timeline:** This study will begin June 30<sup>th</sup>, 2017 and end September 2<sup>nd</sup>, 2017.

Table 1. Project timeline including start date, end date, and duration of major activities or products.

<b>Project Timeline</b>			
<b>Major Activities &amp; Products</b>	Begin	End	Duration (d)
Gather Samples	July 4, 2017	July 18, 2017	14
Extract/Purify DNA	July 5, 2017	July 30, 2017	25
Begin qPCR testing	July 20, 2017	August 5, 2017	16
Analyze Data	July 21, 2017	August 10, 2017	20

Produce Manuscript, Posters, and Presentations	August 10, 2017	October 10, 2017	61
Contribute Blog and Videos	July 4, 2017	October 10, 2017	98

**External Funding:**

No external funds have been procured for this project.

**Budget Justification:**

The funds received from the SEAL grant will be used to purchase reagents and equipment necessary to conduct the sensitive DNA extractions and qPCR reactions. Approximately 4,000 samples need to be analyzed to identify *Symbiodinium* clades via qPCR. This requires the SYBR Green mastermix volume of 50 mL and gDNA extraction kits. It also requires very accurate pipettes, sterile pipette tips, sterile microcentrifuge tubes, sterile 96-well plates, and gloves.

External funding for this project is limited to travel and accommodations in Israel and stems from the NSF International Research Experience for Students in Coastal Zone Research (IRES, NSF 12-551). Any funds not procured will be bared by myself and personal savings. Unfortunately, my NSF Graduate Research Fellowship does not supply research funding.

*Table 2. Major expenses for this study.*

<b>Major Expenses</b>		
<b>Item</b>	<b>Quantity</b>	<b>Cost</b>
SYBR Green PCR mastermix	50 mL	\$2,925.00
gDNA extraction/purification kit	3	\$1,530.00
Pipette	1	\$329.00
Pipette tip	2880	\$327.00
96-well plates	50	\$254
1.5ml microcentrifuge tube	3000	\$675.24
Gloves	100	\$40.00
Round Trip Flight	1	\$1,869

Room & Board	1	\$3,000
<b>Total</b>		<b>\$10,949.24</b>

### References:

1. Bryant, D. et al. 1998, 1-29 p.
2. Ferrario, F. et al. (2014). *Nat. Commun.*, 5, 3794
3. Costanza, R. et al. (1997). *Nature*, 387, 253–260
4. Pochon, X. et al. (2010). *Mol. Phylogenet. Evol.*, 56, 492–497
5. Davy, S.K. et al. (2012). *Microbiol. Mol. Biol. Rev.*, 76, 229–61
6. Muscatine, L. et al. (1977). *Bioscience*, 27, 454–460
7. Muscatine, L. (1967). *Science (80-. )*, 156, 516–519
8. Schmidt, C.W. (2008). *Environ. Health Perspect.*, 116, 292–299
9. Downs, C. a et al. (2013). *PLoS One*, 8, e77173
10. Fitt, W.K. et al. (1995). *Biol. Bull.*, 189, 298–307
11. Hoegh-Guldberg, O. (1999). *Mar. Freshw. Res.*, 50, 839
12. Mendes, J.M. et al. (2002). *Mar. Ecol. Prog. Ser.*, 235, 93–102
13. Bruno, J.F. et al. (2007). *PLoS Biol.*, 5, e124
14. Muller, E.M. et al. (2007). *Coral Reefs*, 27, 191–195
15. Alvarez-Filip, L. et al. (2009). *Proc. Biol. Sci.*, 276, 3019–25
16. Sheppard, C. et al. (2005). *Estuar. Coast. Shelf Sci.*, 64, 223–234
17. Wilkinson, C. *Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville, Australia, 2008, 296 p.*
18. van Hooidek, R. et al. (2016). *Sci. Rep.*, 6, 39666
19. Hume, B.C.C. et al. (2015). *Sci. Rep.*, 5, 8562
20. Cunning, R. et al. (2015). *Proc. R. Soc. B Biol. Sci.*, 282, 20141725
21. Silverstein, R.N. et al. (2015). *Glob. Chang. Biol.*, 21, 236–249
22. Berkelmans, R. et al. (2006). *Proc. R. Soc. B Biol. Sci.*, 273, 2305–2312
23. Rowan, R. (2004). *Nature*, 430, 742–742

24. Stat, M. et al. (2011). *J. Mar. Biol.*, 2011, 1–9
25. Puglise, K. et al. (2007). *Noaa*, 128
26. Correa, A.M.S. et al. (2009). *Mar. Biol.*, 156, 2403–2411
27. Mieog, J.C. et al. (2007). *Coral Reefs*, 26, 449–457
28. Tong, H. et al. (2017). *Sci. Rep.*, 7, 40118
29. Mashini, A.G. et al. (2017). *Mar. Ecol.*, 38, e12424
30. Lampert-Karako, S. et al. (2004). *J. Exp. Mar. Bio. Ecol.*, 311, 301–314
31. Sawall, Y. et al. (2014). *PLoS One*, 9, 1–12
32. Baker, A. et al. (2016). *Protocols.io*, 1–6