STATEMENT OF GRANT PURPOSE

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Symbioses in a Changing World: Nitrogen Pollution and Bleaching in Sea Anemones

In many marine ecosystems, symbiotic relationships form the cornerstone of a healthy and biologically diverse community. Without them, for example, coral reefs would not exist. However, these relationships are being strained due to human activity, which therefore threatens the future of marine ecosystems (Hoegh-Guldberg, et al, 2007). The overarching goal of my research is to determine the effects of a changing environment on symbiotic relationships, and ultimately on the long-term health of coastal marine ecosystems.

Symbiosis, or a close relationship between two unrelated species, is a common feature among cnidarians, a group of animals that includes jellyfish, sea anemones, and corals. In cnidarian symbioses, the animal cells host single-celled algae in a mutualistic relationship: the algae provide the animals with photosynthetically-derived carbohydrates, and in turn, the animals provide the algae with nitrogen, phosphorus (i.e., fertilizer), and a safe habitat. In some cnidarians, up to 95% of the carbohydrates produced by the symbionts are translocated to the animal cells (Wooldridge 2010). This relationship has allowed many cnidarians to thrive in otherwise unfavorable environments. For instance, corals flourish in very nutrient-poor tropical waters because of the tight recycling of matter and energy between the coral hosts and the algal symbionts.

An interesting feature of cnidarian-algal symbiosis is how the relationship fares under environmental stress. For instance, the phenomenon known as bleaching occurs when the algae are expelled when seawater temperature increases. The rise in temperature promotes the growth of the algal symbionts, leading to a build-up of photosynthetically-produced oxygen that can cause oxidative damage to the cnidarian host (Wooldridge 2010; Weis, Davy, et al. 2008). However, bleaching causes the host to become nutritionally stressed and thus, susceptible to diseases and other causes of death (Sheppard 2003).

A recent study highlighted nitrogen pollution as a co-factor for lowering the temperature at which bleaching occurs. It appears that excess nitrogen, like elevated temperature, promotes algal growth, resulting in oxidative damage to the host (Wooldridge 2009). The role of nitrogen in cnidarian-algal symbiosis is particularly intriguing given the growing threat of nitrogen pollution, mainly from fertilizer run-off and poor waste treatment, on the health of coastal ecosystems (NRC 2000). Furthermore, because nutrient pollution is a local stressor, unlike climate change and ocean warming, there are obvious courses for mitigation.

If awarded a Fulbright Grant, I will dedicate ten months to studying the effects of nitrogen pollution on cnidarian symbiosis in the laboratory of Dr. Simon Davy, Associate Professor in the Victoria University of Wellington's School of Biological Sciences. Dr. Davy's research focuses on symbiotic relationships among corals, sea anemones and sponges, and he studies how environmental changes affect the frequency and severity of bleaching events (Wicks, et al. 2010). Dr. Davy's lab is ideal for my proposed work because he is one of the few people in the world with a combined expertise in both bleaching physiology and nitrogen metabolism in symbiosis (Davy, et al. 2002; 2006). In addition, his lab is the only one in the world that studies the New Zealand mud flat sea anemone, *Anthopleura aureoradiata.* This species does not bleach nearly as readily as tropical cnidarians such as corals (Muller-Parker & Davy, 2001), making it a very useful model organism for understanding bleaching resistance. Groundbreaking scientific discoveries have been made using model organisms such as the fruit fly and nematodes. Like these model organisms, *A. aureoradiata* is easily propagated (via asexual reproduction) and maintained in the lab. But more importantly, its resistance to bleaching suggests a physiological mechanism for limiting the production of reactive oxygen, reducing its impacts, or both. Access to this species and the expertise Dr. Davy provides will allow me to carry out a comparative study of factors that affect stability of cnidarian symbiosis. In addition, learning a greater scope of techniques will make me well rounded as a scientist and enable me to develop and test more sophisticated hypotheses as a doctoral student after completion of my Fulbright research.

I will be testing three hypotheses: (1) elevated nitrogen concentrations reduce the temperature threshold for bleaching; (2) species resistant to bleaching have high levels of antioxidants to limit the impacts of reactive oxygen; and (3) symbiotic stability is linked to a continuing capacity for nutritional exchange across a wide range of environmental conditions. To test these hypotheses, I will apply two different but complementary approaches: photophysiology and stable isotope geochemistry. Briefly, photophysiological techniques, which I will acquire in Dr. Davy's lab, will be used to assess overall performance of the anemone-algal symbiosis and to quantify levels of antioxidants. I also plan on carrying out light-dark incubation experiments and stable isotope analysis to quantify the productivity (i.e., photosynthesis minus respiration) and to assess photosynthetic efficiency of the symbiont under varying temperature and nitrogen concentrations. I have developed these skills at the Geophysical Laboratory at the Carnegie Institution of Washington and will be able to send samples to NIWA, a marine research institution in Wellington with stable isotope equipment, for analysis. The experiment will involve using seawater made with isotope tracers, which will allow me to quantify the extent to which these elements are shared between the host and the symbiont under varying conditions.

My experimental design will consist of treating *A. aureoradiata* with three nitrogen regimes (pure seawater, increased nitrogen, and nitrogen-depleted) in individual aquaria. In each nitrogen regime, I will expose the anemones to three temperature treatments – temperatures similar to their natural habitat as well as increased and decreased temperatures. Photophysiology and isotope data previously collected from the non-bleaching species will allow me to highlight physiological differences that contribute to differential resistance to bleaching in cnidarians.

New Zealand's diverse landscape, large coastline, and temperate climate provide an ideal opportunity to study climate change-related issues. The problem of nitrogen pollution is also particularly relevant in New Zealand because, according to the New Zealand Institute (2011), there is a slow but steady upward trend of nitrates in New Zealand's river systems. In contrast, nitrogen pollution has been a key driver of environmental change along the east coast of the US, especially within the Chesapeake Bay, near where I currently reside. Although not yet a critical issue in New Zealand, it is likely to become one in the near future. It is my hope that my research will lend scientific understanding of the threat of nitrogen pollution, especially to an important group of marine organisms.

Working in New Zealand will also expose me to perspectives on environmental issues different than those I have experienced working in Washington, DC. To fully immerse myself, I will volunteer with New Zealand's Department of Conservation. While there are no community projects specific to the marine environment in Wellington, many focus on rivers that empty into the ocean. As rivers are often the greatest source of nitrogen pollution, their protection and conservation is paramount. I have identified several groups to work with including the Wikanae Estuary Care Group, Waitohu Stream Care, and the Friends of Otaki River. I will choose the volunteer group based on feasibility and group need when I arrive in New Zealand. These opportunities will allow me to actively pursue conservation practices in New Zealand while experiencing the country's beautiful and unique ecosystems and culture.