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Symbiosis and Bioinvasive Dynamics of *Durusdinium trenchii* and its Acroporid Coral Hosts

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

Symbiosis and Bioinvasive Dynamics of *Durusdinium trenchii* and its Acroporid Coral Hosts

By

David Craig Lawson

Submitted to the Faculty of
Halmos College of Natural Sciences and Oceanography
in partial fulfillment of the requirements for
the degree of Master of Science with a specialty in:

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ABSTRACT

Biological invasions are a growing threat to coral reef ecosystems, as increasing anthropogenic transport and changing environmental patterns allow invasive species to establish and spread. *Durusdinium trenchii* is a dinoflagellate that has invaded the Greater Caribbean reef system and established symbioses with coral hosts. Establishment and persistence of invasive endosymbionts like *D. trenchii* could indicate a shift in the clade composition of coral holobionts worldwide. Statistical analyses were performed on the GeoSymbio database (Franklin et al. 2012) to determine whether differences in clade composition have occurred over time. Factors that influence biological invasions in marine ecosystems were assessed and analogous fields of study compared with invasion ecology. As no management frameworks currently exist that govern invasive marine microbes like *D. trenchii*, a management framework designed to account for these component fields of study is proposed. Prominent and successful cases of marine bioinvasion management were reviewed and their potential applications assessed. These case studies were synthesized with modern control methods to create a framework for bioinvasion management that is specific to *D. trenchii*. The framework focuses on detection and preventative control of invasive transport vectors.

Keywords: *Durusdinium trenchii*, *Acropora*, bioinvasion, symbiosis, epidemiology, invasion ecology, management framework

1. INTRODUCTION

Primary production refers to the quantification of the biological and chemical processes that result in the formation of organic compounds (Field et al. 1998). These processes form the foundation of food web interactions across ecosystems. One such foundational metabolic process that combines exogenous energy and inorganic molecules to form organic compounds is photosynthesis. Photosynthesis is the process by which solar energy, carbon dioxide, and water react to create the simple sugar glucose. Glucose is a molecule utilized by various metabolic processes as a source of chemical energy for numerous organisms; it is the synthesis of these molecules that distinguish primary producers from higher trophic levels. Molecules synthesized by the metabolic action of primary producers integrate into the biotic components of the ecosystem (Field et al. 1998). This integration has a profound impact on the health, growth, and ecological characteristics of ecosystems. A diversity of organisms across biomes employ photosynthesis as their primary energy production method. It first evolved in cyanobacteria approximately 3.8 billion years ago (Blankenship 1992). Today, photosynthesis can be found in three kingdoms of the Domain Eukarya; Monera, Plantae, and Protista.

Algae is an informal term for some photosynthetic members of the Kingdom Protista that are not true plants (Embryophyta). They are phylogenetically diverse and inhabit a multitude of ecosystems. Algae are responsible for ~50% of global photosynthesis (John 1994, Radmer 1996). Many are independent organisms, but others are symbionts in host organisms. Symbiosis is an ecological system of interspecies relationships in which the participating organisms exchange metabolic products or services (Connor 1995). Symbioses occur across terrestrial and aquatic biomes and can be divided into three basic exchange mechanisms: parasitism, commensalism, and mutualism (Connor 1995). In each exchange mechanism, two or more organisms that inhabit the same ecosystem participate in the symbiosis, whether actively or passively. Mutualism involves organisms investing in each other for shared gain, forming partnerships that shape the fundamental structures of their habitats. In marine ecosystems, mutualistic symbioses play important roles in trophic interactions, and the effects of such symbioses range from improving fitness for single species to forming the basis of ecosystems such as in coral reefs (Muscatine and Porter 1977).

Coral reefs are complex marine ecosystems that are founded on a singular mutualistic symbiosis (Muscatine and Porter 1977). They are characterized by reef-building corals, which are invertebrates of the Phylum Cnidaria, Order Scleractinia. These animals consist of living units

known as polyps that grow as colonies. Polyps are blind sacs or cylinders composed of two epithelial cell layers with a ring of tentacles surrounding the mouth/anus. The lower portion of the outer tissue layer, the ectodermis, secretes a rigid calcareous exoskeleton that accretes using environmentally present component molecules. The interior layer, the gastrodermis, contains digestive cells, and lines the interior gastrovascular cavity, which serves both for digestion and circulation (Goreau, Goreau, and Goreau 1979; Coral Disease and Health Consortium Glossary of Coral Anatomy and Histopathology Terms, NOAA). Larval corals attach themselves to suitable substrate and grow to form colonies that provide habitat for the higher trophic levels as well as other primary producers.

Coral reefs exhibit a roughly equatorial distribution globally, occurring in both the Eastern and Western hemispheres at latitudes ranging from approximately 30°N to 30°S (Andrefouet et al. 2006). Global coral reef surface area is estimated at 527,072 km² (Mora et al. 2006). Coral reefs are estimated to fix CO₂ at a rate of 700x10¹² g C y⁻¹ and produce 20x10¹² g organic C y⁻¹ globally (Crossland, Hatcher, and Smith 1991). Coral reefs can become massive, with colonies of various sizes and species creating intricate structures that can reach high levels of rugosity. Coral colonies come in a variety of growth forms and expand by production of extensive calcium carbonate (CaCO₃) skeletons. Andrefouet et al. (2006) calculated a possible range of 2380-4753x10⁶ kg of CaCO₃ produced by a single coral species (*Hydrolithon onkodes*) across a 504-km² French Polynesian atoll. Reefs are further characterized by high species biodiversity and richness. They occur typically in oligotrophic waters. Their productive capacity therefore extends beyond the primary productive ability of the coral itself.

Coral reef ecosystems exhibit other traits that make them of vital importance to the health of the oceans. The structure itself serves as a protective barrier from oceanographic forces acting on the landmasses adjacent to the reef, as well as to delicate coastal ecosystems such as mangrove swamps and seagrass beds (Reaka-Kudla 1997). The reef structure provides habitat for an enormous diversity of fish and invertebrates, many in vast numbers, that service the ecosystem and nearby human populations (Reaka-Kudla 1997). As an example, Sale (1977) identified 1500 reef fish species in 1977 on Australia's Great Barrier Reef alone. Coral reefs therefore serve as repositories for oceanic biodiversity in a manner analogous to terrestrial tropical rain forests. Reefs provide a major source of protein for human communities via harvesting of resident fish and invertebrates. Additionally, tourism and the aquarium trade have relied significantly on coral reefs

(Moberg and Folke 1999). Foundational to the survival of coral reef ecosystems and their peripheral populations is the symbiotic relationship between the scleractinian coral and its endosymbiotic dinoflagellate microalgae.

The main symbiosis that drives the reefs' high level of primary productivity occurs between numerous species of scleractinian corals and a complex of unicellular photoautotrophic dinoflagellate algae. These dinoflagellates are informally called zooxanthellae and chiefly belong to complex of species formerly the genus *Symbiodinium* (Trench and Blank 1987). The species complex has now been separated into 7 separate genera and the overall complex has become the family *Symbiodiniaceae* (LaJeunesse et al. 2018). They occur as mutualistic endosymbionts within the tissue of the host coral and can be passed along to their offspring (Benayahu et al. 1992). The host and its symbiont are together referred to as a holobiont. The coral gains access to photosynthetic products produced by the microalgae, which contribute to or fulfill the coral's nutritional requirements, supplementing suspension feeding and making the coral/microalgal holobiont a polytrophic organism (Grottoli et al. 2006). Understanding the mechanisms of symbiotic interactions between *Durusdinium trenchii* and its hosts is critical to determining health of coral reef ecosystems.

1.1 Coral/Microalgal Symbiosis

Symbiosis between scleractinian coral and dinoflagellate microalgae involves the dinoflagellates taking up residence within cells inside the coral polyps, where they are bound by membranes generated by both the microalgae and the coral, a "habitat" referred to as the symbiosome (Davy et al. 2012). Microalgal endosymbionts can be transmitted horizontally to new generations of via maternal infection (Davy and Turner, 2003), but coral larvae also commonly capture free-living dinoflagellates from the surrounding water. Coral/microalgal mutualisms are founded on a sharing of ecological benefits. The dinoflagellate gains a stable environment in which it can grow and multiply with enhanced protection from grazing predation. This environment is located within specialized phagosomes of the host's gastrodermal cells. The host coral actively maintains favorable conditions for endosymbiotic photosynthesis by adjusting the pH of the bounded space of the phagosome (Bertucci et al. 2013; Barott et al. 2014). The microalgae have access to nitrogenous waste products generated by the coral, and a consistent supply of solar input, as their hosts are sessile. The coral gains a constant internally-generated supply of photosynthetic

product (amino acids, photosynthetically fixed C) from its endosymbionts to supplement its metabolism. Endosymbionts can, with host facilitation, export up to 95% of their photosynthetic product to the host (Yellowlees et al. 2008). Early research into coral/microalgal symbiosis indicated that the microalgae also increase the calcification capacity of the coral (Gattuso, Allemand, and Frankignoulle 1999). The CO₂ generated through the process of calcification by the coral host was thought to fuel the photosynthesis of the endosymbionts, which in turn produce energetic metabolites that fuel the host's calcium carbonate deposition. Recent studies have yet to elucidate the true nature of the host/endosymbiont exchange that may result in increased host calcification, but have determined that the mechanisms involved are more complex than previously observed (Drake et al. 2013; Bertucci et al. 2015). This complexity is thought to involve host gene expression in the presence of reactive oxygen species (generated in part by photosynthesis) and daylight. Gene expression upregulates production of skeletal organic matrices (SOM) from the increased supply of photosynthate generated by the endosymbionts (Bertucci et al. 2015). Skeletogenesis is reliant therefore on both participants in coral/microalgal symbiosis. To understand how this symbiosis is established, one must begin with the mechanics of the symbiosis itself.

Davy et al. (2012) identified six phases of “establishment and persistence” for coral/microalgal symbiosis in their review of the cellular dynamics of cnidarian-dinoflagellate symbiosis. In order, the phases are: Initial Contact, Engulfment, Sorting, Proliferation, Dynamic Stability, and Dysfunction. Between Initial Contact and Proliferation, a number of cellular recognition mechanisms take place to ensure that the host can cultivate the proper range of endosymbionts. (Davy et al. 2012). This suite of stages that begin and establish the symbiosis have been referred to as “infection,” and the process shares many similarities to innate immune responses in vertebrates (Schwarz 2008). It relies heavily on the processes of molecular signaling (Recognition) that occur between a host and potential symbiont to determine whether or not the microalgal cell is a potential match for the host. Once a matching microalgal cell has been engulfed by the coral host's gastrodermal cells through phagocytosis, the process of establishing endosymbiosis begins. Three major phases occur within the coral gastroderm; Sorting, Proliferation, and Dynamic Stability (Davy et al. 2012). When symbiosis is established within the coral gastrodermis, Dynamic Stability of the microalgal endosymbiont populations is mediated by environmental conditions and physiological responses to change by the host (Titlyanov et al. 1996;

Tivey, Parkinson, and Weis 2020). The host expands symbiont populations with growth, and studies have demonstrated a fairly constant density of microalgal endosymbiont cells within coral gastrodermal tissues under normal conditions (Jones and Yellowlees 1997, Meigo et al. 2009). Despite complex and multiple mechanisms for cellular recognition between symbionts and hosts, some symbiont cells must be expelled, because they do not meet host requirements, and growing populations of cells within the host must be culled to maintain a stable and healthy microbiome. The host removes degraded, dead, and to a smaller extent, healthy microalgal cells through two methods: the extrusion of pellets consisting of microalgal cells held together by mucus (Steele 1977), and expulsion of individual cells (Titlyanov et al. 1996, Meyer and Weis 2012). Healthy concentrations of cells are maintained through periodic expulsion of degraded endosymbionts, and certain corals “farm” their endosymbionts by digestion of healthy microalgal cells that they cultivate (Titlyanov, et al. 1996). This “farming” results in efficient use of C and N by both the host and endosymbiont. While the host does exploit the photosynthetically fixed C generated by the endosymbiont, it also gleans a majority of its N from endosymbiotic production as well (Tanaka, Suzuki, and Sakai 2018). The host also regulates its microalgal populations through nutrient availability and delivery to these populations (Davy et al. 2012). A variety of environmental and internal conditions can lead to interrupted states of coral/microalgal symbiosis, creating the mechanisms inherent to the Dysfunction phase.

Studies of coral/microalgal symbiosis dynamics relative to mechanics and environmental effects have included symbiont switching and shuffling in coral hosts (Jones et al., 2008), thermal tolerance and growth tradeoffs between coral and various microalgal clade associations (Jones and Berkelmans 2010), and measurements of holobiont metabolism under various environmental conditions (Jones and Hoegh-Guldberg 2001). Many of these studies have used corals of the family Acroporidae as model hosts. As an example, Jones and Berkelmans (2010) used Indo-west Pacific *Acropora millepora* as a model host and found that coral fitness varied with different resident microalgal clades under changing climatic conditions. Acroporidae is the most species-rich family of zooxanthellate corals and dominates Indo-Pacific coral reefs (Wallace 1991). Genus *Acropora* includes ~150 species, of which only three (*A. palmata*, *A. cervicornis*, and the hybrid *A. prolifera*) occur in the western tropical Atlantic. Members of this genus host a variety of *Symbiodiniaceae*, including *D. trenchii* (formerly called Clade D1a) (Pochon and Gates 2010).

Recent climatic changes have altered the oceanographic conditions necessary for ideal establishment and maintenance of coral/microalgal symbioses. Rising ocean temperatures coupled with increased acidification stress coral hosts to the point at which they expel their endosymbionts into the water column, a phenomenon called bleaching. This stress response greatly decreases the autotrophic capacity of the host coral (Glynn 1984) and often results in stunted growth and eventual death for the host, given the low-nutrient (oligotrophic) nature of waters surrounding coral reefs (Kuhlmann 1988). Additional interruptions in coral/microalgal symbiosis occur when water temperatures rise. Associations between coral and their endosymbionts that are more purely mutualistic under nominal conditions become increasingly detrimental for the host at higher temperatures. Translocation of endosymbiont-generated photosynthates can become disrupted (Baker et al. 2018), robbing the coral of benefit even as microalgal production increases. Other environmental factors, such as pollution, increased turbidity, and disease outbreaks, may compound the effects of coral bleaching.

The bleaching response to environmental stress may be mitigated by the host ingesting and cultivating new endosymbionts from the surrounding water. This process, known as symbiont switching, can create novel coral/microalgal associations (Lewis and Coffroth, 2004). It differs from symbiont shuffling, in which the proportions of different subclades within the coral host change (Berkelmans and Van Oppen 2006). *Symbiodiniaceae* clades are said to “shuffle” when multiple clades inhabit the same host simultaneously, and concentrations of minority clades grow to usurp the majority clades (Baker 2003). Conditions that lead to symbiont shuffling may include bleaching recovery and seasonal variation in environmental conditions (Berkelmans and van Oppen 2006). Novel coral/microalgal associations occur most readily when stressed coral is exposed to a combination of bleaching conditions and dinoflagellates that are both resilient to the external stimulus and locally abundant (or already present in the gastrodermal tissue) (LaJeunesse, et al. 2010). These novel host/symbiont associations do not often persist past termination of the stress period (Goulet, 2006) due to “tradeoffs” in suitability to environmental conditions inherent to different host/symbiont combinations (Jones and Berkelmanns 2011; Pettay et al. 2015). It is a coral host’s ability to establish different associations with *Symbiodiniaceae* clades (Little, van Oppen, and Willis 2004) that enables recovery in response to short-term exposures to adverse environmental conditions.

1.2 Characteristics of *Symbiodiniaceae*

Symbiodiniaceae species are photosynthetic components of symbiosis in coral reef ecosystems. They use light to catalyze a series of reactions to produce energy and metabolic fuel molecules (Iglesias-Prieto et al. 1992). Their life history consists of two stages: mastigote and coccoid cells (LaJeunesse, Parkinson, and Trench 2012). The mastigote is the motile flagellate cell phase, and the coccoid phase is spherical and nonmotile. *Durusdinium* clades exhibit two types of environmental associations: free-living in the water column and endosymbiotic. Individual *Symbiodinium* clades may include both endosymbionts and free-living forms. Clades that form symbiotic associations are either generalists, which occur in a number of different host species, or host-specific. The latter form associations with fewer taxa than generalists and are not often found outside those symbioses, e.g., Clade A2 associates only with scyphozoans (Baker 2003). The range of microalgal partners with which a host can form a symbiosis is known as its “specificity” (Douglas 2010). The formation of stable microalgal/host symbioses is mediated by the host (Davy, Allemand, and Weis, 2012). The host forms associations through complex host-symbiont signaling and recognition interactions. Because the host controls the establishment of its symbioses, hosts can form associations with a number of different microalgal clades. Different host-microalgal associations produce varying levels of fitness for both host and symbiont when mediated by environmental conditions (Jones and Berkelmans 2010; Cantin et al. 2009). Less well understood microalgal/host associations exist with other invertebrates, including other cnidarians (e.g., octocorals, anemones, hydrozoans), foraminiferans, and mollusks (Baker 2003).

The taxonomy of *Symbiodinium* is in flux. The genus was divided into nine monophyletic clades identified by the letters A to I; Pochon and Gates (2010) identified the nine clades using Next Generation genome sequencing techniques, specifically by isolating distinct ribosomal DNA sequences. These sequences act as phylotypes—taxonomic markers used to establish similarities among clades. Phylotypes distinguish approximately 409 distinct but genetically similar sub-clades (Franklin et al. 2012). These sub-clades are organized phylogenetically, an approach that differs from the older morphometric/morphological basis of classifying *Symbiodinium* species (Baker 2003, Lee et al. 2015). The phylogenetic classification model for *Symbiodinium* favored an alphanumeric scheme for naming distinct types, but they have been given species names as they have become better understood, e.g., *D. trenchii* was named as a species in 2018 after being previously treated as *Symbiodinium* Clade D1A (LaJeunesse et al. 2014). The various types and

species of *Symbiodinium* demonstrate physiological differences as well, both individually and in conjunction with their host species. *Durusdinium trenchii* is unique in that it maintains photosynthetic efficiency across a greater range of temperatures than some of its congeners (Baker et al. 2004). Continued study of the phylogeny of the genus *Symbiodinium* has determined that the variety of morphological, physiological, and genetic diversity merits the separation of clades into their own distinct genera (LaJeunesse et al. 2018). LaJeunesse et al. (2018) reorganized the genus/clade structure of *Symbiodinium* into a multi-generic structure that separates the genus *Symbiodinium* (now restricted to Clade A) into 7 component genera. High-resolution morphological and genetic analyses have allowed for these distinctions to be made. Clade D and the dinoflagellate formerly known as *Symbiodinium trenchii* have been grouped into the genus *Durusdinium* (LaJeunesse et al. 2018).

Symbiodiniaceae most diverse and abundant in the tropics but occurs almost worldwide (Baker 2003). Coral reefs in the Caribbean Sea primarily harbor B and C clade representatives (Baker 2003), whereas the Pacific and Indian Ocean reef are largely dominated by Clade C and, secondarily, Clade D (Baker 2003). Franklin, et al. (2012) assembled a worldwide database of *Durusdinium* phylotypes and their corresponding environmental locations across coral reef habitats, but no formal comparison with older studies that compile similar results (Baker 2003; Lee et al. 2015) has been undertaken.

Another aspect of novel coral host/symbiont associations is the potential for invasion of alien habitats by non-indigenous symbiont clades. Host corals are sessile, and oceanographic barriers such as current patterns and turbulence (Wolanski and Kingsford 2014) limit dispersal of coral larvae. Limited larval energy reserves (Wilson and Harrison 1998) also limit dispersal. By contrast, many *Durusdinium* clades can survive and grow in the water column, which increases their potential distributional ranges. Anthropogenic assistance through vectors (Stat and Gates 2008) can also increase microalgal distribution ranges. Non-native clades are considered invasive if they become abundant enough in new areas to disrupt local habitat dynamics (Mack, et al. 2000).

1.3 Characteristics of Acroporid Corals

The coral family Acroporidae Verrill, 1902, originally included many genera but now contains only four: *Montipora*, *Anacropora*, *Astreopora*, and *Acropora* (Fukami, Omori, and Hatta 2000). The family is named for *Acropora* (from the Greek “akros” for “summit” or “peak”, and

“poros” for “hole” or “passage”) based on the tall calice at the tip of each branch. Acroporidae is the most species-rich of scleractinian families (182 known species) and is restricted to tropical and subtropical latitudes. Most species occur in the South Pacific and Indian Oceans, while only three occur in the Atlantic (Wallace and Muir 2005).

Because acroporids exhibit a wide variety of colors and growth forms, and colonies of the same species can be visually disparate, field identification to the species level is often difficult. The majority of acroporids are table-forming or branching (Pratchett et al. 2015). The variety of growth forms and their abundance in reef systems makes these corals important contributors to three-dimensional reef structure. They act as both habitat for other reef organisms and provide in-shore relief from forces such as currents and wave action.

Acroporids are hermaphroditic, and most species release gametes in large-scale synchronous spawning events driven by multiple environmental factors, including lunar phase, water temperature, and the presence of spawn from nearby coral colonies. The gametes undergo sexual recombination and develop into free-swimming planula larvae. The larvae settle under specific environmental conditions and metamorphose into juvenile corals, which accrete and grow before reaching sexual maturity. Acroporids do not transmit their endosymbionts horizontally from colony to ovum but obtain them from their environment during the planula larval stage (Schwarz et al. 2008). These corals form associations with a number of different *Durusdinium* clades, and are considered generalist hosts (Putnam, Stat, Pochon, and Gates 2012). Associations are largely regulated by the clade and abundance of endosymbiont present in the water column while the coral is in its planula stage. Pacific and Indian Ocean acroporid species host symbionts dominated by members of clades C and D, while Atlantic species chiefly harbor clades B, C and A (Baker 2003). With rising global sea surface temperatures (SST) and increasing ocean acidification (OA), opportunistic clades like D1a (*D. trenchii*) can enter coral tissues from the environment and establish novel symbioses with corals. The usurpation of more established clades has implications for the future health and growth of acroporid-dominated coral reef ecosystems worldwide.

1.4 Principles of Biological Invasion with Emphasis on *Durusdinium*

The native range of a given species is often not the only habitat suitable for that species' survival, establishment, and proliferation (Mack, et al. 2000). The competitive nature of species that inhabit similar trophic levels creates the ability for organisms to expand their range. Hulme et

al. (2008) identified three overarching mechanisms by which species establish new populations in areas previously uninhabited by them: natural spread (e.g., across neighboring ecosystems), human importation, and the appearance of a transport vector. Each is subdivided into specific pathways used by invasive species. The increased globalization of trade and transport since the Industrial Revolution has led to a marked rise in biological introductions and invasions (Hulme 2009). In addition, Mack et al. (2010) noted that the factors affecting invasions appear to be conserved regardless of the scale of invader size, whether microbial parasites or megafauna.

The process of biological invasion involves a confluence of factors, although the sheer number of possible factors make it difficult to provide overarching explanatory principles (Mack 2000). Blackburn et al. (2011) described a dichotomy of frameworks that has developed as the field of invasion ecology developed. Richardson et al. (2000) presented invasions as a “series of barriers that a species negotiates to become either naturalized or invasive. (page 94)” In contrast, Williamson and Fitter (1996) view invasions as a sequence of stages through which a nonindigenous species must pass in order to establish in a novel environment: Transport, Introduction, Establishment, and Spread (Figure 1). Both schemes involve factors that govern success or failure in a given biological invasion. Environmental conditions that may stymie a potential invader during the Transport stage include: scale of transport, pattern of dispersal, availability of the transport/dispersal vector, and condition of the species before and after transport/dispersal. Environmental factors may also constitute one of the barriers described by Richardson et al. (2000). Examples that form barriers to survival include the presence/density of potential predators, resource availability, and differences between the origin and destination ecosystems. Recently, Blackburn et al. (2011) proposed a framework that encompasses both previous schemes into one that accounts for the presence of barriers as a part of the process of progression to each of the stages described by Williamson and Fitter (1996).

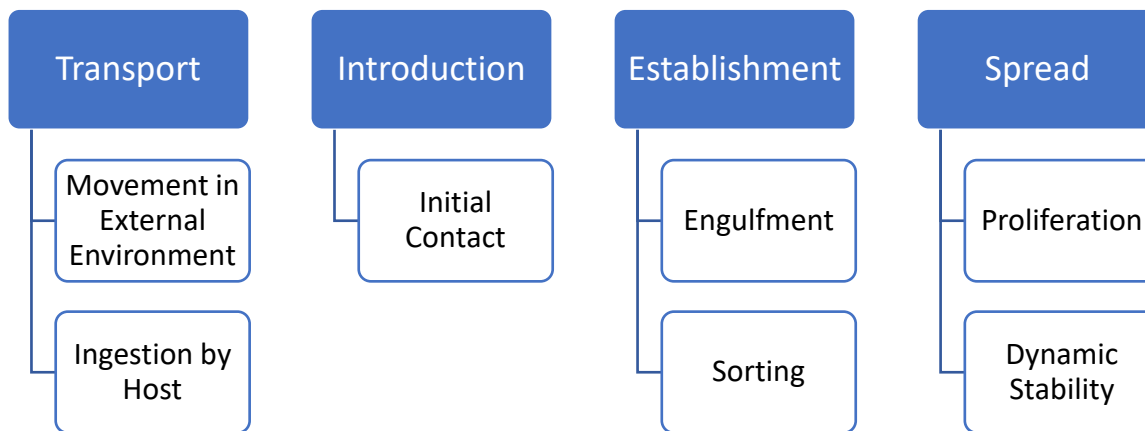


Figure 1: Stages of biological invasion (blue rectangles) according to Williams and Fitter (1996) matched with their closest analogues from Davy, Allemand, and Weis (2012) (white rectangles). The Transport phase did not match with stages denoted in Davy et al.

Biological invasions can have wide-ranging effects. They can impact ecosystems at multiple ecological levels and also affect human populations. The mechanisms that define and characterize biological invasions are conserved across the size scale of the invading organism (Mack et al. 2000). As a result, detection and management of biological invasions becomes more difficult as the size of the invading organism decreases. For an aquatic microbe such as *Durusdinium*, examination of its invasive capacity is a challenge. In addition to its small size, other characteristics make this dinoflagellate a difficult invasion ecology research subject. Because *Durusdinium* “species”, clades and subclades exhibit limited morphological differences, which can obscure the presence of an invasive among native populations, genome sequencing techniques are required for differentiation. Their ability to either form symbiotic associations or proliferate in the water column/substrate widens the area that investigators must search to determine the presence/establishment of an invasive species. Their global distribution introduces difficulties in distinguishing native populations from non-natives. Additionally, exposure to transport vectors is widespread in the form of ships taking on and releasing ballast water. These factors together present a concerning invasive profile for *Durusdinium*.

1.5 Biological Invasion Mechanisms and Analogues

Parallels can be drawn between coral/microalgal symbiosis, and epidemiology. Direct similarities exist in the vocabulary used to describe endosymbiotic establishment in

coral/microalgal associations and epidemiology. Examples of direct similarities between the two phenomena include a beginning state of colonization, opportunistic species, and endosymbiotic interactions (Casadevall and Pirofski 2000). Casadevall and Pirofski (2000) identify a symbiotic state of certain bacterial pathogens, in which the pathogen inhabits the host without causing damage after a commensal stage. This habitation within a host cell is comparable to coral/microalgal association in that neither partner is disadvantaged. The manner in which *Durusdinium* cells enter and proliferate inside their coral hosts bears strong resemblance to bacterial or viral infections in more anatomically complex organisms (Fransolet, Roberty, and Plumier 2012; Weis, 2008).

The establishment, maintenance, and proliferation of endosymbiotic dinoflagellate microalgae in scleractinian coral hosts can be described as a form of facilitated infection, or assisted biological invasion on a micro scale (Fitt and Trench 1983). Davy, Allemand, and Weis (2012) identify six stages of symbiotic establishment and persistence in cnidarian-microalgal symbiosis. Similarities among these six stages of coral-microalgal symbiosis and the stages of biological invasion proposed by Williamson and Fitter (1996) provide clear analogues between the ecology of biological invasions and the dynamics of coral-microalgal symbiosis. Further similarities become apparent when comparing coral-microalgal symbiotic dynamics with bacterial infections. Bacterial infections in mammalian cells often begin first with the pathogenic bacteria binding to and entering the host cells through phagocytosis (Finlay and Cossart 1997). Phagocytosis is defined as a form of ingestion whereby a larger cellular body subsumes a smaller object or cellular body. The gastrodermal cells of coral hosts engulf ingested microalgal endosymbionts through phagocytosis (Mohamed et al. 2016). The distinction between the two processes occurs after the engulfment phase. In pathogenic bacterial symbioses, the parasitic bacteria reproduce inside host cells at the host's expense, eventually lysing out of the cell and spreading. Engulfment in coral/microalgal symbioses is highly specialized, and the membrane of the engulfing symbiosome is tailored to extract photosynthetic product from the endosymbiont while preventing their degradation and supplying them with nutrients (Mohamed et al. 2016).

Once established within the host, the similarities between coral-microalgal symbiosis and biological invasion become less direct. The environmental conditions of the holobiont are regulated by the coral host rather than through the interactions with abiotic ecosystem components. The outcomes of the Establishment phase differ greatly between biological invasions and coral-

microalgal symbioses. Species in a biological invasion either persist or die off after becoming established in a new environment, but endosymbiont establishment within a coral host can result in several different possible states, and the endosymbiont can persist when external populations succumb to external stressors. The mechanisms that hosts use to regulate their endosymbiont populations are only now beginning to be understood.

In the case of *Durusdinium*, proliferation into alien environments has resulted from both natural spread and anthropogenic transport. Stat and Gates (2008) found that a *Durusdinium* Clade D symbiont was present in ship ballast water in Hawai'i, documenting an anthropogenic transport vector for the genus. Pettay et al. (2015) discovered that the Clade D species, *D. trenchii*, invaded colonies of the merulinid coral *Orbicella faveolata* in the Caribbean. A suspicion that these *D. trenchii* populations were non-native coupled with previously observed invasive behavior of *D. trenchii* (LaJeunesse et al. 2009; Jones and Berkelmans 2010; LaJeunesse, Low, and Trench 2009) led Pettay et al. (2015) to undertake a genotypic analysis of the Caribbean *D. trenchii* population. A comparison of multilocus genotypes (MLG) of Pacific and Caribbean populations revealed that the eight sampled Caribbean populations included lineages derived from a small set of Pacific clones (Pettay et al. 2015). The small number of clonal lineages indicates that *D. trenchii* in the Caribbean was likely a transplant from Indo-Pacific communities of the clade. LaJeunesse, Forsman, and Wham (2015) identified a species of *Siderastrea* coral (Siderastreidae) that had been introduced to the Bay of Panamá from the Atlantic by determining the clade makeup of the coral's microalgal symbionts. The introduced species contained *Durusdinium goreau* and a strain of *D. trenchii* identified as a clone of the population that had invaded the Caribbean and Atlantic reefs. These examples document invasion of dinoflagellate symbionts in coral reef ecosystems, and the disparate locations of the invasions imply that this phenomenon may be widespread. The occurrence of this phenomenon in relation to observed environmental changes associated with global SST change and ocean acidification merits further study into the dynamics of microalgal invasion in coral reef ecosystems.

1.6 Research Objectives

The investigation was divided into four parts, each of which was devoted to one of the research questions. First; does evidence exist for global shifts in the clade composition of *Durusdinium* associations with acroporid corals? To answer this, statistical comparisons between

clade compositions over time were made to determine whether a clade phase shift has occurred in coral reef biomes. As symbiont population composition within coral hosts can influence coral growth and survival (Jones and Berkelmans 2010), a clade shift in overall symbiont composition within coral hosts worldwide could be a predictor of a change in reef ecosystem health/survival. Franklin et al.'s (2012) GeoSymbio database was used to determine whether populations of symbiotic microalgae are changing in coral reefs. GeoSymbio permits comparison between sample years, so by examining the dominant phylotype at a single site over different years, any statistically significant changes over time can be identified. I used 2-tailed T tests to determine differences between associations at different times. Significant differences in coral/microalgal associations might reveal an ongoing clade shift in major ocean systems that are relatively isolated from one another.

Second; what factors play a role in the establishment and maintenance of invasive coral/microalgal symbioses? A summary of the invasion of parts of the Caribbean Sea by *Durusdinium* Clade D acted as a case study. Pettay et al. (2015), which provided the bulk of the information on the Caribbean invasion of 2012, was thoroughly examined, along with other invasion investigations of the coral microbiome (e.g., LaJeunesse, Forsman, and Wham 2016; Lajeunesse et al., 2015) to identify possible factors that may have influenced the introduction and spread of *D. trenchii* in the Caribbean. These factors were divided into several broad categories, which included Host Species Stressors, Invasive Species Characteristics (e.g., morphological, genetic, tolerance), Environmental Factors, Spread, and Facilitation. These categories and their factors provided a list of the processes involved in a potential global microalgal symbiont phase shift. Establishing similarities among mechanisms for biological invasion can advance the field by providing groundwork for further studies into both marine microbial invasions and coral microalgal symbioses, where a dearth of information exists on the underlying forces that influence and allow novel associations to occur and persist.

Third; do the multitude of factors involved in biological invasions by microbes such as *Durusdinium* have parallels in other fields of study? Microbiological invasions present a unique set of challenges to the fields of detection and management that are not present in the detection and management of invasions by larger species. The differences between these invasions are presented and the current state of research on microbiological invasions summarized. The summary of microbial invasion research also provides a background introduction to invasive

management principles. Well-documented epidemiological principles and methods were assessed for parallels with Pettay et al.'s (2015) study. Principles of megafaunal invasions were introduced to provide background for, and comparison with, the 2012 microbial invasion in the Caribbean. The analyses were expanded to incorporate the implications of invasion events for analytic methods and management, as management strategies for both megafaunal and epidemiological invasions are well-studied.

Fourth; what goes into the management of the biological invasion by a marine dinoflagellate? Management policies for preventing introduction, mitigating invasions as they occur, and assessing marine environments for vulnerability to invasion are presented. Case studies from different invasions and management processes (e.g., Hulme et al., (2008); Hulme (2009); Snow et al., (2007); Whitfield (2002)) are presented to establish a background. Then, the factors assessed in these cases were compared to those developed later in the section, followed by a summary of current management policies and methods specific to marine macroalgae. The factors gleaned from earlier parts of the research were used as a basis for creating a specified method for first predicting, then determining the presence of microbial algal symbionts in a given marine environment. The final product consisted of three components. The first is a detailed guideline for predicting areas of high probability for microalgal symbiont invasion. The prediction method includes map figures with overlays representing areas where factors that contribute to increased invasive potential are present. The second component is a basic standard operating procedure for detecting microalgal symbionts that may be present in the water column or inside coral colonies. Methods for detection were drawn from previous studies such as Pettay et al. (2015) and presented together. Finally, the sourced management techniques and technologies were combined to develop a proposed methodology for controlling invasive populations of microalgal symbionts once they have become established in non-native regions.

2. THE POTENTIAL FOR CLADE SHIFTS IN ENDOSYMBIONT COMMUNITIES

A large amount of data is required to detect the presence of a global shift in the composition of *Durusdinium* clades in scleractinian coral hosts. Limitations still exist in constructing a ground-truthed atlas of *Durusdinium* clades even with modern advances in genetic sequencing technology. If comparison with previous research sufficiently indicates that a difference exists between clade compositions of the past and present, it follows that an accounting of current wild symbiont clades

could yield important results. The GeoSymbio database (Franklin et al. 2012) contains an expanding body of research that attempts to map the locations of identified *Durusdinium* clades. It uses data accumulated from studies to mark the place and time in which clades were observed in the wild. It includes data on the symbiotic association of the clade (if any), genetic isolate used in identification, and the depth of collection, when available. As the GeoSymbio database contains collection information on a large range of *Durusdinium* clades and their time-stamped associations, it was subjected to simple statistical analyses to determine if any differences appear between early studies of location/association and more current studies.

2.1 Site Selection

As GeoSymbio is a database of studies that involved genetic sequencing of *Durusdinium* samples, a geographical bias is unavoidable. Certain sites across the globe have received greater research attention than others, and many clades listed in the database were cultured at laboratories and identified away from their native ranges. The database contains a number of geographic locators for each entry, listed in descending scale: Ocean, Country, StateRegion, SubRegion, Locale, Latitude, and Longitude (Franklin et al. 2012). It contains references from the Pacific, Indian, and Atlantic Oceans, with separate sub-categories of Mediterranean, Red Sea, and Caribbean Sea also listed under oceans. The vast majority of entries in the database fall under the Pacific Ocean category (2989), with Caribbean (922) and Indian (567) being the next most populous (Franklin, et al. 2012). In order to select sites that represent the greatest sample size, SubRegion sites from these three “Oceans” were used. A total of 86 SubRegions are identified by GeoSymbio, but only 85 represent geographic locations. The most populated SubRegion is “N/A,” representing clades that were identified under laboratory conditions. Additionally, the number of observed host associations with *Durusdinium* clade D1a (= *S. trenchii*) were counted and separated by date of collection.

2.2 Data Analysis

The database was queried to determine the earliest appearance of Clade D1a in time, where it appeared globally, and its various coral associations. By pulling from numerous studies undertaken at different times, a history of the occurrence of a given clade can be generated across geographic regions. This information can indicate the native range of *D. trenchii* and its invasion

history as it appears outside its native range. For this study, we can examine the occurrence history of *D. trenchii* associations with acroporid coral hosts both globally and in the different coral reef ecosystem regions. Differences in associations could indicate changes in the major clades of microalgal endosymbionts inside acroporid coral hosts. One-Way ANOVA was used to determine differences between the groupings of data in the GeoSymbio database (Franklin et al. 2012). The raw counts were normalized to account for differences in the number of collections made during the sample timeframes. When dealing with change over time, the study used the year in which the collection began as the indicator for time. These “start collection years” represented the earliest possible time for detection within the bounds of the database’s information. When possible, the start collection years of the database were divided into 3-year cohorts to provide groupings for the ANOVA. Associations that occurred during the cohort were counted for use in the statistical testing. Data were tested for normality (Shapiro-Wilke) and homogeneity of variance (Brown-Forsythe). If necessary, square-root transformations were applied to the data in order to meet assumptions of normality, or Kruskal-Wallis ANOVA on ranks tests were utilized. All statistical analysis was carried out using Sigmaplot 14.

To determine if differences in the state of symbiotic equilibrium occur at different times, the frequency of recorded microalgal clade and type associations with *Acropora* across start collection years were analyzed. Additionally, the frequency of coral species that associated with *D. trenchii* during the available start collection years was analyzed. Finally, the frequency of geographic SubRegions (database designation) that had recorded *D. trenchii* associations during the available start collection years was analyzed. Information on all sites at all dates was often unavailable, as the studies in the database had limited geographic and temporal scales. Examination of changes in the clade composition of geographic areas over time can establish that different states of symbiotic equilibrium have occurred. Such differences justify further research into the states of equilibrium and the underlying causes of the changing states.

2.3 Results

The GeoSymbio database contained 228 records of *D. trenchii* sequenced between 1980 and 2010. Few associations were recorded before 2000, with a subsequent sharp upturn, both worldwide and in each component Ocean. Because the database was published in 2012, little information is currently available after 2010, although the database is currently being updated. The

upturn in recorded associations of *D. trenchii* with host species is of interest, because it occurs globally, not only inside the native range of the species.

No statistically significant difference occurred in microalgal clade association with acroporid coral between any of the 3-year groupings (One-way ANOVA, $F=1.333$, $p=0.349$) (Figure 2). No statistically significant difference occurred in microalgal type association with acroporid coral between any of the 3-year groupings (One-Way ANOVA, $F=3.124$, $p=0.109$) (Figure 3). No statistically significant difference was detected between 3-year groupings of coral species associated with *D. trenchii* (Kruskal-Wallis ANOVA on ranks, $p=0.460$) (Figure 4). Finally, no statistically significant difference was observed in number of Sub-Regions containing *D. trenchii* associations across 3-year groupings (One-way ANOVA, $F=0.250$, $p=0.859$) (Figure 5).

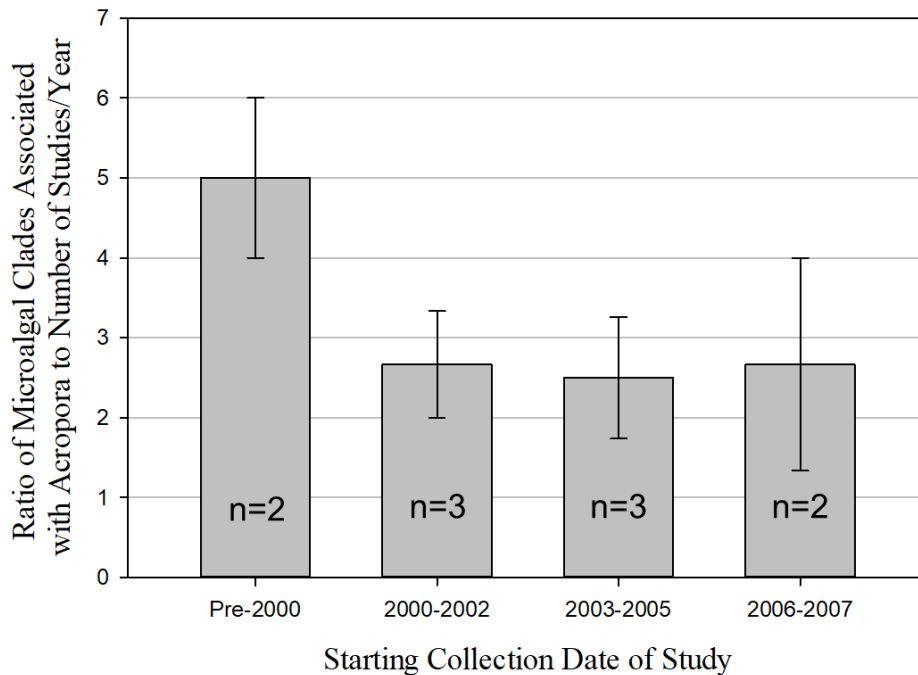


Figure 2: Number of microalgal clade associations with Acroporids in GeoSymbio Database.

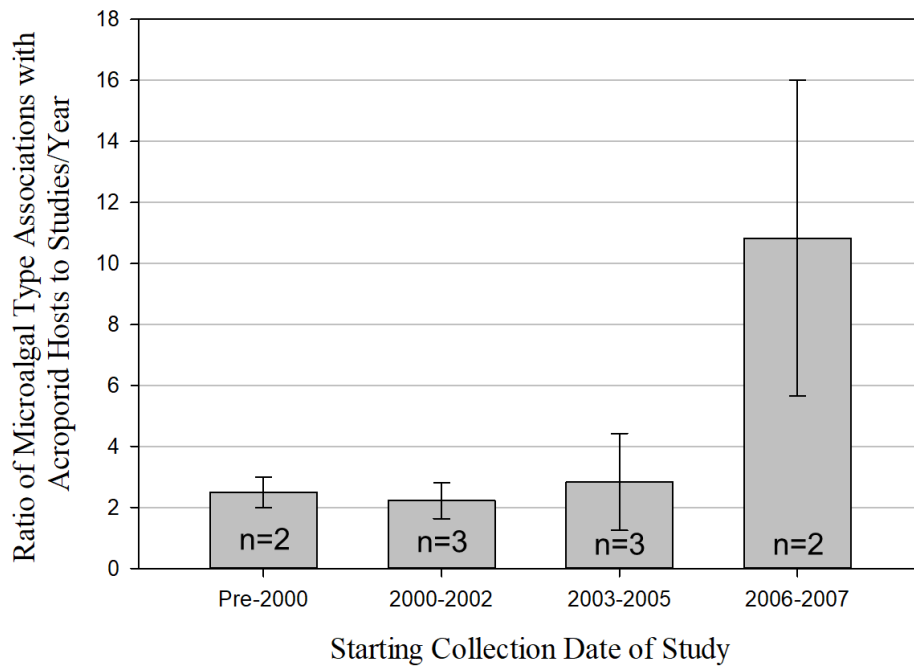


Figure 3: Number of microalgal type associations with Acroporids in GeoSymbio Database.

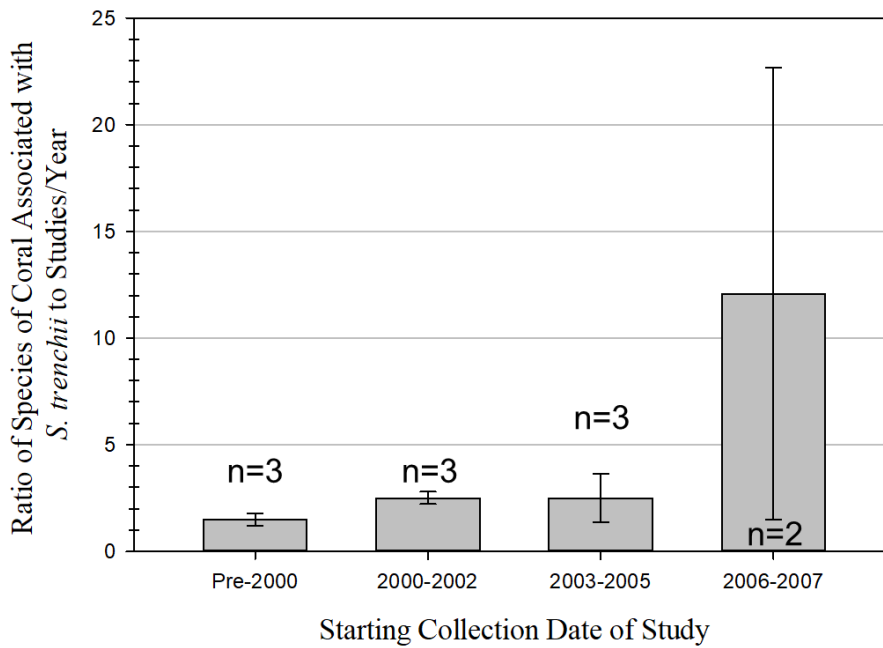


Figure 4: Number of coral species associated with *D. trenchii* in GeoSymbio Database.

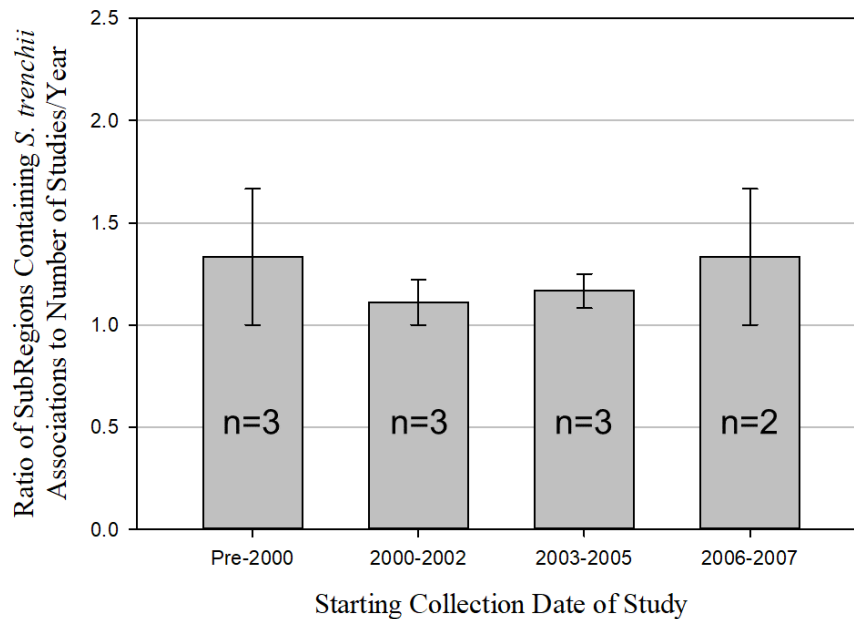


Figure 5: Number of subregions containing *D. trenchii* associations in GeoSymbio Database.

2.4 Implications and Conclusions

The results do not support the existence of a clade shift in acroporid endosymbionts between 1985 and 2007. Conversely, they demonstrate that acroporid corals during this time display specificity to established symbioses, and that range expansion or contraction for *D. trenchii* was unlikely to have occurred. This result is in contrast to previous studies, which indicate that acroporid corals are symbiotically flexible (Putnam et al. 2012). Corals demonstrate varying degrees of fidelity with respect to their endosymbiont associations (Jones and Berkelmans, 2010); however, even corals that have nominally narrow phylogenetic ranges of endosymbionts still tend to accumulate small populations of different clades (Silverstein, Correa, and Baker, 2012). Alterations in symbiont clade associations have implications for biological invasions. These corals are also currently being exposed to rising SST and OA, which is altering the efficacy of their symbiosis with their previously established endosymbionts. Such environmental changes also may provide circumstances for a potential clade shift, as they create ideal conditions for growth of thermophilic endosymbiont clades like *D. trenchii*. Together, environmental disruption of symbiosis and favorable conditions for invasive growth increase chances for invasive success of *D. trenchii* if introduced to such environments.

A statistical variation over time in species associations with *D. trenchii* could indicate the observed increase in associations. Establishing statistically that the number of coral species associating with *D. trenchii* is different during different years supports the previous conclusion that coral associations with *D. trenchii* are often opportunistic and short-term. This physiological aspect of *D. trenchii* associations has ramifications for clade shifts. An endosymbiont able to take advantage of compromised symbioses in their hosts might proliferate if presented with abundant compromised symbioses.

Finally, determining that geographic areas contain different numbers of *D. trenchii* associations at different times could indicate that movement of microalgal populations are occurring between reef ecosystems and reflect changing environmental conditions. An increase in the geographic range of associations could indicate that temperature-induced bleaching events are allowing the establishment of new *D. trenchii* in compromised colonies. Conversely, decreases in the number of areas with these associations imply that previously occupied areas are now free of *D. trenchii*, reflecting enough recovery in some areas from bleaching events that normal associations re-establish and phase out *D. trenchii*.

While the evidence presented by the statistical analysis does not support the existence of a clade shift in the global populations of *Durussdinium*, a number of confounding elements to this analysis exist. The temporary nature of most *D. trenchii* associations due to growth and calcification tradeoffs mean that *D. trenchii* is not the ideal symbiont for most corals under normal environmental conditions. Coevolution between corals and available endosymbionts retards the process of novel association establishment by physiologically favoring historically established symbioses (Pettay et al. 2011). Until global SST reaches such a consistently high point that most established endosymbiont associations break down, a permanent clade shift to thermally tolerant endosymbionts is unlikely. Additionally, the concept of coral host specificity and preference for symbiont clades has become a matter of debate. Silverstein, Correa, and Baker (2012) found that 68% of all coral colonies surveyed for their experiment hosted multiple clades. They noted that the concept of host specificity is an “artificial dichotomy,” and that the natural state of most coral holobionts is more likely to involve multiple successful clade associations (Silverstein, Correa, and Baker 2012).

Despite the data indicating that differences in coral/microalgal symbiosis do not occur, the data set and the statistical tests have limitations. The sample sizes for the clades, types,

associations, and years of data collection were relatively small, with the GeoSymbio database consisting of studies published before the database publication in 2012. Additional temporal limitation occurred at the establishment of the database, as genome sequencing technologies were not developed at the collection start date for some of the earliest studies. Finally, a dramatic difference exists in representation between temporal delineations (decades). Far more data exists for 2000 to 2010 than for any other represented decade. This might reflect climatic overturn in these regions during that period that resulted in genuine changes in association. Conversely, it could be due to a larger number of studies. Geographic limitation was also evident, with the majority of studies originating from the Pacific and Indian Ocean regions early on, and the collection from the Caribbean later. Finally, the database was limited by incorporating data from previous studies that were not uniformly carried out and were not conducted with the purpose of establishing the GeoSymbio database. This has limited the database by potentially not providing all of the fields that can be used for outside analysis. Such data limitations, the mutable composition of the holobiont, and environmental variations justify new studies to further investigate these research questions.

3. FACTORS THAT MAY INFLUENCE A MICROALGAL ENDOSYMBIONT CLADE SHIFT

Biological invasions can cause wide-ranging alterations in the functional capacity and makeup of a given ecosystem. Their consequences have been widely documented across a number of ecosystems, with earlier studies focusing on terrestrial megafauna and flora (example REFS). As scientific technologies and techniques have progressed, organisms across a larger spectrum of sizes have become available for research. Additionally, the scale of invasion research has greatly increased. Research into biological invasions in marine ecosystems presents unique challenges, as marine ecosystems tend to exhibit greater natural connectivity between local habitats, have fewer physical barriers separating them, and are utilized by humanity in ways that greatly differ from terrestrial ecosystems.

Goreau and Hayes (1994) identified the “Central Atlantic,” as one of ten global “Coral Reef Provinces”, spanning most of the Greater Caribbean Sea, Western Tropical Atlantic Ocean, and the Gulf of Mexico. They demonstrated that, as of 1991, the area became a “hot spot” for coral bleaching, as water temperatures exceeded global long-term averages by more than 1°C during the year’s hottest period. During the period between 1979 and 1990, 60 major coral bleaching events

occurred in the Central Atlantic province (Glynn, 1992). Later studies found that the sea surface in that area spent a greater amount of time warming from 2000-2005 than any recorded decade preceding it (Donner, Knutson, and Oppenheimer, 2006). During this time, warming sea surface temperatures (SST) combined with record temperatures in 2005 to produce a massive coral bleaching and mortality event (Wilson and Souter, 2008). This event left surviving Caribbean coral populations in a weakened physiological state at best, having expelled large portions of their symbiotic algae. At worst, it killed large tracts of coral colonies ranging from Northern Brazil to Florida. It is the weakened, bleached coral colonies that provided the environment for the invasion of alien symbionts to occur.

The phenomenon of symbiont uptake into bleached coral from the environment allowed the 2012 invasion described by Pettay et al. (2015) to take place. The corals that survived the 2005 bleaching event needed to re-establish symbioses with microalgal endosymbionts in order to resume normal metabolic function and growth. While corals have the ability to quickly re-cultivate populations of endosymbionts from remaining healthy cells within their gastrodermis (Kemp et al. 2014), their heterotrophic capabilities provide an opportunity for exogenous populations of *Durusdinium* to establish new symbioses with hosts. An invader species, already present in the environment prior to the bleaching event, proved able to establish and maintain a symbiosis with coral outside of its native range.

Most coral endosymbionts are dinoflagellate microalgae species of the genus *Durusdinium*. *D. trenchii* is a species of *Durusdinium* that is native to the South Pacific and Indian Oceans. It has a proven thermal tolerance that has allowed corals that harbor it and other Clade D species to survive bleaching events with fewer ill effects than corals harboring populations of algae in different clades (Stat and Gates, 2011). Additionally, *D. trenchii* and other Clade D symbionts have a documented history of behaving as opportunists, infecting recently bleached coral hosts and proliferating quickly to become the dominant microalgal phylotype among a bleached coral's symbiont library (Grottoli et al., 2014). As global SST's now consistently exceed coral bleaching thresholds for larger portions of the year, thermally tolerant species of *Durusdinium* become more prominent as corals that harbor the symbionts experience greater survivorship than their counterparts. This phenomenon allowed *D. trenchii* to establish a foothold in the Caribbean, outside its native range. The following case study focuses on the establishment of *D. trenchii* in Caribbean corals during recovery from the bleaching event of 2005. The symbiotic association of

this dinoflagellate with scleractinian corals, coupled with the effects of climate change and ocean acidification, make this case study a landmark reference in the mechanics of a climate-facilitated microbial invasion in a marine environment.

3.1 Case Study: Summary of Pettay et al. (2015)

Pettay et al. examined *Durusdinium* population diversity in the coral *Orbicella faveolata*. Colonies harboring *D. trenchii* survived the 2005 bleaching event with greater frequency, motivating analyses of symbiont population makeup in these corals. As Clade D rarely dominates endosymbiont communities outside the Persian Gulf (Stat and Gates, 2011), Pettay et al. (2015) hypothesized that those that dominated the microalgal community in these *Orbicella* colonies were not native to the Greater Caribbean. Genetic analyses of Caribbean versus South Pacific *D. trenchii* (and other species) from reefs at the same latitude in their native ranges found that populations from the latter region were genetically diverse and exhibited a variety of genotypes that differed between individuals and across geographic space. *D. trenchii* is the only Clade D species found in the Caribbean Sea and the Atlantic Ocean, which led Pettay et al. (2015) to explore the genotypic diversity of *D. trenchii* samples collected from the Caribbean.

Pettay et al.'s (2015) genomic analysis of *D. trenchii* samples collected from *O. faveolata* in eight different Caribbean and Gulf of Mexico localities (Florida Keys, Flower Garden Banks, Yucatan Mexico, Belize, Panama, Curacao, St. Croix, and Barbados) found that the strains in these colonies were clonal, i.e., they originated from and maintained genetic characteristics of a single genetic line of *D. trenchii*. One clonal genotype, designated α , was found in every Caribbean sampling site and ~42% of all samples taken (Pettay et al., 2015). They concluded that genetic diversity of *D. trenchii* was very low in the Caribbean, which supported the hypothesis that the species was non-native and recently introduced to the region. In invasion ecology, the phase of Introduction or the overcoming of the barrier of Captivity or Cultivation (Richardson et al., 2000; Williamson and Fitter, 1996; Blackburn et al., 2011) is often noted to result in a reduced number of individual invaders that survive to reproduce following transport to a non-native environment. This reduction in population size can lead to a genetic bottleneck. In the case of an organism with multiple methods of reproduction (such as dinoflagellate algae) (LaJeunesse, Parkinson, and Trench, 2012) it can also result in a highly clonal population of individuals over time. This

evidence supports the theory that *D. trenchii* is a non-native invader species in the greater Caribbean.

3.1.1 Implications and Impacts

Establishing that the populations of *D. trenchii* in Caribbean corals are invaders creates a number of implications and unanswered questions. The nature and severity of effects on the Caribbean reef systems following establishment and spread of this organism are of concern. The mechanics of the introduction of this species are also of interest, given that reconstruction of the events that began the invasion can assist in prevention of further introductions. Management and mitigation processes are also important, as additional research should establish whether *D. trenchii* negatively impacts Caribbean reefs. Pettay et al. (2015) indicate that more research needs to be conducted regarding the biological invasion of the Caribbean by *D. trenchii*.

One of the first questions raised by the discovery of an alien species in a new environment is often: “How did this organism get here?” The Panama Canal is a major avenue for anthropogenic transport between the Greater Caribbean region and Eastern Pacific Ocean. Completed in 1914 and expanded in 2016, the Canal spans the Isthmus of Panamá and permits the crossing of between 13,000 and 14,000 vessels per year (Muirhead et al. 2015). Pettay et al. (2015) referenced studies dating to the 1990’s, when *D. trenchii* was first observed in Atlantic corals near Panamá and noted that the greatest genetic diversity of *D. trenchii* occurs in major shipping destinations in the Caribbean, most notably Panamá itself, Barbados, and Curaçao. Shipping hubs are convergence points for invasive transport vectors, and introductions through ballast water exchange are common (Stat and Gates, 2008). Tracing the points of establishment back to these first observations, it logically fits that a native Pacific species that was introduced to the western Atlantic could have been transported chiefly through the Panama Canal.

Pettay et al. (2015) determined that a population of endosymbionts that originated in the Pacific was transported to the Greater Caribbean region and established in multiple locations across that region. The success of this bioinvasion can be attributed to the symbiotic behavior of *D. trenchii* and the environmental conditions that persisted over the course of the invasion. The clonal nature of a number of the endosymbiont populations pointed to a single introductory population, and the close proximity to a major inter-ocean transport hub supports the hypothesis of anthropogenic transport of the original population. The symbiotic behavior of *D. trenchii* is

opportunistic and thermophilic (Stat and Gates 2011), a combination that made its invasion of the Greater Caribbean successful due to the prevailing environmental conditions in the region and their impact on native coral symbioses. Large-scale bleaching events brought on by rising SST cleared out the previously established symbionts inhabiting the species of *Orbicella* in the region. *Durusdinium trenchii* that were transported to reefs adjacent to major shipping hubs were thus able to colonize local corals and form novel symbioses. The global rise in SST contributed to the continued success of these associations, as the disturbed conditions that led to the initial associations never fully abated. Associations that would have normally been temporary due to the growth and calcification tradeoffs common to *D. trenchii* were longer present, as SST's remained elevated from previous norms. Pettay et al. (2015) established that a contributory suite of factors permitted the transport to and successful invasion of a novel ecosystem. Focusing on these factors will be useful in developing a management protocol for future marine microbial invasions.

3.2 Factors

Crucial to the development of a comprehensive invasion management program for marine microalgal endosymbionts is the identification of factors that influence the invasive potential and success of species. The field of invasion ecology has already made advances in this sub-genre of the study of invasion pathways (Hulme et al., 2008), but the field is relatively new. Additionally, identifying the conditions that contribute to the success of a biological invasion is made more difficult by the ecological complexity of an alien species that establishes itself in a novel environment. Hulme et al.'s (2008) Simplified Framework for Categorizing Invasion Pathways can serve as a basis for establishing the initial introductory method of the invasive species, but the framework only provides the raw definition of the type of invasive and the means via which it arrived in its new environment. It does not identify or account for the invasion vulnerability of an environment, nor the particular characteristics that endow a species with the ability to invade. Though the Framework does arrive at a generalized assignment of management responsibility, it requires specifics before it can be expanded into a comprehensive management protocol. Using the invasion of Caribbean scleractinian corals by alien *Durusdinium* endosymbionts as an example, the following system of invasion factor identification is proposed.

In the scenario of a species of symbiotic microalgae invading a non-native coral reef ecosystem, the various factors can be divided into three broad categories. The first deals with any

biological characteristics of the invasive alga and its coral hosts and covers factors influencing the coral/microalgal holobiont. The second is potentially the broadest, as it encompasses the characteristics of the invaded environment that make it a suitable habitat for the microalga to establish itself. The third addresses the anthropogenic activities that contribute to the establishment and success of the invasive species. To address the need for specifics, this study examined *D. trenchii* as the invasive microalgal endosymbiont and the genus *Acropora* as the invaded host species. To match the case study of Pettay et al. (2015), the environment is the Greater Caribbean coral reef system.

3.2.1 The Holobiont Factors

The major biotic components that affect the invasive potential of the microalgal species and its coral host make up the first category, which can be divided into two major sub-categories: 1) characteristics of the microalgal endosymbiont and 2) characteristics of the scleractinian coral host (Figure 6). The two species coexist in mutualistic symbiosis, forming the holobiont unit that reacts to environmental stimuli, and Van Oppen et al. (2008) found that it may respond more strongly to changes in its environment than either of its two component species (Van Oppen, et al., 2008). This is especially true for *D. trenchii*, which has been described as a generalist symbiont that can either associate with hosts or free-live (LaJeunesse, et al., 2009). In contrast, Caribbean acroporid corals associate with only four of the nine general clades of *Durusdinium* endosymbionts, and with relatively few distinct species/clades within those general clades (Baker and Rowan, 1997). The two species groups were assessed for factors that may impact the invasive success of *D. trenchii*.

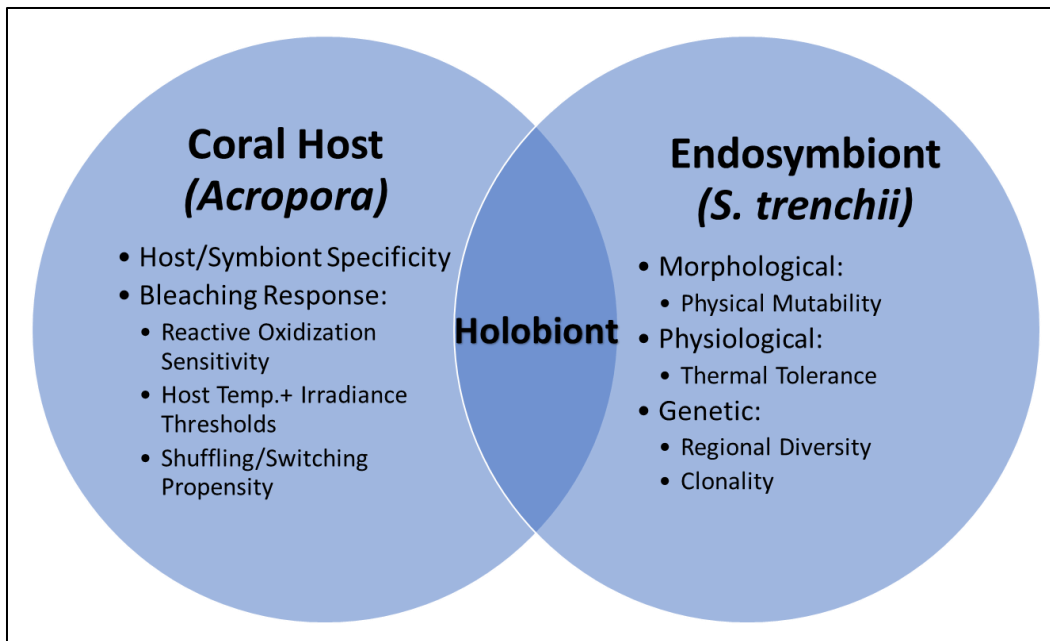


Figure 6: Overview of Coral/Microbial Holobiont Factors that can be used to determine Invasion Vulnerability in a given environment.

3.2.1.1 *Durusdinium trenchii* Factors

Several species traits make *D. trenchii* a successful invader in environments to which it is not native. These factors can be loosely grouped into three subgroups: morphological, physiological, and genetic. Morphological traits refer to the physical aspects of the microalgal cell that may allow it to outcompete other strains to become dominant in the endosymbiotic populations of *Durusdinium* within coral cells. Physiological traits refer to the attributes of the endosymbiont that are not manifest physically, but impact the survival and growth of the cells, populations, and holobiont as a whole. Finally, the genetic factors refer to the population-level gene dynamics that play a role in the success and survival of the *D. trenchii* populations in Greater Caribbean reef systems.

The morphologies of various *Durusdinium* clades are largely the same across ecosystems and phylogenies (LaJeunesse, Parkinson, and Trench, 2012). The coccoid phase of the microalgal cell, the only life cycle phase that occurs inside the gastrodermal cells of its coral host, varies little between the genetically distinct phylogenies of *Durusdinium*. In *D. trenchii*, morphological differences are not as readily apparent as in Caribbean-native species. However, *D. trenchii* can photosynthesize at a greater range of light intensities than can Caribbean-native species, although this response appears physiological rather than related to cell size or internal concentration of chloroplasts (van Oppen, et al., 2009). With reference to Blackburn et al.'s (2011) unified

framework for biological invasions, the wide range at which this species can photosynthesize would assist it in the Introduction, Establishment, and Spread phases. In the Introduction phase, the dinoflagellate would be exposed to its new environment (the Greater Caribbean) and overcome the invasion barrier of captivity. For a microalga, its presence in the ballast tanks of a ship in port provide an example of exposure. Ballast water discharge and release of the microalga into the surrounding water could overcome the captivity barrier. After the Introduction, the dinoflagellates would have to survive and reproduce to complete the Establishment phase. Survival of the colonizer population and successful reproduction are barriers to Establishment. Finally, the stable population of dinoflagellates would have to colonize adjacent environments to complete the Spread phase, renewing the invasion cycle on a near-field scale. The increased photosynthetic range of *D. trenchii* would allow it to more easily overcome the barriers of Survival, Reproduction, and Spread through an ability to use downwelling light effectively at different depths. The only other grey-area morphological trait that potentially sets *D. trenchii* apart from the species it displaces upon invasion is its propensity for physical mutation that can sometimes result in adaptive evolution under new conditions (van Oppen, et al., 2009). An ability to evolve under novel circumstances would also aid in the aforementioned stages, when coupled with physiological traits that assist it in establishing and spreading.

Van Oppen et al. (2009) state that, since morphology is largely conserved across the various clades of the *Durusdinium* genus, their physiological capacities and genetic makeups become much more important when assessing the distinctions between clades. Physiological traits vary widely among the clades of *Durusdinium* (van Oppen, et al., 2009), and most often involve interactions with the host species with which they associate. Types of physiological interactions that make a clade of *Durusdinium* a more or less effective endosymbiont include but are not limited to heat tolerance (and by association, bleaching resistance), effect on host calcification, and growth rate within the host cells. Host-endosymbiont interactions vary between clades of endosymbiont that associate with the same host species (van Oppen, et al., 2009). Additionally, a single endosymbiont clade can form associations with different host species that vary in effectiveness, e.g., *D. trenchii* interacts with several different scleractinian hosts in unique ways. When it is the dominant endosymbiont, it confers upon its host coral a degree of thermal tolerance by maintaining stable photosynthetic production across a greater thermal range compared with other clades. This allows the coral to resist bleaching when environmental water temperatures would

cause most of its locally occurring symbionts to reduce photosynthetic efficiency. However, this tolerance does come at a growth tradeoff to the host, as the calcification capacity of the holobiont decreases when *D. trenchii* dominates. In addition to decreased calcification, *D. trenchii* dominance may reduce coral fecundity (Little et al., 2004). The coral thus gains a slight edge in survivability but loses out on growth and reproduction. While longstanding associations of coral with *D. trenchii* allow the coral to be out-competed by faster growing colonies under normal conditions, the distinct survival edge imparted by the physiological factors of *D. trenchii* make it an effective colonizer in ecosystems where the shift to higher surrounding temperatures renders normal associations less than effective for the coral holobiont. This shift in endosymbiont population makeup results in the genetic conditions that open the Greater Caribbean to potential invasion by this microalgal endosymbiont.

The genetic factors that improve the ability of *D. trenchii* to invade a novel ecosystem exist on a higher ecological scale than the individual genes and chromosomes within a single microalgal cell. They involve the genetic diversity of the entire Caribbean reef system and the changes that it is currently undergoing. Van Oppen et al. (2009) noted that the genetic diversity in clades of Caribbean *Durusdinium* is lower than in the Pacific. The geographic isolation of the Greater Caribbean and the proximity of the major reef systems to each other explain this smaller diversity. Average genetic divergence between native clades is only 17.1% (Pochon and Gates, 2010). When environmental conditions become unsuitable for normal coral-microbial associations (i.e., thermal bleaching), the low diversity of the region results in large populations of coral hosts without adequate endosymbiont populations. The introduction of a new, more tolerant species of endosymbiont is thus made easy by allowing invaders a plethora of new hosts to colonize. Following the 2012 invasion of the Caribbean by *D. trenchii*, clonality in endosymbiont populations varied across different spatial scales (van Oppen et al., 2009). However, the clonality of *D. trenchii* is consistently high across habitats in which it occurs. This pattern can potentially be explained by the introduction of a single strain of endosymbiont during a period of thermal stress on the region's coral. Once ensconced within a coral host, the microalgae could proliferate and spread from colony to colony by increasing survival in the hosts it occupied. The features of the coral host that affect invasive potential cannot be ignored.

3.2.1.2 Acroporid Coral Host Factors

Scleractinian corals exhibit several physiological attributes that affect their symbioses with the various clades of *Durusdinium* that they harbor. Those attributes specific to establishing and regulating endosymbiont populations can be important in determining invasion vulnerability. For this investigation, the Greater Caribbean and its resident acroporid species (*Acropora cervicornis*, *A. palmata*, and *A. prolifera*) (Fogarty, 2012) will be examined as examples. Main factors will include acroporid specificity and physiological responses of acroporids to bleaching conditions.

Van Oppen et al. (2001) found that in the acroporid-rich reefs of the Pacific, clades of *Durusdinium* and species of *Acropora* formed and continued associations based on three major determinants: environmental availability of symbiont clades, coevolution of new species/clades, and coral host specificity. In Greater Caribbean reef systems, genetic diversity of both coral hosts and endosymbionts is lower due to the region's geographic isolation and oceanographic patterns. Lower genetic diversity of hosts means that the successful associations are more genotypically specific than in larger, less constrained reef systems. While the Greater Caribbean has been geographically isolated for an extended period of time, symbioses between resident acroporids and their endosymbionts are not the result of coevolution between local microalgal strains and coral hosts. Rather, associations between acroporids and dinoflagellate endosymbionts do not appear to be entirely random. Instead, other factors (physiological, biogeographical) may be involved in determining the success of coral/microalgal associations between these species (Van Oppen et al., 2001). As acroporids do not transmit endosymbionts vertically, that is, from generation to generation, microalgae within a coral colony phylogenetically reflect the surrounding exogenous populations.

Microalgal symbiosis in acroporid corals is further constrained and complicated by the physiological response of the coral host to stress. Densities of microalgal clades harbored within scleractinian corals can vary over time in response to seasonality, stress, or changes in water quality through a process known as symbiont shuffling (Baker, 2003). Populations of certain microalgal endosymbionts can also be entirely replaced with new microalgal colonizers through a process known as symbiont switching (Baker, 2003). These responses can occur after exposure of the coral host to stresses such as physical damage (e.g., storms, currents, impacts), disease, pollution, and water parameter changes. Bleaching is a primary example of an acroporid stress response and occurs when environmental conditions exceed a threshold beyond which the

microalgal endosymbionts can no longer optimally provide the host with photosynthetic product. Under these conditions, the endosymbionts begin to produce greater-than-normal concentrations of reactive oxygen species (Weis, 2008). These types of oxygen differ from the species produced under ideal photosynthetic conditions, and their presence within the thylakoids—the membranes in which photosynthesis takes place—of the microalgal cell can lead to damage of the thylakoid (Weis, 2008). When concentrations of these reactive oxygen species reach high enough levels, they overwhelm the antioxidant defense mechanisms of the endosymbionts. They then affect the coral host cellular tissue, resulting in bleaching. Bleaching mechanisms appear to vary, but four major mechanisms have been described: 1) coral hosts can suffer gastrodermal cell death, or apoptosis; 2) symbiont-containing cells can detach into the gut cavity and be expelled; 3) the host can degrade and digest symbiosomes, and 4) microalgal endosymbionts of varying condition can be exocytotically expelled from the host (Bieri et al. 2016). Without the endosymbionts to provide the coral with autotrophic capability, the coral's growth and calcification capacity greatly diminishes, and it relies on suspension-feeding heterotrophy to survive. While coral hosts often regrow endosymbiont populations by using exogenous nutrients to feed their remaining microalgal endosymbionts (Morris et al. 2019), corals can, under bleaching conditions, establish new associations with exogenous microalgal endosymbionts and recover from bleaching events via this method (Buddmeier and Fautin, 1997). These new associations provide opportunities for non-native microalgal endosymbionts to establish themselves in a novel environment by colonizing available coral colonies. A number of host physiological traits, in this case for *Acropora*, need to be assessed to determine the invasion vulnerability of a reef system dominated by members of this genus. Such traits include host sensitivity to reactive oxygen species, temperature bleaching thresholds, and irradiance bleaching thresholds that are independent of photosynthetic production of its endosymbionts. Also important is the propensity of the host to establish new combinations of endosymbionts through shuffling or novel symbiotic associations through symbiont switching. As a number of these host factors are directly dependent upon environmental conditions, the reef environment itself needs to be assessed for factors that may contribute to a biological invasion of its coral species.

3.2.2 The Environmental Factors

Like all producer species, acroporids and their dinoflagellate endosymbionts are dependent upon environmental conditions for their ability to survive, grow and reproduce. Even slight variations in reef water quality can affect growth and survival of coral colonies and populations. Reef water quality depends upon many oceanographic and atmospheric factors. However, this analysis focuses on a few key features of the Greater Caribbean, divided here between two overarching categories: Trends in Oceanographic Conditions, and Area Dynamics (Figure 7). Trends in Oceanographic Conditions include patterns in temperature and water chemistry that change with respect to historical norms. Area Dynamics consist of the more constant oceanographic features that are characteristic of a particular region (e.g., the Greater Caribbean or Western Atlantic). Connectivity, a third set of factors illustrated in Figure 5, is a component of Area Dynamics that is distinct in that several of its component factors are biological and not solely oceanographic.

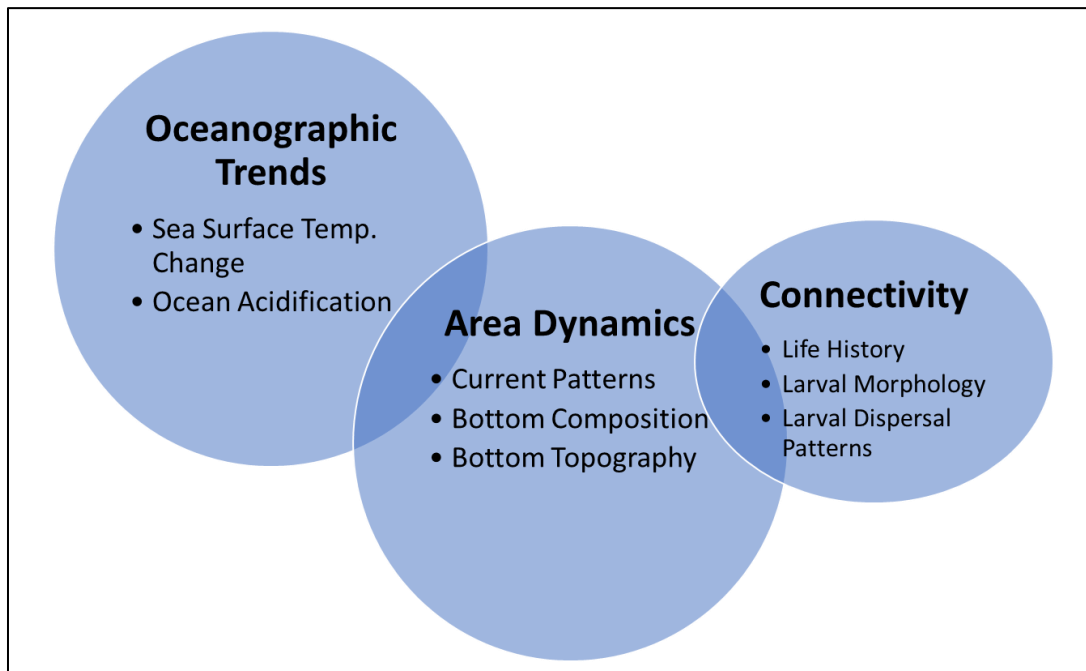


Figure 7: Overview of Environmental Factors that can be used to determine Invasion Vulnerability in a given environment.

3.2.2.1 Trends in Oceanographic Conditions

Two major trends in marine chemistry threaten the health and survival of coral reefs globally. The first, rising sea surface temperatures (SST), reflects warming in global surface water. The second is the recent decrease in pH of ocean water worldwide, known as ocean acidification

(OA). These trends are the result of changes in the gaseous composition of the atmosphere that increase its heat-retentive capacity. The heat retained by the changed atmosphere disrupts the complex interplay between the atmosphere and the oceans, which act as a heat and gas sink for the atmosphere. The specific effect that SST and OA have on coral reef ecosystems is to promote coral bleaching conditions, whether by driving temperatures above the ideal range for photosynthetic action by the endosymbionts. Because these are global phenomena, their effects need to be assessed and quantified when creating a framework that identifies invasion vulnerability of a given area.

Most of the major reef-building scleractinian coral species inhabit the upper reaches of the photic zone of the world's oceans. The majority of acroporids live in shallow water and are exposed to changes in sea surface water quality. Globally, mean sea surface temperatures have been rising at accelerating rates over the past three decades (Iz, 2018; Houghton et al. 1990). Past a certain threshold, SST can cause coral bleaching. Examining a given area's mean SST, number of historical bleaching events, and capacity for colonial coral recovery from such events can create a picture of the resiliency of coral reef ecosystems in that area. These factors can also reflect the invasion vulnerability of the area, as reefs that are continually exposed to bleaching conditions and exhibit pronounced colonial recovery could exhibit more numerous instances of novel coral/microbial symbiotic associations than reef systems where bleached corals simply died after such exposure. While SSTs alone have caused bleaching events across reef ecosystems, it is rare for a single water quality parameter to be the sole influence on life in a marine ecosystem. SST acts in tandem with ocean acidification to exacerbate stresses on corals and to indirectly promote the spread of introduced dinoflagellate endosymbionts.

Ocean acidification is the result of increased concentrations of carbon dioxide gas in the atmosphere. This gas, when in contact with surface ocean water, transfers from the atmosphere into the surface water to equilibrate concentrations between the two media (Doney, et al., 2009). When seawater contains higher concentrations of CO₂, its pH decreases as the gas proceeds through the chemical reactions of the oceanic carbonate cycle, ultimately forming hydrogen (H⁺) and bicarbonate (HCO₃⁻). High H⁺ concentrations drive the pH of seawater down from its normal state of about 8.1. For calcifying corals that inhabit the near-surface photic zone of the world's oceans, exposure to lower-than-average pH can have numerous detrimental effects. Scleractinian corals rely on a complex series of chemical reactions to accrete new skeletal calcium carbonate in the form of aragonite from the dissolved inorganic carbon (DIC) present in the water and the CO₂

derived from heterotrophic metabolism of the coral's food (Pearse, 1970). These reactions require a specific pH range; pH outside of this range can significantly impact coral calcification. However, this is not all of OA's impact on coral reefs. Anthony et al. (2008) demonstrated that excessive CO₂ induced bleaching in an acroporid by disrupting the productivity of the endosymbiont's photosynthesis, and Doo, Edmunds, and Carpenter (2019) demonstrated that OA encourages alterations in benthic community structure in reef ecosystems. In combination with elevated SST, OA can also severely compromise diversity of the coral microbiome (Grottoli et al. 2018). When coupled with other conditions, such as increased temperature, the threat to reefs posed by OA is serious. It serves as a factor indicating the invasion vulnerability of a given reef system by denoting geographic areas of coral stress, and how, under stressful conditions, the corals are presented with the opportunity to form novel associations with endosymbionts.

3.2.2.2 Area Dynamics

Other oceanographic features of a given reef system lend themselves to the establishment and spread of an invasive species such as *D. trenchii*, which is motile in the water column and sediment when not in a symbiotic association. Spread to new regions is therefore largely a function of water and sediment exchange between localities. The small size and limited mobility of *D. trenchii* mean that individuals will not travel far under their own power. Van Oppen et al. (2001) demonstrated the effects of biogeographical factors on coral/microalgal associations, especially in the Pacific, where diversity of microalgal symbionts is greater and the distances between sampling sites are larger. Historical evidence demonstrates the importance of environmental impacts on coral/microalgal symbioses. Baker and Rowan (1997) found that large-scale climate shifts affected the survival and success of *Durusdinium* clades in the Western Atlantic, and those that survived were more likely to be incorporated into symbioses with corals. As climate has already been mentioned as a factor, two other oceanographic features will be assessed as factors that influence invasion vulnerability: current patterns and connectivity between reef systems.

While current patterns refer to the most consistent large-scale water movements across a given ocean region, connectivity is a more conceptual framework that integrates multiple oceanographic factors to quantify the biological exchange among ocean regions. Current patterns directly influence connectivity, especially with respect to larval dispersal and other functions involving the spread of limited-mobility microbiota. A distinction is made between the two concepts because, while current patterns reflect water motion in an area and are purely a function

of physics, connectivity analyses demonstrate the scale and reality of biological exchange between localities and account for non-oceanographic factors as well.

Current patterns of the Greater Caribbean are strongest near the surface (<1200 m depth) where they are driven by wind (Gordon, 1967). Apart from several exceptions where they are interrupted by large land masses, they proceed in a northwesterly direction past the small chains of the Southern Caribbean and through the larger islands further north until they reach the Gulf of Mexico. When these currents reach the Gulf of Mexico, they are influenced by the Loop Current, which forms the Western boundary current of the North Atlantic Ocean. This current enters the Gulf area through the Yucatan Channel and proceeds Northeast to the Straits of Florida, where it leaves the Gulf (Yang et al. 2020). This pattern influences life histories of Caribbean marine species, as it generally predicts the larval dispersal of reef fish and coral larvae (Roberts, 1997; Shulman and Bermingham, 1995). Notable exceptions to this general rule include areas with locally variable current patterns such as gyres, and larval physiological traits that either limit or extend dispersal duration and range (Roberts, 1997). Caribbean current patterns are also an effective transport vector for disease, the most notable example being the mass mortality of black sea urchins (*Diadema antillarum*) that occurred throughout the Caribbean between 1983 and 1984. Lessios, Robertson, and Cubit (1984) noted that the progression of the die-offs almost exclusively followed the known patterns of surface water currents in the region. Due to this pattern of spread, the completeness of effect on *Diadema* populations, and the fact that both open water reef systems and aquaria with sea-water inlets were affected by the mortality events, Lessios (1988) theorized that the mortality was caused by a water-borne pathogenic agent. If this is the case, although the pathogen was never identified, the *Diadema* mortalities of 1983-1984 serve as an exemplary case study for biological invasion of a microbial, water-borne species. Current patterns, especially in well-mapped regions like the Greater Caribbean, serve as a contributory metric for determining the invasive potential of an area. For example, compare two geographic locations in the Caribbean relative to general current pattern: a theoretical reef on the western end of Grand Cayman and one on the eastern end of Martinique (Figure 9). Martinique's eastern coast is exposed to influx from the equatorial Atlantic and from the coast of Brazil. Grand Cayman, especially its western coast, is exposed entirely to water that has not only passed around Martinique, but passed the majority of the Caribbean South American coastline (Figure 9).

By referencing the currents in the area, the species composition of the location may be predicted based on the sources of its water. If an invasive species was introduced only on the reef at Grand Cayman, it could be reasonably expected that the upcurrent Eastern Martinique reefs would be unaffected for some time (Figure 9). Conversely, an invasive established at Martinique would likely reach Grand Cayman relatively rapidly. This type of analysis, examining the direct influence of one geographical point on another, is known as a connectivity analysis.

Ecological connectivity generally refers to the movement of populations and resources between environments that results in the dynamic equilibrium of ecosystems (Kindlmann and Burel, 2008). It is well-studied in terrestrial ecosystems and is becoming more widely researched in marine biology. Ecological connectivity takes many forms, but most important to the current investigation is population connectivity, a measure of the ability of individuals of a population to disperse between spatially distant localities (Roughgarden, Gaines, and Possingham, 1988). With respect to marine ecosystems, population connectivity takes into account ocean current dynamics as well as biological and other oceanographic factors, e.g., ocean current seasonality, bottom composition and topography, pelagic larval duration, larval morphology, and species life histories (Trembl et al. 2008). Information on population connectivity between disjunct species populations coupled with assessments of ocean current patterns would be useful in predicting the path and timeframe of biological invasions. Connectivity should be considered as a factor in a framework for assessment of an area's invasion vulnerability.

3.2.3 The Anthropogenic Factors

Humans have spread to every ecosystem on the planet, and they affect environments in a variety of ways. Common anthropogenic interactions with marine ecosystems include trade (transport of goods across ocean surfaces), direct exploitation (fishing, mining ocean floors, farming), and indirect exploitation (tourism, pollution, alteration of natural state through construction or coastal activities). When assessing a reef system for invasion vulnerability, it is important to consider such anthropogenic activities. Their nature, proximity to the reef system, and their scale all contribute to the overall effect on the reef environment. Additionally, humans play a large role in the introduction of invasive species to novel locations. For the purposes of this investigation, the factors that result from anthropogenic activity in coral reef ecosystem vicinities will be simplified into two overarching categories: Spread and Facilitation.

3.2.3.1 Anthropogenic Spread

Historically, human maritime activities have served as transport vectors for different species (plants, animals, microbiota) from their native source environments to novel ecosystems (di Castri, 1989). As transportation and trade have increased in frequency and scale (mirroring human population growth), the number of species introductions and invasions have increased concurrently (Mack et al., 2000). Transportation of invasives to novel environments through human agency can be either accidental or deliberate. Although introductions made through anthropogenic transport do not necessarily result in successful biological invasions, repeated introductions increase the likelihood of establishment of introduced species. In marine biological invasions, the potential invasive is often transported accidentally via shipping and introduced repeatedly through established shipping routes/hubs. For this reason, assessing the invasion vulnerability of a reef system should consider proximity to major ocean transport routes/hubs.

3.2.3.2 Facilitation

Anthropogenic facilitation of biological invasions can take numerous forms. Human activity that disrupts the naturally occurring functions of a marine ecosystem stresses that environment. When functions of a marine ecosystem are disrupted, the potential for colonization by alien species increases. As an example, when a natural benthic mussel and oyster community on breakwaters in the Adriatic Sea was disturbed by replenishment and maintenance activities in the early 2000's, the resident colonies of sessile invertebrates were replaced by macroalgae, which included a non-native species (Airoldi and Bulleri, 2011). This case represents an example of a situation in which humans facilitated an invasion through direct intervention via active disruption of the seafloor. Human facilitation of marine biological invasions can also be less direct, through activities such as point source pollution, overfishing, and large-scale alterations to global climate patterns. As an example of indirect anthropogenic facilitation, Piola and Johnston (2008) found that increasing levels of heavy metals in Botany Bay and Sydney Harbor, Australia, decreased the species richness of native sessile marine invertebrates but did not affect the diversity of non-native species in either area. This study demonstrates that anthropogenic stressors placed on a marine environment can sometimes create opportunities for invasive species to exploit unhealthy niches. Human activity in a region surrounding reef ecosystems should be assessed as a contributing factor to invasive vulnerability.

Assessing these factors for their impacts and assigning values to their effects on invasion vulnerability permit initial development of a framework for management protocols. Helpful to the creation of such protocols are comparable systems of identification and control that have been proven effective in analogous situations. A common analogue to biological invasions is epidemiology.

4. COMPARISONS BETWEEN INVASION ECOLOGY AND EPIDEMIOLOGY

From the onset of research into the ecology of biological invasions, comparisons were made between their characteristics and those of disease epidemics caused by parasitic organisms. The study of these disease-causing organisms and their effects on populations is known as epidemiology, a much older and better-researched field than invasion ecology. Epidemiology assesses a number of different factors when examining the impact of a disease-causing pathogen, and these factors can be extrapolated in scale to fit components of biological invasions. Factors mentioned previously that have ramifications in both fields of study include: vectors that transfer the organism, host biology, invasive organism biology, invasion environment conditions, and effects of introduction/establishment of the invasive organism on its environment. Several steps are required when examining similarities between epidemiological and invasion ecological studies. A general overview of basic epidemiological principles will introduce the concepts that are used in comparing these research fields. The comparisons are then explained in detail, with the approximate equivalent concepts in invasion ecology divided into two categories based on scale: 1) the specifics of invasion biology with respect to coral-microalgal symbioses, with the focus of the comparisons being the cellular dynamics of coral-microalgal symbiosis biology, and 2) the larger-scale characteristics of invasion biology and their epidemiological analogues, with emphasis placed on introduction and spread of invasive species in a novel environment. Finally, mitigation/eradication principles in epidemiology will be outlined, and any comparable concepts in invasion ecology will be elaborated upon.

4.1 Basics of Epidemiology

MacMahon and Pugh (1970, page 3) define epidemiology as the “study of the distribution and determinants of disease frequency.” In a medical context, this directly applies to the study of disease in human individuals and populations. Disease, as it relates to MacMahon and Pugh’s

definition, refers to adverse effects on the health of an organism or population caused by interaction with a pathogen. Additionally, epidemiology studies the spread of pathogenic interactions within populations of organisms. This brief summary of the nature of this extremely diverse medical field will include the following components: history, concepts, and methods of study. The latter two categories will form the basis of the comparisons between the fields of epidemiology and invasion ecology.

The basis of disease in an organism often has its roots in a form of symbiosis known as parasitism, which occurs when one organism (the parasite) gleans biological benefit at the expense of its host organism. The primary concepts of epidemiology revolve around three major aspects of disease—distribution, determinants, and frequency—which form the basis of every epidemiological observation and prediction (Hennekens and Mayrent, 1987). Early studies of disease involved making use of available data to quantify the “existence or occurrence of disease,” measuring its frequency and distribution in populations (Hennekens and Mayrent, 1987). John Snow’s study of cholera outbreaks in London in 1854 was novel for its time in that it attempted to isolate causal factors of a disease outbreak, i.e., its determinants. To that end, Snow used data that demonstrated the frequency and distribution of the cholera outbreak. He developed a pattern of observation and analysis that became known as epidemiological reasoning (Hennekens and Mayrent, 1987). This form of reasoning informs[?] the various research strategies of modern medical epidemiology, which include descriptive, analytic, observational, and interventional studies (Hennekens and Mayrent, 1987). As research methods progressed over the centuries, the study of infectious diseases like cholera developed its own lexicon, which is today used to describe the interactions between pathogenic organisms and their hosts (Casadevall and Pirofski, 2000). This lexicon has direct comparative implications with invasion ecology, especially in the context of coral-microalgal symbiosis. Many of the terms used to describe pathogenic disease are direct analogues for symbiotic processes that occur at the cellular level of coral-microalgal symbiosis. As methods of studying the distribution and frequency of disease have advanced, so has the ability to map the spread of disease through geographic areas. This ability correlates well with the field of invasion ecology, as invasive species entering/establishing into a new geographic area behave in patterns similar to pathogenic organisms invading hosts (Mack, et al., 2000).

The types of epidemiological studies mentioned above lend themselves to comparison with the field of invasion ecology. Descriptive studies of disease tend to examine the details of

frequency and distribution of individuals with diseases in a population (Hennekens and Mayrent, 1987), which have obvious commonalities with species richness surveys, and community composition studies in ecology. In the same way that a researcher examines the demographics of diseased individuals and their geographic distribution, a researcher interested in the potential invasion of an alien species can examine both the geographic distribution of said species outside its native range and the species composition of invaded environments. Analytic epidemiological studies involve comparisons of distinct groups for risk factors that determine susceptibility to disease, as well as observations of diseased populations and experiments on in-progress outbreaks. Ecological analytical studies vary, but include similar susceptibility studies in terms of invasion or community composition/disturbance. Between the basic characteristics of epidemiology (e.g., lexicon, methodology) and microbial invasion ecology, the beginnings of a direct comparison can be made.

4.2 Building the Comparison between Microbial Invasion Ecology and Epidemiology

Microbial invasions in marine ecosystems represent a historically recent branch of ecological research. Mack et al. (2000) identified a number of factors that serve as commonalities between pathogen/host dynamics and biological invasions entry/transport vectors, alteration of host environment as a result of invasive introduction, and mitigation of population establishment and spread. Common also to both disease epidemics and biological invasions, preventative action has been demonstrated to be a more cost-effective and efficient method for mitigation than post-introduction/establishment methods. Examples of ecological mechanisms that closely mirror epidemiological phenomena include macro-scale mechanisms like symbiosis and environmental invasion. On a smaller (micro-scale) scale as well, similar mechanics are found in intracellular interactions between microorganisms and the invasion of host cells by foreign organisms.

Epidemiological organism associations that most closely parallel symbioses generally involve pathogenic microbes or viruses that exploit the cells of a larger host. Finlay and Cossart (1997) discuss the myriad ways in which bacterial pathogens make use of existing mechanisms inside their host cells to replicate and move, and these strategies have parallels in other forms of microbial symbiosis. While parasitism (and occasionally commensalism) are the forms of symbiosis that have the greatest parallels in epidemiology, microbial mutualisms can utilize similar mechanisms to form foundational symbioses. A prime example of these similarities is the methods

that pathogenic bacteria use to establish associations with mammalian hosts and the interactions between scleractinian coral and their dinoflagellate microalgal endosymbionts. Pathogenic bacteria and dinoflagellate endosymbionts share a number of cellular interaction mechanisms, such as recognition, adhesion, and the suite of interactions that result in cellular invasion. Cellular recognition occurs in many intercellular interactions between symbiont cells and their hosts. Basic recognition between host cells and endosymbionts in both pathogenic bacteria and dinoflagellates both involve a host cellular membrane that is receptive to a number of cues from the incoming endosymbiont (Finlay and Cossart, 1997; Davy et al., 2012). In cases of mammalian cells accepting parasitic bacteria, host membrane cell receptors that normally recognize functional compounds generated by the host can be misled to adhere to the parasitic endosymbiont (Finlay and Cossart, 1997). This self-promotion of adherence occurs in coral-microalgal symbiotic establishment as well. Exact chemical exchanges and interactions are poorly understood, but Davy et al. (2012) found that microalgal endosymbionts stimulate receptors in the solitary hydrozoan polyp *Hydra* that actively recognize certain species/clades of endosymbiont. The specificity of host membrane receptors and the requirement that endosymbionts make use of those receptors form the basis of commonality in areas of cellular recognition. The similarities between pathogen/host infection mechanisms and coral/microalgal symbiosis establishment also extend beyond the signaling/recognition stage. Specifically, the mechanisms that govern cellular invasion in both coral/microalgal mutualisms and pathogen/mammalian parasitism have direct correlations.

Scleractinian coral/microalgal symbioses are founded on the establishment and retention of stable populations of the symbiont within the host's cellular tissue. Comparably, in many bacterial parasitic symbioses with mammalian cells, the pathogenic bacteria gains entry to host cells and replicates inside the protection of the host cell's membrane. Finlay and Cossart (1997) noted similarities between pathogenic bacterial invasions of mammalian host cells and the cellular uptake method of phagocytosis. Phagocytosis describes the variety of pathways that a large number of different clades use to intake and store foreign materials (Stuart and Ezekowitz, 2008). Material incorporated by phagocytosis is housed in intracellular vacuoles, which are membrane-bound organelles that can either hold the material or digest it. Mammalian pathogens such as bacteriophages reproduce within vacuoles and enter their host cells through methods similar to phagocytosis (Finlay and Cossart, 1997). Coral gastrodermal cells take up recognized dinoflagellate endosymbionts through direct phagocytosis (Davy, Allemand, and Weis, 2008). The

key differences between parasitic mammalian pathogenic bacteria and mutualistic symbiotic microalgae lie mainly in the regulation of endosymbiont populations by the host. What makes the symbiosis between pathogens and their mammalian hosts parasitic is the manner in which the pathogen obtains benefits. Their reproductive strategy consists of invading host cells and converting to a phenotypically different form in order to lyse out and infect new host cells, and is predicated upon the inability of the host to recognize, reject, or expel the parasite (Oliva, Sahr, and Buchrieser, 2018). Conversely, the basis of the metabolic success of a coral host is largely dependent on its ability to uptake, regulate, and contain their endosymbionts. In the case of coral/microalgal symbiosis, the host is an active participant that works to retain and cultivate stable microalgal populations, rather than a resistant medium for reproduction. Cellular invasion by the endosymbiont is the goal in coral/microalgal symbiosis rather than an event to be prevented. Despite the differences between the outcomes of the symbioses, the pathways to entry into the host cells remain markedly similar. Further similarities become evident when the characteristics of intracellular life for the endosymbionts are considered.

Both in the case of pathogenic invasion of mammalian cells and in the case of coral/microalgal mutualisms, the endosymbiont exists for a large portion of its life cycle within the cellular matrix of the host. In the case of mammalian pathogenic bacteria, their entrance into host cells usually results in the encapsulation of the endosymbiont by a host vacuole. Once the parasite has entered the cell, the characteristics of the intracellular life of the parasite are diverse and specific to different host/parasite associations (Finlay and Cossart, 1997). One common method that pathogenic bacteria use to take advantage of the relative safety of the vacuole's interior is to manipulate the chemical composition of the vacuole membrane. The endosymbionts block the incorporation of chemicals responsible for lysosomal digestion of vacuoles and their contents (Finlay and Cossart, 1997). With digestion of their vacuole inhibited, the incorporated pathogens can reproduce with relatively little interference from the host. While this inhibition of host cellular function acts detrimentally on the host in parasitic symbioses, a similar retention of endosymbionts by the host occurs in coral/microalgal mutualisms. Venn et al. (2009) found that symbiosomes (endocellular vesicles containing symbiotic dinoflagellates) in certain coral species have lower pH's than comparable vacuoles within coral cells, indicating a manipulation of host cellular processes by the endosymbiont. Such manipulations of host cellular processes to discourage

endocellular digestion are common to both pathogenic bacterial symbioses and coral/microalgal symbioses.

Endosymbiont proliferation also occurs in both types of symbioses. In the parasitic bacterial/mammal model, the proliferation of the pathogen within the host cell occurs as a homogenous culture as the pathogen exploits the function of the host. Regulation of pathogenic colonies by the host cells is impeded by numerous types of bacterial intervention, all with the goal of allowing the invaders to reproduce and extract more benefit from the host (Finlay and Cossart, 1997). Host regulation of endosymbionts in coral/microalgal mutualisms occurs at a cellular level and is key to long-term success and growth of the coral/microalgal holobiont. The specialized containment cells of the host gastrodermis actively detect their contained endosymbionts and regulate a blend of symbiont clades with which they form associations. *Acropora* polyps obtain their endosymbionts by taking water into their gastrovascular cavity that contains a diverse group of potential dinoflagellate endosymbionts. Once inside, the gastrodermal cells can not only recognize the clade composition of their multiple endosymbionts but can adjust the composition through extrusion of pellets held together by mucus that contain rejected endosymbionts (Davy, Allemand, and Weis, 2008). They can also recognize deterioration in the cellular composition of their endosymbionts and extrude these deteriorated cells (Davy, Allemand, and Weis, 2008).

The gastrodermal cell can actively degrade and assimilate endosymbiont cells before expelling material that the cell cannot absorb. Under non-stressful conditions, corals can expel degraded and functional endosymbionts to maintain endosymbiont populations (Fujise, et al., 2013). The bleaching response of many corals involves the large-scale release of *Durussdinium* cells from the gastrodermal cells without digesting them (Fujise, et al., 2014). Coral mechanisms for endosymbiont expulsion under thermal stress are different from the mechanisms of expulsion at normal temperatures (Fujise, et al., 2013). Finally, the host can allow the limited growth and division of endosymbionts by manipulating availability of inorganic micronutrients and light (Meyer and Weis, 2012) to the dinoflagellates. Utilizing these mechanisms, the coral host exercises control over the endosymbiont population. So, while the mechanisms that govern intracellular life between the host and endosymbiont differ in the two forms of symbioses, both exhibit important underlying similarities. Further similarities between epidemiology and the study of biological invasion ecology become apparent at larger scales.

A number of coincidental factors make the study of invasion ecology directly comparable to epidemiology. These factors include, but are not limited to: transport vectors, population establishment/proliferation dynamics, and the fates of the invader and its environment (Mack, et al., 2000). The key and major differentiation between the invasion of a single host organism by a pathogenic parasite and the invasion of an ecosystem by a non-native species is scale. However, disease epidemics do have a “macro” scale. Individuals within a population can act as transport vectors for disease, and their movements allow the disease to invade new populations. A prime example is the 2014 Nigerian Ebola virus outbreak. During this disease outbreak, one ill individual served as the transport vector that allowed the virus to spread from Liberia to Nigeria. According to reports in Shuaib et al. (2014), this individual flew into Lagos and exposed 72 people to the virus at the airport alone. This case provides an analogue for a species invading an area to which it is not native. A small population (viral load of the ill individual) is ferried to a location outside its normal range (Lagos International Airport), where it is exposed to a variety of potential new host environments (people at the airport). This basic pattern often occurs, whether in epidemiological or invasive ecological cases, in hubs of transport across the world. Transport hubs offer a confluence of factors that make transfer and establishment of various invasions possible. The incoming and outgoing traffic form individual transport vectors, allowing the possibility for crossover of stowaway organisms or disease. The communities adjacent to transport hubs are thus exposed to a number of potential colonizers. For these reasons, it is critical to understand transport networks and their roles in epidemiology and invasion ecology. Crossover between the studies of epidemiology and invasion ecology can occur in the study of transport hubs, as in Floerl, et al. (2009). Their work applied epidemiological methods, specifically Susceptible-Infected-Resistant (SIR) modeling, to study the ecology of benthic invasive species. Floerl, et al. (2009) examined the efficacy of toxic antifouling paints on the hulls of recreational yachts in preventing the spread and establishment of a hypothetical fouling marine invasive species in New Zealand. By analyzing factors such as surface area of potentially invaded marinas, levels of vessel traffic in each marina, the frequency of resistance application (antifouling agent), and number of vessels utilizing the resistance, the team demonstrated the seemingly stochastic nature of biological invasions. They also created a predictive model that spells out the process by which these invasions occur.

Studies like Floerl et al. (2009) demonstrate a need for predictive modeling and understanding the factors that promote a successful biological invasion. They underscore the

crossover and similarities between ecological invasion study and epidemiology and are a valuable tool for assessing invasion vulnerability. Effective management policies make use of predictive models such as these to assess risk and determine course of action when dealing with ecological invasions. Combining an understanding of the factors involved in a biological invasion with the analogous fields of study such as epidemiology creates a holistic perspective for management protocols.

5. BIOLOGICAL INVASION MANAGEMENT PRACTICES WITH FOCUS ON MARINE MICROALGAL SYMBIONTS

Many cases of biological invasion occur as a direct result of anthropogenic transport, agriculture, and habitat alteration. The effects of these invasions have proven detrimental to the native species of invaded ecosystems and often to human activity in the region. Consequently, a variety of mitigation strategies have been designed and implemented to ameliorate the damage caused by invasive species. The development, establishment, and application of these strategies is the process of management of the invasive or its host ecosystem. Management is of key importance to the eradication of non-indigenous species outside of their home range and to the remediation of invaded ecosystems. The progression of management policies and programs have closely followed the growth of the scientific field of invasion ecology.

5.1 History of Invasion Ecology and Invasive Species Management

The study of non-native species impacting novel ecosystems has informed the development of management protocols and practices. Research into invasion ecology is ideally applied to invaded ecosystems through management practices, although policies, practices, and research are still developing. Invasion vectors are becoming more diverse, and environments are both more disturbed and more connected by human activity. The development of management will be reviewed and its various deficiencies and strengths mapped. Areas of weakness will be discussed upon, and a management framework will be proposed for marine microorganisms that accounts for the current state of the field of invasion ecology.

Invasion biology stems largely from studies in ecology, the field that deals primarily with the interactions between organisms and their environment. The science of ecology began largely in the late 18th to early 19th centuries, with the descriptive work of naturalists like Darwin, Buffon, and de Candolle, and advanced by the turn of the 20th century to a point at which basic concepts

of invasion biology became apparent (Cadotte, McMahon, and Fukami, 2006). A dissociation between ecology and invasion biology became apparent early. Ecological studies largely focused on describing patterns of species distribution within environments. The precursor work to invasion biology attempted to explain the described distributions. Studies resembling modern invasion biology first appeared in the 1930's, with ecologists like Clements noting changes in dominant species within their ranges and attributing the dynamics of species distribution to "succession" (Cowles, 1899). Clements noted that human activity could in some cases impact species succession. The development of niche ecology underscored the ways invasive species could become established and impact their environments. Lotka (1924), D'Ancona (1954), and Volterra (1978) discussed predator/prey interactions and their effects on population densities. They provided an analysis framework that was extrapolated to determine an invader's effect on these interactions. Studies specific to the cause, process, and effect of biological invasions were rare until 1964, when the International Union of Biological Scientists convened to discuss the growing problem of non-native species. The resulting publication (Baker and Stebbins, 1965) proved to be a foundational treatise on invasive species. Another group promoting interest in the ecological dynamics of invasive species was founded in 1969. The Scientific Committee on Problems of the Environment (SCOPE) was formed by the International Council for Science to research various environmental topics, including non-native species invasions. During the 1980's a spike occurred in studies centering on biological invasion by terrestrial plants. The study of invasions grew in geographical scope during this period, as the well-established ecological schools of Europe and America inspired work by conservation ecologists in South Africa and SCOPE conference attendees in Australia. Invasion ecology "rose to ascendance," as a topic of study in ecology (Lockwood, Hoopes, and Marchetti, 2007). From 1989 to the present, the topic of invasion ecology has been popular in research, with the number of authors and studies too large to enumerate (e.g., Carlton 1996; Davis, Thompson and Grime 2001; Richardson and Pysek 2008). During this time, the first political regulating bodies with explicit jurisdiction over biological invasions began to coalesce. Governmental edicts like Executive Order 13112 (Clinton, 1999), signed into law in 1999, created management infrastructure for regulating American conservation authorities to prevent the introduction and spread of invasive species in the United States. In addition to the formation of regulatory bodies, scientific research was translated into legislative protocol. In 1990, the United States legislature passed the Nonindigenous Aquatic Nuisance Prevention and Control

Act (16 USC 4701-02) in response to the invasion of the Great Lakes by the zebra mussel (*Dreissena polymorpha*). An example of translation of ecological theory to managerial terminology occurs in the publication of the proceedings of the United States National Research Council (2002), which outlined criteria required for a predictive system for biological invasions. These were the beginnings of the history of management of biological invasion.

Predictive systems became one of three major focal points of invasion biology from a management perspective. The other main points of emphasis for management bodies became response/control methodologies and prevention protocols for biological invasions. The emergence of cohesive management can be explained by the advancement of the science of invasion ecology. The field progressed to a point at which prediction, detection, eradication, and prevention became practical possibilities.

5.2 Current State of Marine Bioinvasion Management: Case Studies and Recent History

Management of invasive species is complex, as various factors can complicate the interplay between research and application. Environments worldwide are increasingly disturbed as human activity increases across ecosystems. Anthropogenic climate change is beginning to impact more remote and pristine areas. This demands more research into novel invasion sites and species, as well as repeat studies in areas of prior research, as the changing climate places new demands on ecosystems already exposed to invasive species. Management of invasive species is further complicated by the fiscal and political dynamics. Balancing the various priorities of environmental governance has proven difficult even for highly directed and regimented management bodies. Remediation methods for biological invasions are often both costly and effort intensive. The combination of long-term planning, stringent monitoring, and deliberate action that invasion remediation requires are often unattractive to management bodies due to the variety of demands placed upon them. Of special difficulty in management are biological invasions in aquatic ecosystems, particularly marine ecosystems, which possess a number of unique traits that distinguish their biological invasions. Their largely unbounded geographical nature creates opportunities for invasive species to spread, while also increasing the difficulties inherent to monitoring and detection (Hewitt, et al., 2004). While previous studies and management policies focused largely on determining the impacts of biological invasions, current research and policy involves a greater emphasis on prevention and environmental remediation. Additionally, advances

in statistical modeling have permitted more attempts to establish predictive systems for environments and species. To develop a more complete set of protocols tailored to specific marine species, several recent case studies are summarized.

Culver and Kuris (2000) were among the first to document a complete eradication of a marine invasive species. Hallmarks of this study included thorough assessments of the involved species, transport vectors and environment. They utilized principles from different scientific disciplines and a multi-strategy approach to eradication. The invasion began in 1993 with the discovery of a previously unidentified marine polychaete parasite in abalone mariculture facilities in California (Culver and Kuris, 2000). The assessment process that eventually resulted in its successful eradication began with several studies that identified the polychaete and studied both its biology and effect on hosts (Culver and Kuris 2000, and citations therein). The initial studies involved comparing behaviors of the parasite in its native and novel environments. Shortly after the detection of the parasite, a population was discovered outside of mariculture facilities. In 1996 the California Department of Fish and Game introduced policies intended to prevent further spread. A plan was drafted for control and eradication that relied on the implementation of principles first pioneered in the field of medical epidemiology. Specifically, the plan utilized the Kermack-McKendrick theorem of threshold density for transmission of the parasite, which states that, in order for a population of parasites to be transmissible to other hosts, a specific density of hosts must exist (McKendrick, 1940). This principle was applied to these polychaete parasites by creating a plan that called for the large-scale removal of species identified as susceptible to infection. The theory was that removing the potential hosts would raise the threshold for transmission to a point where populations of the parasite would become unable to find new hosts (Culver and Kuris, 2000). Implementation required cooperation between the various management groups responsible for the affected environment. Academic researchers and their technicians identified the problem and proposed solutions. The research team monitored the progress of the eradication by implementing new detection studies throughout the process. The eradication solution required the cooperation of the abalone mariculture facilities that were the origin of the invasion. Screening systems were installed on the outflows of the mariculture facilities to prevent further release of the parasite into the environment (Culver and Kuris, 2000). Finally, public sector input from the California Department of Fish and Game came in the form of regulation that governed the eradication process, and labor contributions to apply the framework laid out by the

researchers. It was recognized that it takes both stringent monitoring and continuous applied effort to control a biological invasion (Culver and Kuris, 2000). The onus of these two keys to success often falls on the management bodies, and lapses in either or both can stymie eradication or allow establishment.

Hoey et al. (2016) provide another example of marine management research and application. This study specifically attempted to utilize management practices to control the crown-of-thorns sea star (*Acanthaster planci*) on Australia's threatened Great Barrier Reef ecosystem. This species can occur in numbers large enough to cause severe damage to coral reef ecosystems by eating the reef-building coral colonies. This species is native to the Great Barrier Reef, but mirrors invasions via population "blooms" approximately once every 17 years (Pratchett, et al., 2014). These blooms result in the second largest single-stressor-attributed loss of coral on the Great Barrier Reef. Control methods have been studied and applied ever since these outbreaks became widely known (circa 1960). To combat the effect of the most recent *A. planci* population explosion, which began in 2010, the Australian government commissioned a study to develop alternatives to individual removal of individual sea stars from the ecosystem through euthanasia (Hoey et al., 2016). The study eventually concluded with an entirely new management framework that was centered around biosecurity practices (Hoey et al., 2016). Six keys to pest management were agreed upon:

- 1) Establishment and Maintenance of a Knowledge Base: The life history of *A. planci*, its physiology, the ecology of the Great Barrier Reef, and its abiotic dynamics are key for any proposed control method. However, the concept of a knowledge base was extended to include the management element. Study of previous responses to *A. planci* outbreaks, other pest management bodies, and their various protocols revealed that a reactionary approach was commonly implemented to control outbreaks. Funding for management was often assigned after the outbreak had been identified and was withdrawn once the outbreak had abated. This "Issue-Attention Cycle" (Downs, 1972) was determined to be an incomplete strategy for controlling and reducing the impact of *A. planci* outbreaks that could over time become more expensive than a more focused long-term approach (Hoey et al., 2016).
- 2) Surveillance and Detection: Proactivity in identifying areas of risk and outbreak-favoring conditions were found to be keys in this stage. It was determined that design of an effective surveillance and detection system to combat *A. planci* outbreaks would require "early

detection and rapid response.” Such a system would require sensitivity to “spatial and temporal components” such as seasonality of sea star aggregations and potential refuge areas where populations of the sea star occur in constant numbers (Hoey et al., 2016). It was concluded that the system would need to be cost-effective as well, as cost-intensive systems may be unattractive or simply unavailable to management organizations.

- 3) **Rapid Response:** A critical step in the control process of any pest is the action taken once a problem aggregation of the pest has been identified. The nature of the marine ecosystem and the pest species were considered when designing the requirements of a framework to control blooms of *A. planici*. The most important feature seemed to be connectivity of sites in the Great Barrier Reef (Hoey et al., 2016). The proposed strategy centered on the use of epidemiological principles with specific attention paid to hub and spoke modeling, which assigns value to vector strength and pathway connection between sites where an invasion is occurring. This allows users to map and determine the likely course of the invasive’s spread (Azmi et al., 2015). In the context of response to a bloom in *A. planici* populations, the hub and spoke model would allow managers to identify source reefs and the currents that form the pathways for larval dispersal of the sea star (Hoey et al., 2016). The rapidity of the response was also important, as any lag time between detection and intervention can result in secondary establishment of a pest colony or greater distribution of affected areas (Hoey et al., 2016).
- 4) **Monitoring:** Monitoring is distinguished from surveillance by its comparison to an arbitrary statistical threshold, rather than to deliberate management action (Hoey et al., 2016). Monitoring programs measure spread of a species of interest, map their distributions, and mark the progress of invasions. Successful monitoring programs make inventive use of all available resources while minimizing cost to management bodies. The example cited in Hoey et al. (2016) was the use of social media-based citizen science groups to undertake swim surveys of sites identified by earlier steps in the framework. The contribution of the citizen scientists allowed staff to focus on response protocols. It was concluded that consistency in monitoring was key, and programs were needed to span both boom and bust periods in the pest cycle to gain efficacy in later cyclic periods.
- 5) **Communication and Preparedness:** Often overlooked as an important aspect of effective invasion management, public awareness and education regarding invasion issues play a

major role in community preparedness for invasion/pest events (Hoey, et al., 2016). Community preparedness is a hallmark of a pro-active approach to management, which is often both more cost effective and successful than a reactive approach. Educating and involving the community can decrease response time in the event of an invasion by building a collaborative network of involved stakeholders that can accurately identify abnormalities in the environment (Hoey et al., 2016). This can be key for the surveillance and monitoring stages of the framework, serving as an insurance policy for the management body against being blindsided by invasive impacts.

- 6) Funding Models: Fiscal planning plays an essential role in the outcome of an invasion event. A common flaw in management framework design is the financial structure, which can suffer from shortsightedness due to competing management priorities. The phenomenon of funding or management focus not being allocated until the effect of an invasion is undeniable is termed “fiscal pragmatism” (Hoey et al., 2016). An effective financial strategy attempts to direct funding to employ preventative methods rather than restorative solutions once an environment has been adversely impacted by an invader. In addition to funding prevention, ease of access to finances is key once a framework protocol has been activated. Streamlining the process of obtaining funding for response and monitoring can drastically reduce the effect of an invasion (Hoey et al., 2016).

This study advocated for a major shift in the outdated modes of thought regarding invasion management. Previous structures centered around reactive applications of restorative measures need to be changed to reflect a more proactive, preventative, and responsive system of biosecurity for marine ecosystems.

Crafton (2015) applied advanced statistical modeling to the field of invasion biology. Statistical modeling can be implemented to predict outcomes for different stages of the invasion process. Modeling is often utilized by management bodies to assess risk or project the result of differing management strategies. This study was undertaken using free software and methods common to invasion biology to demonstrate application to different invasion environments. It used modeling in the context of a marine ecosystem invasion to attempt to determine “where a new species might arrive and how likely that species is to survive in that new environment.” In order to model these likelihoods statistically, Species Distribution Modeling (SDM) was implemented, and included two main categories: “environmental suitability and availability of transport vectors.”

Crafton (2015) selected five marine crab species to model for invasion risk, each chosen specifically for their different invasion characteristics, including (but not limited to): transport vectors, previous invasion history, life history, biology, and native range. Environmental suitability of global coastal habitats was mapped for each species. Introduction likelihood was graphed as a function of distance from a port when compared to percentage of occurrence of each species. Crafton (2015) determined that “significant overlap” existed between environmental suitability for invasion and introduction likelihood in a number of high-traffic ports across the globe. It is important to note that any statistical prediction of biological invasions is inherently limited by the availability of data on the distribution of the subject species. The distribution modeling performed was useful for predicting spatial parameters of invasive transport, but not for predicting the timing of the potential range expansion. Crafton (2015) also noted the “spatial bias,” of the analysis and recognized that some of the environments received low scores in both environmental suitability and/or introduction likelihood because of their greater distance from a port. While the majority of introductions of the five crab species do occur near ports as a result of trade-based transport, the timing of introductions is important to consider. Old or constant introductions to an area can allow an established population in a port locality to disperse to more distant environments. Crafton (2015) concluded that, with the application of the proper type and amount of data to readily-available modeling programs, relevant results can be generated that can form a verifiable predictive system for biological invasion. The results stressed the importance of understanding the limitations of models generated in this manner, including the limitations of the data and potential biases of the model outputs.

These three studies on current hot topics in invasion management research and application have similar bases. They all use marine invertebrates that disperse via planktonic larvae as their model organisms. They represent a broad scope of the practices of marine bioinvasion management, but focus can be narrowed to examine the management of microbial marine bioinvasions. The study of aquatic microbes such as *D. trenchii* and their propensity for bioinvasion has evolved concurrently with technology and the field of invasion ecology. Aquatic microbial invasions have been a specific research topic since the late 1980's (Drake, et al., 2007). The increasing globalization of trade has made the imperative for effective control apparent. Trade expansion is important to the management of marine microbes, because global maritime transport networks act as invasion vectors for many marine species, especially marine microbes.

Marine microbial bioinvasions have a relatively short history of scientific study and an even shorter history of management. Marine bioinvasions are often the result of anthropogenic transport of a founder population, and a majority of human maritime activity involves movement via boats and ships. This movement, especially involving mass transit of trade goods between countries, permits the movement of small populations of species as stowaways. A major management protocol enacted on a global scale to combat ballast-assisted bioinvasions was a measure put forth by the International Maritime Organization (IMO) in 1991. As an agency of the United Nations, the International Maritime Organization has jurisdiction over international shipping, and its 1991 action called for mid-ocean ballast water exchange in an attempt to minimize direct exposure between port environments (Lockood, Hoopes, and Marchetti, 2007). This action was to be performed on a voluntary basis, which placed obvious limitations on effectiveness of the regulation. However, in 2004 the IMO adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (Ballast Water Management Convention-BWMC), which required vessels that ratified the treaty to develop and maintain plans for exchange and management of ballast in addition to holding certifications in ballast management (Hess-Erga, et al., 2019). Since its adoption, a majority of worldwide fleets have ratified the treaty and are participants (Hess-Erga et al., 2019). This method of overarching management of marine transport pathways can be effective when considering the attributes of marine microbes that maritime shipping can harbor.

5.3 Prediction of Areas Vulnerable to *Durusdinium* Invasion

Aside from simple observational studies, another method for data collection that can be useful for building a knowledge base regarding biological invasions is predictive analyses. Such analyses utilize statistical modeling and available data on a number of invasion predictors to generate outcomes that approximate distributions of invasive populations. Predictive analyses can often be generated at low cost using publicly licensed software but have a number of restrictions that can lessen their utility. The stochastic nature of invasion features can also confound certain statistical modeling programs. Predictive systems therefore need to utilize the factors presented in Part 2 to effectively account for variations in the component structures that make up an invasion. Key to predicting environments that may be vulnerable to invasion are a thorough understanding of the factors involved in successful invasions.

Biological invasions are more often successful when the invader can integrate into the existing ecological framework. In the case of *Durusdinium*, its place in the ecological framework involves the niche of its host species as well as its own niche. The potential invader has a global distribution and an existing niche in many coral reef ecosystems. When considering areas that are vulnerable to invasion by *Durusdinium*, the range of close ecological analogues should be considered as well. Predicting where an invasion can occur in the Greater Caribbean reef system first requires an understanding of the locations and distributions of vulnerable coral holobiont populations (Figure 10).

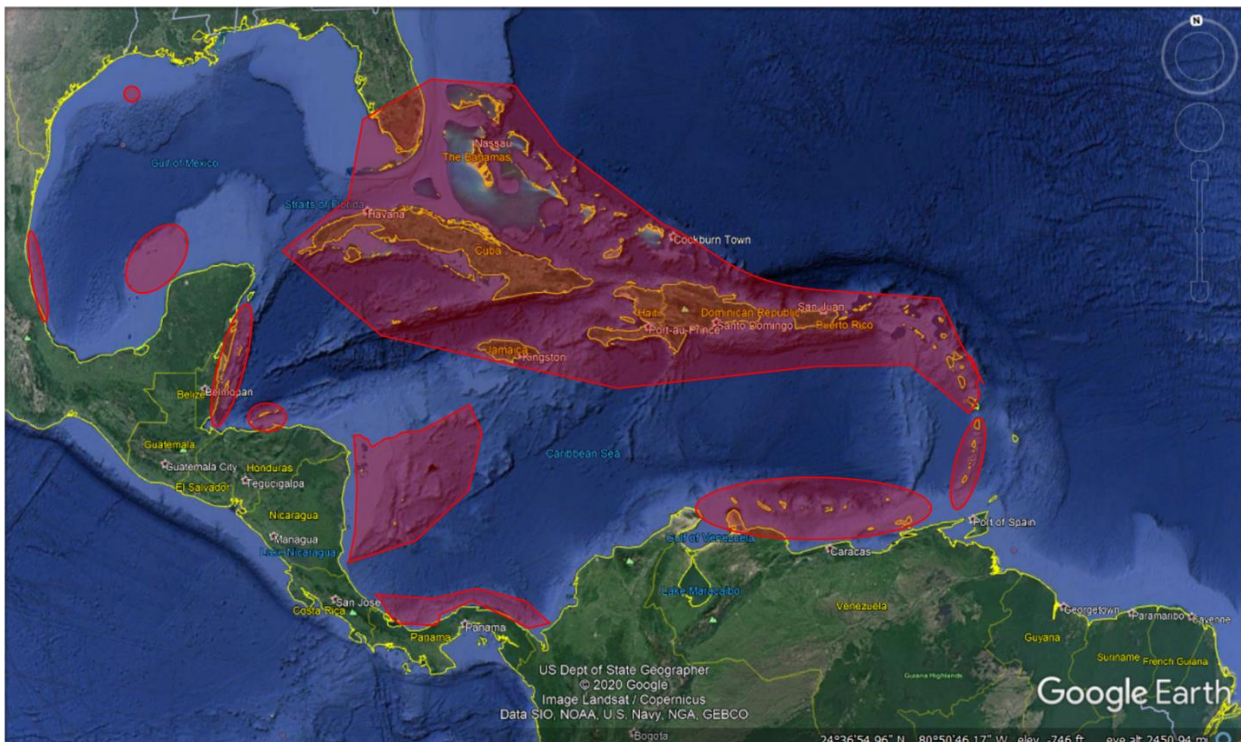


Figure 10: Approximate Geographic Ranges of Coral Reefs in the Greater Caribbean Reef System. Map Data: Google, Landsat/Copernicus, SIO, NOAA, U.S. Navy, GEBCO (Robertson et al, 2015).

Environmental perturbations, such as Sea Surface Temperature Anomaly, can be used to determine an area's predisposition to future coral bleaching. Sea Surface Temperature Anomaly refers to the temperature difference between a current area's temperature and long-term averages (NOAA Coral Reef Watch). Mapping these disturbances (Figure 11) can identify problem reefs if the anomaly is consistent over time.

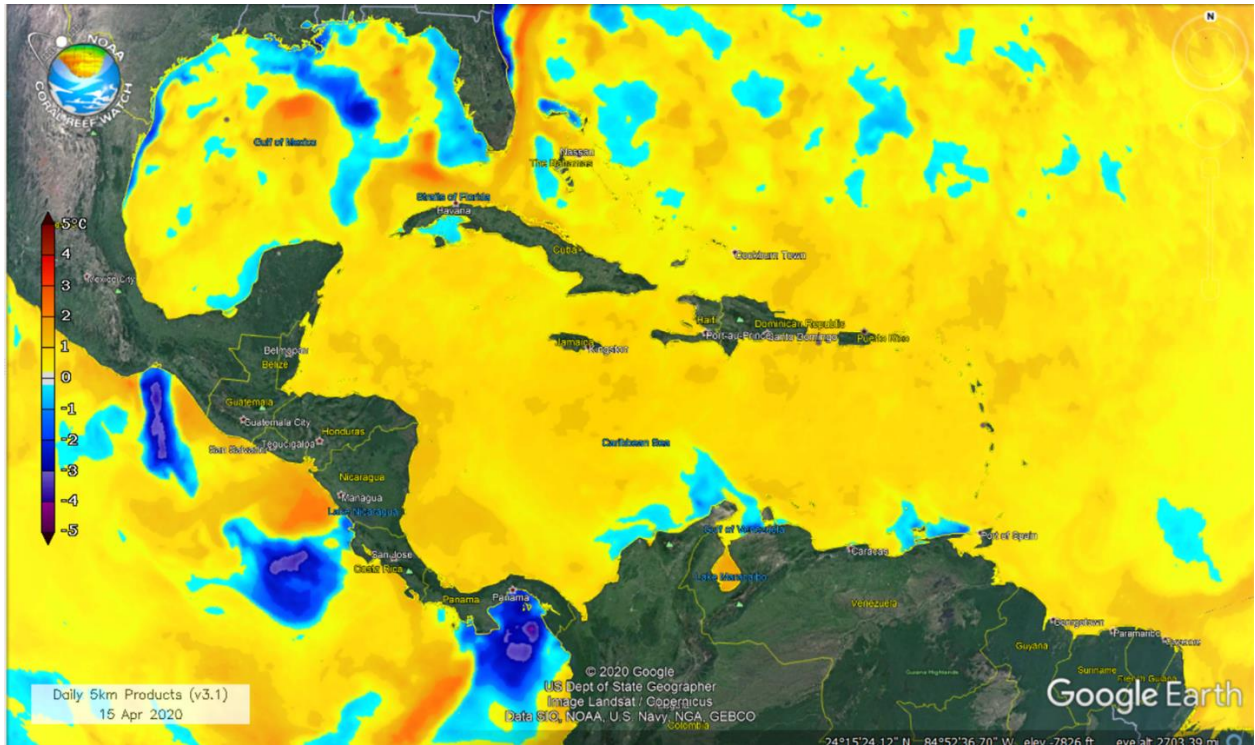


Figure 11: Sea Surface Temperature Anomaly in the Greater Caribbean Region, Daily Measure 4/15/20. Map Data: Google, U.S. Dept. of State Geographer, NOAA, SIO, U.S. Navy, NGA, GEBCO (NOAA Coral Reef Watch, 2020).

Finally, determining anthropogenic activity in the area permits researchers to focus their assessments for invasions. For a species known to be transported by maritime shipping, shipping hubs in the area of interest must be assessed (Figure 12). Founder populations would be introduced into these areas, and adjacent habitats would be the first to be invaded.

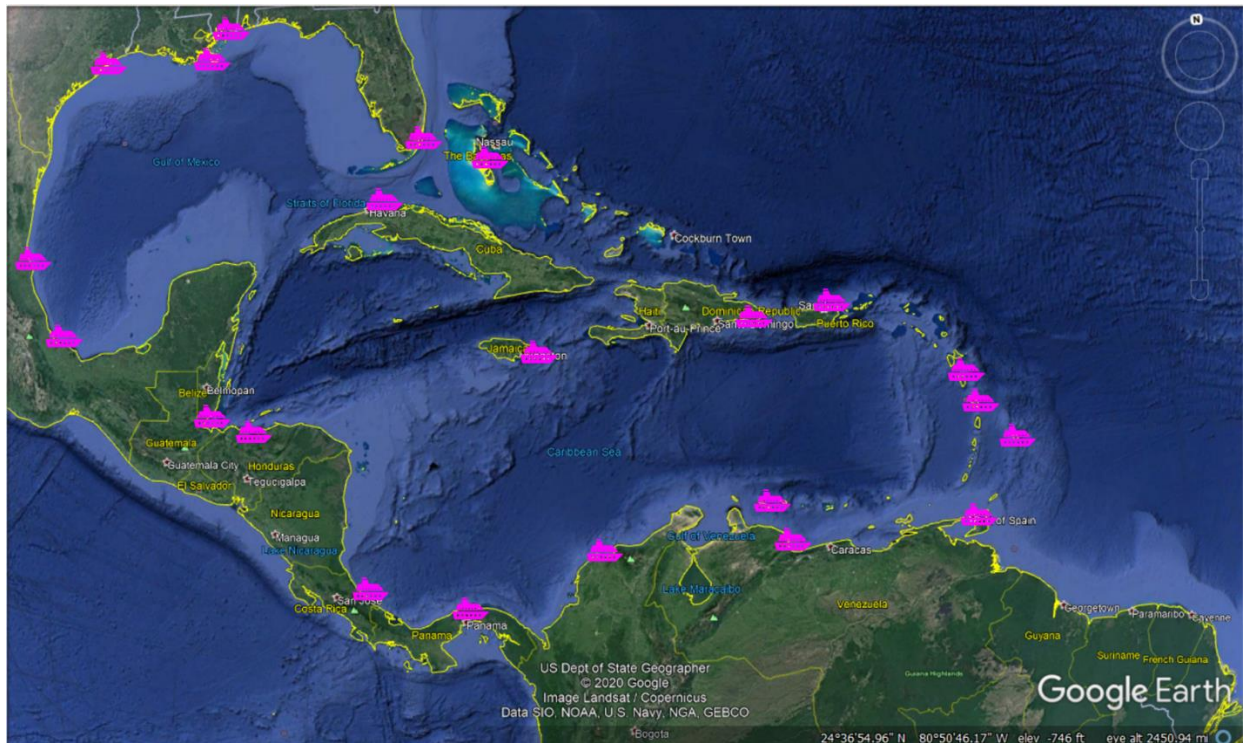


Figure 12: Approximate Location of Major Maritime Shipping Ports in the Greater Caribbean Region (Begot Buleon and Roth, 2001). Map Data: Google, Landsat/Copernicus, SIO, NOAA, U.S. Navy, NGA, GEBCO.

Combining these predictive factors builds a multilayered picture of the areas most vulnerable to invasion. While simplistic, the graphics presented in figure 10 below do allow for some prediction involving invasion vulnerability. Heavy anthropogenic activity, such as in the triangle between Havana, Freeport, and Miami (Figure 13), could potentially affect the reefs in this part of the Caribbean, given that much of that subset of the region is exposed currently to SSTs that deviate from the norm. The layering of predictive factors and the determination of their interactions are basic to the development of predictive systems for biological invasions.

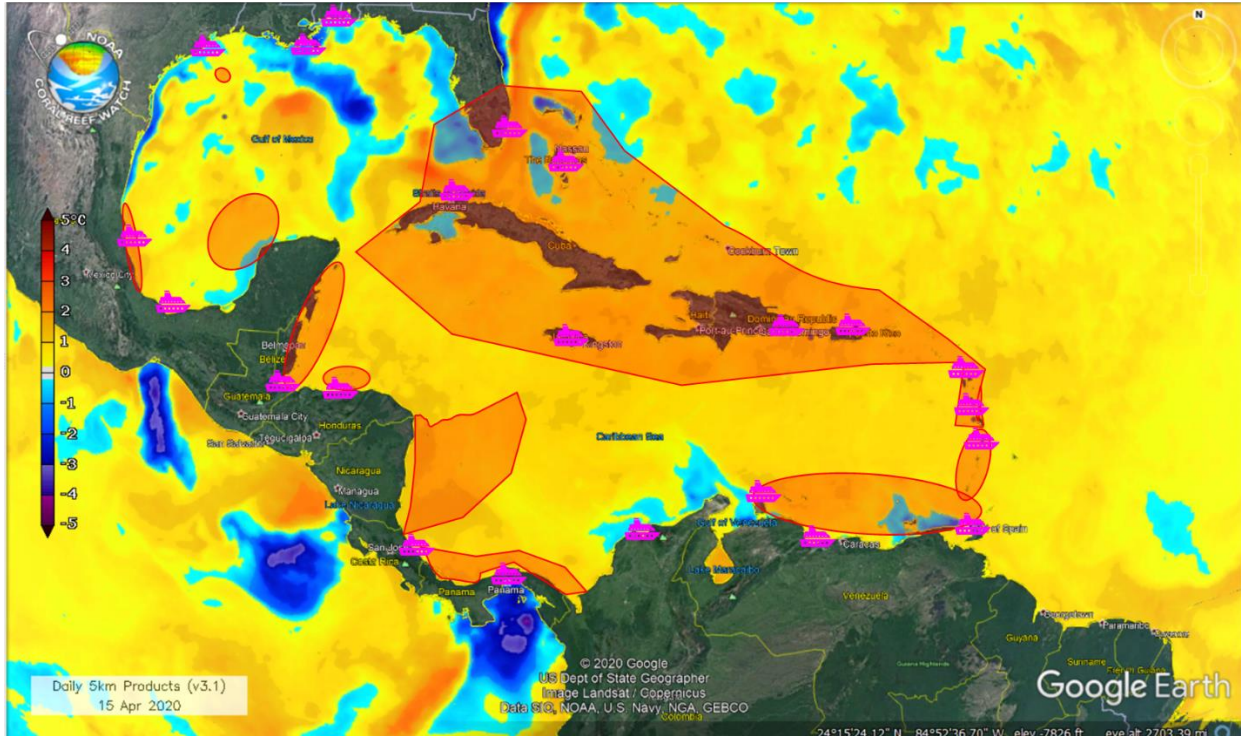


Figure 13: Multiple Overlay Map Indicating Areas of Anthropogenic Activity that Correspond to Coral Reef Distributions and Anomalous SST. Map Data: Google, U.S. Dept. of State Geographer, NOAA, SIO, U.S. Navy, NGA, GEBCO (Begot Buleon and Roth, 2001; NOAA Coral Reef Watch, 2020; Robertson et al, 2015)

5.4 Designing a Management Protocol for *Durusdinium*

Durusdinium species, including *D. trenchii*, possesses a number of characteristics that inform its population behavior both inside and outside its native range (i.e., during a bioinvasion). The management of marine microbial bioinvasions requires specific considerations that accommodate these characteristics. These dinoflagellates are small, with normal coccoid cells 6-13 μm in diameter (LaJeunesse, Parkinson, and Trench, 2012). Their microscopic size and dual cellular forms (mastigote and coccoid) affect their ability to invade. As with many marine microbes, they are often transported outside their native range via ballast water. Large numbers can be passively extracted from their native range when pumped with environmental water into ship ballast tanks. These populations can then be transported at little to no metabolic cost to the dinoflagellate. Their two life-history forms allow them to persist within ship ballast tanks as both free-living coccoids and as endosymbionts inside larval invertebrates (Stat and Gates, 2008).

Durusdinium can reproduce asexually via mitosis, and the process consists of darkness- and light-dependent phases (LaJeunesse, Parkinson, and Trench, 2012). This method of

reproduction lends itself to invasive behavior, as a single cell can reproduce alone and effectively sustain a clonal population as long as environmental conditions are favorable. Of particular interest is the separation of light and dark processes. Theoretically, an individual cell could undergo the obligate dark process during transport in the lightless environment of the ballast tank and emerge into a new environment ready to undergo the light-requiring cytokinetic processes that would immediately result in two individuals. Population genetics data gathered on various clades of *Durusdinium* show a level of variation that closely approximates the level of variation associated with sexually reproductive populations (Baillie et al., 2000). While observational evidence of the process of sexual reproduction in these dinoflagellates is scarce (LaJeunesse, Parkinson, and Trench, 2012), the genetic variation could indicate a method for sexually recombinant reproduction. A variety of reproductive strategies would help populations of introduced *Durusdinium* avoid the loss in viability that an entirely clonal population could incur. This variety could also magnify the effects of invasion over the course of multiple introductions, as individuals from different genetic lineages of *D. trenchii* could be introduced by different ballast water exchanges. This would bolster the genetic variability of populations near introduction points, lessening the founder effect and allowing populations to stabilize before expanding through further passive transport.

Vulnerability to invasion, current invasion conditions, and connectivity of the introduction point to other sites all play large roles in the establishment and propagation of invasive populations. Each of these aspects contains so many potential contributing factors as to make the course of a species invasion seem almost stochastic in behavior (Freckleton, Dowling and Dulvy 2006). Certain environmental attributes are major indicators of invasive establishment or spread through environments. Local currents determine the direction of possible invasive spread for passively transported microbial invaders. Native species that occupy the same ecological niche as an introduced species can indicate invasion vulnerability, especially if the native species' populations are under stress (Vasquez, 2005). Non-native *Durusdinium* species can readily occupy the ecological role of displaced native species due to their global distribution, e.g., the "opportunistic" tendencies of *D. trenchii* permit it to displace other clades as primary endosymbionts in health-compromised coral hosts (Stat and Gates, 2011). These clades have overwhelmingly similar morphologies (Lajeunesse, Parkinson, and Trench, 2012), which makes distinguishing native vs.

non-native populations difficult. Genetic techniques are often required to discern the genetic makeup of *Durusdinium* populations.

The problem of identification is one of the key difficulties that management bodies face when attempting to curtail biological invasions, especially of microbes. Other management issues include identifying the transport vector, identifying source populations, detection, eradication, and consistency with monitoring and mitigation action. Following the proposed framework in Hoey et al. (2016), the issues that retard management success are present in all six Pest Management Keys.

Issues confounding management protocol development for *D. trenchii*

- 1) Establishment and maintenance of a Knowledge Base: Lack of globally standardized/enforced methods with mutual agreement (BWMC notwithstanding),
- 2) Surveillance and Detection: organism size, morphological similarities, multiple niches,
- 3) Rapid Response: Lack of verified control method research, application, documentation,
- 4) Monitoring: Issue-Attention Cycle deficiencies, lack of standardized method for sampling/identification, advanced genetic techniques required for identification,
- 5) Communication and Preparedness: Lack of education and outreach regarding issue of microbial invasions to public (IMO has provided outreach to vectors),
- 6) Funding Models: Lack of advancement in allocation of funding for biological invasions in general, no specific plan formulated for microbes.

The paucity of research into all six Keys to Pest Management demonstrate the need for a modified management framework that deals specifically with marine microbes like *D. trenchii*. Research on marine bioinvasions is growing in scope and volume but lacks a definitive accounting of the numbers and names of introduced marine species (Geller et al., 2010). A comprehensive assessment of invasive species could contribute to predicting future bioinvasions by establishing comparisons between successful invasive species and their phylogenetically close relatives. Additionally, the changing state of Earth's climate is driven principally by anthropogenic activity, which provides further justification for the creation of an adapted framework. Previous frameworks were developed during a more climatically stable period. Maritime trade activity is growing by an average of 2.1% annually (Barki and Deleze-Black, 2017), which results in greater frequency of potential introduction, as ships move between ecosystems more often. Finally, greater frequency of introduction can lead to increases in establishment of non-native species, which can

have ecosystem-scale effects (Pettay et al. 2012). For these reasons, the following proposes a marine microbe-oriented management framework specifically to address *D. trenchii* invasions.

5.5 Management Framework for *Durusdinium* Invasions

The proposed management framework considered previous studies as templates and modified existing structures to match the characteristics of a *D. trenchii* invasion. Specifically, the keys for pest management of *A. planci* in Hoey et al. (2016) were used as a basis and altered to reflect the differences between the macroinvertebrate *A. planci* and the microbe *D. trenchii*. Actions proposed were targeted to the different invasion stages of the Unified Framework for Invasion (Blackburn et al., 2011). The different invasion stages represent opportunities to arrest the progress of bioinvasions; targeting management policy and action to specific invasion phases should increase cost-effectiveness of the proposed solutions. The management framework should provide solutions across the spectrum of invasion stages to provide multiple individual actions that can be grouped or used singularly.

*5.5.1 Framework Step 1: Acquiring Knowledge Regarding *D. trenchii* Invasions and Applications to Management*

The first key to pest management involves the construction and maintenance of a knowledge base on the pest species (Hoey et al., 2016). This requires both assessment of previously collected data and collection of new data once areas of deficiency have been identified. Review of previously collected data can be undertaken by a prospective management body with little initial cost. Three broad research topics are important when establishing a knowledge base for a potential biological invasion: basic information on bioinvasions, the non-indigenous species of concern, and the potentially affected environment. All three require both review of existing studies and commission of new ones.

Potential Topics for Review:

1. Bioinvasions: Effective management of any biological invasion requires understanding of the history and current state of the field of invasion ecology. Special attention should be paid to invasions of marine ecosystems, focusing on the study of potential vectors, previous management strategies, and predictive systems. The invasion history of the ecosystem of concern should be thoroughly researched. Any previous studies regarding environmental

disturbance, anthropogenic impacts on the ecosystem, and locally introduced species are of interest. Research into previously effective management policies and practices is also important, as it can provide a template for protocols to deal with future invasions.

2. *Durusdinium trenchii*: A review of research on biological invasions must include the non-indigenous species of concern, in this case *D. trenchii*. The ecology and biology of this potential invader must be thoroughly understood in order to develop effective countermeasures in the event of an invasion. Studies of the species in its native range can help determine its behavior if the target environment bears ecological similarities to its native habitat. Its life history, including those of its suite of known symbiont hosts, needs to be researched to understand its potential establishment patterns. Local analogues to its hosts and occupants of its ecological niche should be identified so they can be incorporated into potential surveillance/monitoring programs. Preferred habitat research can also be incorporated into these programs, as the generalist nature of *D. trenchii* creates multiple sinks where populations can subsist (Stat and Gates, 2011). Aside from its biology, any studies involving transport of dinoflagellates should be collected to better understand the population dynamics and range expansions of closely related species.
3. Environment: Finally, understanding attributes and dynamics of the potential invasion ecosystem is essential. Most aspects of the invasion environment can be divided into two larger categories: transport and ecology.
 - a. Transport factors relate to species import into and export from the environment. Maritime shipping travels through ports, so research into ports local to the invasion environment must be undertaken. Shipping volume, frequency, destinations, and sources all should be studied. Local estimates of shipping traffic can be of value when considering whether multiple introductions may occur during an invasion. A review of current local ballast water management practices and protocols can inform the development of new frameworks. Studies involving local regulations and compliance should be assessed, and in their absence new ones should be performed. Public awareness of the issue of invasion is important to later management keys. Any studies that involved public outreach or usage of the public as an asset to management should be reviewed to inform later practices. Finally, impacts of transport on the local environment need to be properly understood in

order to tie the transport vectors into the local ecology. Studies that demonstrate the effects of transport hubs, vectors, and regulations on the environment must be assessed.

- b. The ecology of an environment is a major component of the study of biological invasions. Studies of its past and current ecological health need to be reviewed, as their results can help determine the impacts of various stressors. Identifying disturbance events, whether anthropogenic or natural, can provide insight into the various reactions of an environment to disruptive ecological stimuli. Connectivity studies are also essential, as they can inform the likely path of an invasion once an invasive becomes established. Assessments of available habitat can be useful determinants of the locations where an invasion can begin. Numerous other aspects of the environment can provide insight as well, such as climate, local species analogues, and invasibility assessments. In summary, all ecological research topics relevant to a potential invasion site should be reviewed, as biological invasions can be affected by a myriad of ecological factors.

While the value of review of extant research cannot be overstated, previously collected data has glaring limitations that should not be overlooked when developing a management framework for biological invasions. Such data does not provide a complete picture of the current state of an ecosystem, so additional data must be collected when establishing a body of knowledge in preparation for management of biological invasions. Data collection can be both expensive and time consuming, and arguably the two most important resources for management bodies are funding and time. Thorough review of extant data is thus made more important by any potential fiscal and temporal limitations imposed by higher regulatory bodies. With these limitations in mind, the majority of proposed data collection methods for this particular framework should be observational. Such studies are used in epidemiological investigations to passively determine disease dynamics; they can be effective in gathering data on the health of an environment as well. Observational studies can be performed on an environment to compare against previous environmental conditions or other similar invasions. To examine transport dynamics and to possibly determine the presence of potential invaders, water sampling from ballast tanks could occur as specified in Ruiz, et al. (2000). Surveying biosecurity control methods for ship ballast tanks could both help develop new frameworks and inform investigators on the effectiveness of

ship-borne control methods in preventing invasions. To determine the existence/extent of community awareness of biological invasions, simple questionnaires could gauge public involvement and identify areas of public concern. Finally, to determine environmental conditions, several basic ecological assessment strategies could be employed. Trained citizen scientists or student researchers could survey species richness to assess diversity and to look for signs of invasive occurrence. These surveys can document species richness and signs of disturbance or other anthropogenic impacts on the environment.

Combining a review of extant studies and new data collection to build a knowledge base has useful implications at all stages of Blackburn et al.'s (2011) Unified Framework for Biological Invasions. Review of extant research can provide information that can inform management of each of the phases and can be useful in developing solutions that make the barriers between phases insurmountable. With respect to proposed data collection, ballast water sampling and biosecurity measure surveys can provide management solutions that affect the Transport phase. The proposed species richness surveys that include public outreach and education can help prevent the Establishment phase by providing baseline data that can assist in preparedness for an invasion event. Finally, predictive analyses and review of extant research can reveal invasion pathways and elucidate dispersal patterns. This has direct implications in prevention of the Spread phase. Once the knowledge base has been consolidated, management focus can shift to surveillance and detection of potential invasive species.

5.5.2 Framework Step 2: Identifying Invasions in the Environment

Surveillance and Detection methodologies are essential for managing and mitigating biological invasions. Without proper surveillance, understanding of environmental norms cannot be comprehensive. A lack of effective detection methods can result in misidentification of an invasive species or failure to recognize an introduction or establishment event. With improper surveillance and detection methods, cryptic invasions—those that involve markedly similar non-indigenous and indigenous species—can become a problem (Carlton, 2009). Cryptic invasions cause problems for management by displaying ecological symptoms of bioinvasions without the obvious presence of a morphologically distinctive invader. *Durusdinium* invasions would be considered cryptic, as multiple species of the dinoflagellate are likely to occur together, and morphology is largely conserved between species of *Durusdinium* (LaJeunesse, Parkinson, and

Trench 2012). Effective surveillance protocols specific to a *Durusdinium* invasion of an ecosystem have a number of requirements. The uniqueness of the ecosystem in question requires that the protocols be heavily informed by research. Through review of literature and data collection, the surveillance program needs to accurately identify transport vectors, potential population sources, potential population sinks, and invasion pathways. Surveillance and mitigation procedures need to be proactive and preventative to quickly respond to the presence of an invasive species, should it be detected. The surveillance design needs to consider both spatial and temporal components of an invasion. The greatest introduction risk would be ascribed to geographic areas with direct contact with non-native water sources, like ports and ballast disposal sites. Detection methods need to be concentrated in these geographical areas, and the frequency of application of these methods need to be greater as well to account for the potential of multiple introductions. Invasion pathways such as currents need monitoring as well to determine the magnitude and direction of invasive spread. Temporal considerations include environmental condition fluctuation as a result of seasonality, nearness in time to bleaching events/other stressors, and anthropogenic transport schedules. Sensitivity to seasonality could be important for opportunistic endosymbionts like *D. trenchii* which can take advantage of bleaching/stress periods to establish novel associations with corals outside its native range (Stat and Gates, 2011). Summer disease season for scleractinian corals or the weeks after a temperature-induced bleaching event would both provide temporal windows for free-living non-native populations of *D. trenchii* to establish in a reef ecosystem. Anthropogenic transport schedules also need to be understood and accounted for as part of a surveillance methodology, as an influx of shipping would provide multiple possible introduction events. A management surveillance protocol that centers around *D. trenchii* would therefore benefit from concentrating detection efforts at these times. Water samples taken from identified areas/times of risk need to be genetically analyzed to determine the species present. In addition to water samples, sediments from potential sinks and host organism tissue samples could be taken and analyzed in the same way. In order to maintain the cost-effective requirement of a limited management framework, concentrations of samples would be taken from the times and places listed above. Sampling activity being more limited outside of the previously described spatial and temporal parameters would reduce excess expenditure on sample collection and processing. Surveillance and detection have management implications across all of the stages of Blackburn et al.'s Unified Framework (2011). Methods for surveillance and detection could not only identify

the presence of invaders in the Transport, Introduction, Establishment, and Spread phases, but may also be sensitive enough to pinpoint the stage of a given invasion. Detection methods aboard ship ballast tanks would aid in control of the Transport and Spread Phases. As ships are known to be prominent transport vectors, it is logical to focus surveillance efforts on them as known carriers of non-indigenous species. Environmental sampling and analysis are useful in identifying an invader in samples taken from vulnerable environments and can thus point out a species in the Introduction and Establishment phases. Once the foundational management aspects of learning about the problem through development of a knowledge base and identifying its presence the environment through surveillance have been accounted for, the next phase of management begins.

Detection is essential for successful management. Without an appropriate methodology, an invasive species will be difficult if not impossible to detect. The progress of an invasion is also nearly impossible to document. In the case of *D. trenchii*, detection is essential, because different clades are so difficult to distinguish visually, the microbe occurs in a variety of coral reef niches, and even aggregations are not visible to the naked eye. *Durusdinium* is detected in its environment via two major methods: collection and genetic analysis. Collection methods involve sampling populations for analysis. Clade composition is determined via genetic analysis of sampled populations. Samples must be collected from the three parts of a reef environment where these dinoflagellates occur: within a coral host, or free-living in the substrates (sediment, rock, other living surfaces) and the overlying water column. Each of these habitats normally harbors a variety of native *Durusdinium* clades, and all could simultaneously harbor invasive clades, making assessment of each of the habitats essential to effective detection. Collection methods and how they are distributed differ for each habitat.

Collecting endosymbionts from coral cells allows analysis of the endosymbiotic population structure at the time of collection. Such collections require clipping off a small piece of the colony and preserving it. Samples are typically transported in lightless, low-temperature containers and storage in a dimethylsulfoxide-buffered salt-water solution (Pettay et al. 2015). Multiple samples are taken from different portions of each colony (Figure 14) to account for variabilities in environmental exposure (Pettay et al. 2011).

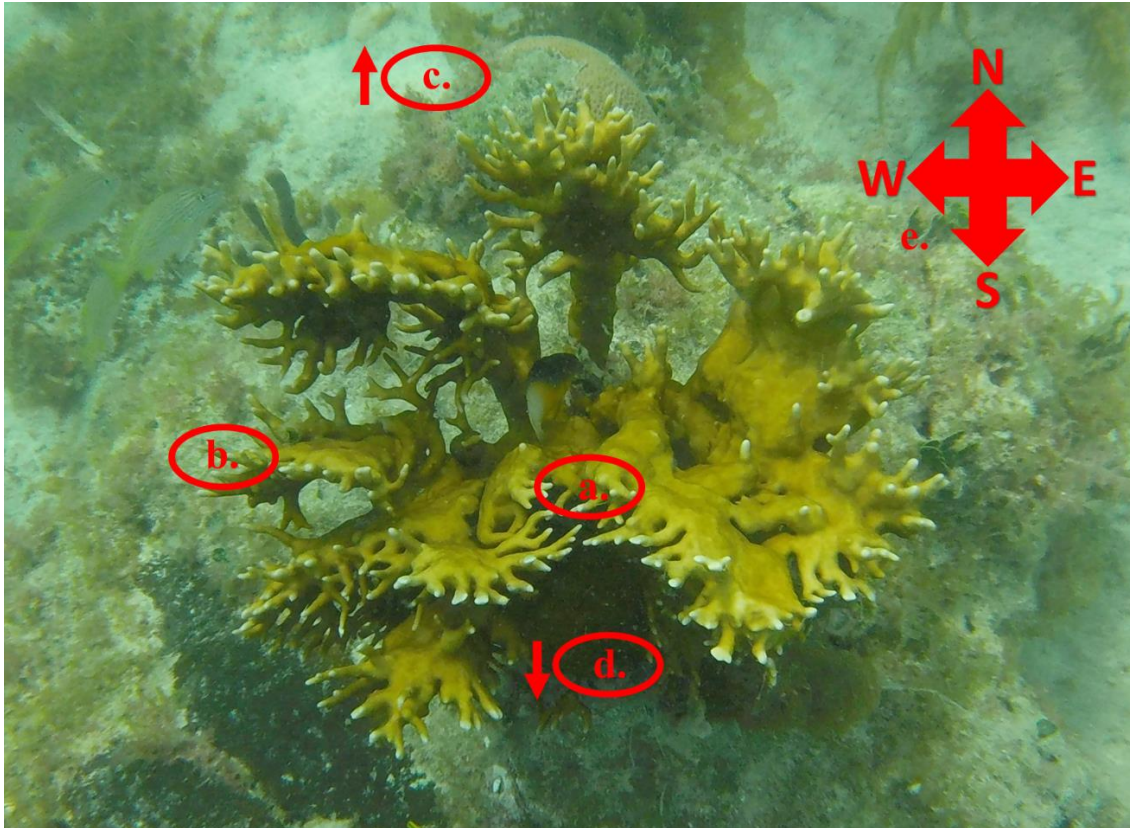


Figure 14: Coral Biopsy Collection Zone Examples on a colony of *Millepora alvicornis*. Biopsy clipping sites include samples from the central (a.), distal (b.), surface-facing (c.), bottom-facing (d.), and directional (e.) portions of the colony. Photo Credit: David Lawson, 2018.

Geospatial methods are important for collecting biopsy samples for endosymbiont analysis. Determining the best collection method depends on the purpose of the investigation, the type and distribution of host colonies, and environmental factors of the reef. Detection methodologies for management either involve assessing a reef on which *D. trenchii* has not been identified (surveillance) or determining the extent to which *D. trenchii* has become established (monitoring). Each of these conditions requires unique geospatial methodologies. For example, monitoring protocol could involve sampling all colonies within randomly assigned quadrats on a reef tract to assess the diversity and distribution of clades. Surveillance protocol would be more regimented, with sample tracts being chosen with greater attention to invasion vector proximity. Geospatial methods for surveillance would further extend to sampling all colonies along repeated reef transects to get a more comprehensive picture of where the invasion has established in relation to oceanographic features such as currents or unique bottom topographies. While these methods are

essential to collection of associated populations of *D. trenchii*, they also can be applied to collecting free-living forms of this dinoflagellate.

Reef substrates and water column support biomes of microbial life that include free-living *Durusdinium*. Both habitats must be searched in order to detect this generalist coral reef microbe. Collection methods are simpler for free-living *D. trenchii* but the means of obtaining genetic information are similar for both coral biopsy samples and free-living dinoflagellates. Sample preservation is largely the same, with transport in lightless low-temperature conditions. *Durusdinium trenchii* is collected from the water column simply by obtaining samples of reef water, either from near the surface (Takabayashi, Adams, and Pochon 2012) or from a variety of depths (Yamashita et al. 2012), depending on the purpose of the investigation. Samples from multiple depths account for sub-surface currents and differences in light transmission. Substrate collection involves similar techniques, with some distinct considerations. Sediment from aerobic surface layers should be collected, as *Durusdinium* do not survive in deeper anoxic levels of reef substrate (Takabayashi, Adams, and Pochon 2012). Geospatial collection methods are again important, as current patterns influence sediment settlement rates and distributional patterns on coral reefs (Quigley, Bay, and Willis 2017), e.g., the reef flat and backreef areas are less exposed to offshore currents than the reef crest. Sampling only reef flat sites would provide an incomplete picture of the species composition of a reef. Current patterns are obviously important to the clade composition of the water column as well.

Genetic analysis of samples determines the population structure of the *Durusdinium* communities from the collection sites. Such analyses vary widely in scale and purpose, and studying *Durusdinium* in reef environments makes use of this variety to tailor the analysis to the research objective. Most important to the detection steps in management frameworks is identification of the clades present and their relative abundances. When detecting an invasion from a specific geographic area, distinguishing native versus non-native taxa is also important. Genetic analysis begins with the extraction of DNA from samples. DNA extraction has been simplified by the development of kit-based extraction techniques applied in concert with next-generation sequencing (Weber, DeForce, and Apprill, 2017) but can prove difficult for samples taken from complex symbiosomes such as coral, because a much of the extracted DNA comes from the coral host. Also, the efficacy of kit chemical components designed for lysis of cells to release DNA are often retarded by the host cellular structure and heterogenous composition of the coral microbiome

(Weber, DeForce, and Apprill, 2017). However, endosymbiont DNA can be differentiated and amplified to determine the clade makeup of the endosymbiont population. The primary method for DNA amplification is Polymerase Chain Reaction (PCR), which uses DNA primers to locate known sequences of sample DNA and copy them exponentially (Ochman, Gerber, and Hartl, 1988).

Since its invention, PCR techniques and applications have advanced to a point at which identified DNA sequences can be selected and amplified to determine composition within a sample. Yamashita et al. (2012) used this specialized PCR by amplifying the 28s rDNA region in a sample of *Durusdinium* and employed primers specific to clades A-F; after amplification they compared the relative amounts of clade-specific amplified DNA present through gel electrophoresis. Pettay et al. (2015) used a similar method of amplification and electrophoresis to establish the clade of the dominant resident endosymbiont in colonies of *Orbicella faveolata* but amplified a different region of DNA: the Internal Transcribed Spacer Region 2 (ITS 2). This region of DNA is common to analyses involving *Durusdinium* because PCR of different clade ITS 2 regions yield fragments with different base pair lengths (LaJeunesse 2002). Pettay et al.'s (2015) and Yamashita et al.'s (2017) investigations also differed in scope. While detection of component *Durusdinium* clades from the samples in Yamashita et al. (2017) proved sufficient for their objectives, Pettay et al. (2015) sought to establish that a non-native clade was establishing symbioses in their region, which required additional genetic analyses. The fragments of DNA they gleaned from PCR were constructed into multi-locus genotypes (MLG) to differentiate clade D species from one another (Pettay et al. 2015). Repetitive pieces of DNA known as microsatellite loci were isolated in previous studies (Pettay et al. 2011), amplified through PCR and used to identify clade D endosymbionts in their samples (Pettay et al. 2015). Alleles in these microsatellites are unique to certain geographic areas, and analysis of their presence and numbers in their samples identified the genetic origin of the clades present (Pettay et al. 2015). These methods are relevant to the detection of *D. trenchii* in a reef environment.

5.5.3 Design of a Detection Program Methodology for *D. trenchii*

Effective management protocols place emphasis on the two steps of surveillance and monitoring. Specific to *D. trenchii*, a detection program would consist of the following parts (Figure 15): 1) Baseline Establishment should focus on understanding the reef environment that

is vulnerable to invasion; 2) Initial Surveillance u be implemented once the possibility of an invasion has been established, and its results should determine the next steps; 3) Post-Detection Monitoring should occur if the results indicate that an invasion is beginning or imminent. The process should return to Baseline Establishment if invasive *D. trenchii* has not been detected.

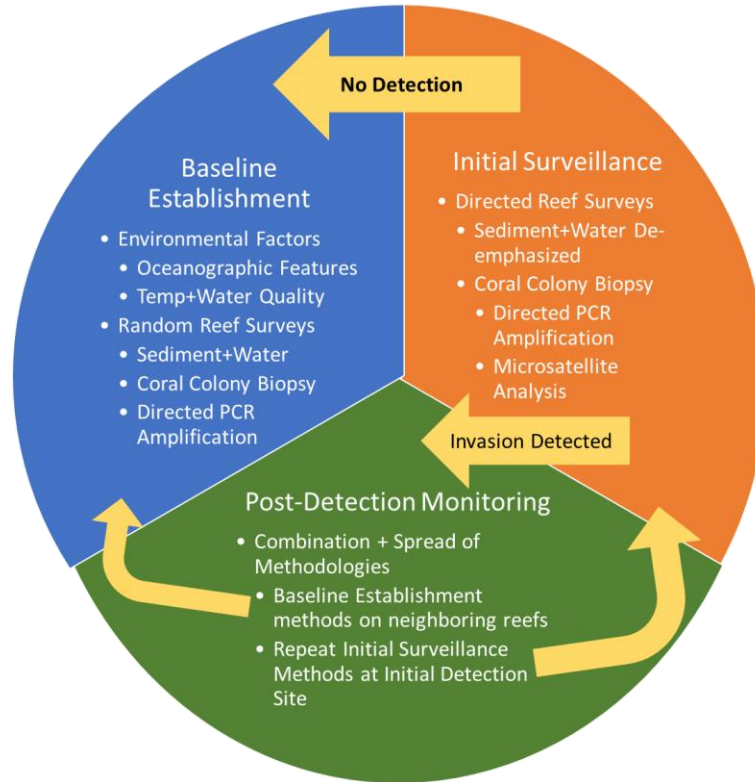


Figure 15: Basic Process of Proposed Detection Methodology.

Baseline Establishment involves a thorough assessment of the reef system and surrounding environment that are deemed vulnerable to a *D. trenchii* invasion. Assessing the surrounding environment consists of oceanographic mapping of the reef system, including bottom topography and real-time measurements of current patterns. Connectivity analyses also need to be commissioned to measure the impact of the reef system on its neighboring reefs. Major nearby hubs of human activity must be factored into the connectivity analyses, as they often represent the source of invasives. After the environment has been surveyed, the initial reef assessment can begin. These surveys need to encompass all three *D. trenchii* niches. However, if funding is insufficient, focus can be narrowed to the environmental rather than symbiotic niches. Depending on the physiological condition of the reef’s coral colonies and the thermochemical condition of the environment, free-living *D. trenchii* may or may not be present. If coral colonies are healthy, and

SST is not in a bleaching range, the probability of symbiotic association of *D. trenchii* is lower, due to the growth tradeoffs inherent to associations with this symbiont. Surveys should consist of randomly assigned, large (~10 m x 10 m) quadrats laid out along the reef tract. Within these quadrats, 100-mL water samples are taken within 1 m of the surface, as well as at 4-m depth increments until the bottom is reached. At least 10 random sites on the bottom would be assigned, and 100-mL samples of aerobic surface sediment collected. Finally, if practicable, coral colonies within the quadrat are biopsied using Figure 11 as a guideline. DNA will be extracted from samples and amplified using PCR following methods laid out by Yamashita et al. (2012). The analysis would focus primarily on determining the clade compositions of *Durusdinium* in the different reef niches.

If the analyses performed in Baseline Establishment determine that clade D *Durusdinium* are present, microsatellite analysis like those outlined in Pettay et al. (2015) should be undertaken to determine their origin. These analyses form the first part of Initial Surveillance, after which more reef surveys follow. These reef surveys will be distinguished from those in Baseline Establishment by their geospatial strategy and focus on coral biopsy. The focus is on detecting the establishment of novel symbioses with native corals rather than initial detection. The new geospatial strategy is thus more directed and features repeated regular transects across the reef tract rather than random quadrats. Coral colonies intersected along 25-m transects will be sampled using figure 11 as a guideline. The water and sediment samples will continue as well but with reduced emphasis. The genetic analysis will remain the same, as PCR amplification with specialized primers is necessary to continue to monitor the reef system's clade composition. Microsatellite analysis on any clade D symbionts discovered remains essential as well. The results of these genetic analyses will determine the next course of action.

The next step shifts from Surveillance and Detection (Hoey et al. 2016) to Monitoring. If no symbioses are detected, the process reverts to the Baseline Establishment phase. If no *D. trenchii* symbioses can be identified, its physiological effects are not present, although the environment may still have been invaded. Study can commence on the effect of unassociated *D. trenchii* on its environment, and the environment can be monitored to assess the spread of the invasion. Detection of symbiosis requires implementation of Post-Detection Monitoring, which can be viewed as a combination of methods used in previous phases. It involves a widening of focus from the initial detection area and applying the Baseline Establishment phase to un-surveyed

neighboring reefs, using the random quadrat method and emphasizing all niches. In the affected reef, the Initial Surveillance methods should be repeated regularly to measure the progress of the invasion. The process should repeat throughout the subsequent steps of the management framework.

5.5.4 Framework Step 3: Mitigation Activities

The most immediately obvious aspect of many management bodies are the activities they undertake to mediate human impacts on the environment. In the case of biological invasions, these activities constitute Hoey et al.'s (2016) third key to pest management: rapid response. Rapid response entails timely approaches to curtail the population growth, and thereby the environmental impact of, an invasive species outside its native range. Application of rapid response to bioinvasions has successfully eradicated marine invasives (Culver and Kuris, 2000) and has also resulted in reducing associated ecological damage. Developing action plans as invasion response protocols requires consideration of the different forms of response measures. Blackburn et al.'s (2011) management portion of the Unified Framework for Biological invasions breaks possible management action down into four categories which correspond to their stages of invasion. Prevention is any action that occurs before an invasive species enter the Establishment phase (Blackburn et al., 2011). Once the species becomes established, the management action is limited to eradication methods, which fall into the categories of containment or mitigation, depending on whether the dispersal barrier has been overcome in the spread phase (Blackburn et al., 2011). Eradication is a difficult and potentially expensive course of action for most management bodies, so, to respect cost-effectiveness, this framework should focus on preventative actions.

Preventing biological invasions involves both an understanding of invasion mechanics and pathways and vigilant surveillance of the environment by targeting potential invasives even before they arrive by applying the transport and introduction phases of Blackburn et al.'s unified framework (2011). In the case of a potential invasion by *D. trenchii*, preventative action would largely target transport vectors and entail applications of ballast water biosecurity methods aboard maritime trade vessels. These vector vessels can employ a number of biosecurity measures, most of which involve treatment of the water carried in ballast tanks. Some are quite simple, e.g., removal of particulates via filtration or ballast water exchanges while underway rather than in port (Anil et al. 2002; Lockwood, Hoopes, and Marchetti, 2007). Such physical methods have

limitations and drawbacks, including decreased effectiveness of physical filters as they become clogged with particulates, and incomplete exchanges that may permit organisms to persist in water exchanged while underway. More intensive options include chemical additives to treat ballast water, e.g., adding a simple biocide, use of a disinfectant such as ultraviolet radiation or ozone, or gas supersaturation of the ballast water (Anil, et al. 2002). These options have their own drawbacks aside from chemically altering the water, including potentially precluding release of ballast water after use, expense, and complexity of system design. To maximize efficiency of removal, a combination of these biosecurity treatments should be employed, e.g., ultraviolet sterilization of water as it is taken aboard, followed by mechanical filtration (drum or sand filter) to remove particulates (including *D. trenchii* mastigotes and coccoids) before the water reaches the tanks, and mid-ocean ballast exchange

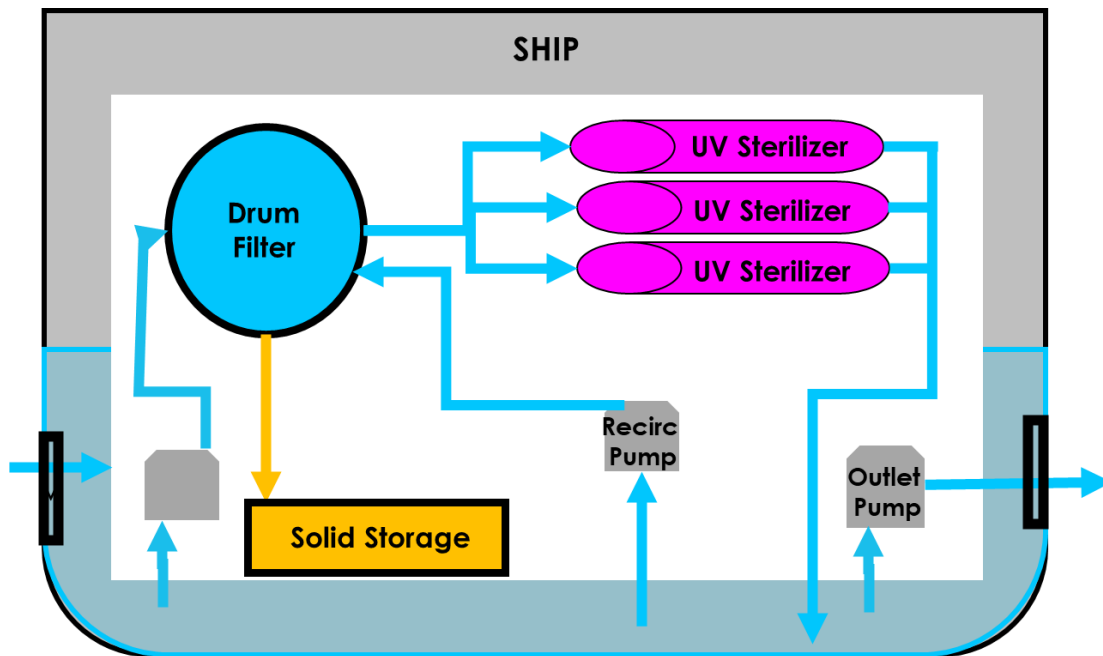


Figure 16: Basic detail of proposed ballast water treatment system

Once the vessel is underway, the water in the tanks would be recirculated through the ultraviolet sterilization unit. This disinfects the water without altering its chemical composition (avoiding a biocide) and provides elevated exposure time to the disinfectant without altering the ballast's ship stabilization processes. However, alteration would occur mid-voyage, as the ship enters the third stage of the biosecurity protocol and performs a mid-ocean ballast exchange, utilizing the onboard disinfection previously detailed. It would also continue to sterilize new water acquired mid-voyage. The vessel would arrive with less water from the potential source of the

invasion, and any water present in the tanks upon arrival would have been thoroughly exposed to the disinfectant. Mid-ocean ballast water exchange and the treatment of ballast water as it is brought on board constitutes a pre-border prevention (Keller and Lodge, 2010), which would reduce the possibility of bioinvasion by dealing with potential invasives before they arrive anywhere near the target environment. However, this biosecurity-based method for preventing a *D. trenchii* invasion has some drawbacks. It requires the cooperation of a sizable portion of the maritime transport community. Proposing a mechanical design change to a community as diverse and cost-centric as the maritime transport community would require political leverage to ensure compliance. However, the downside of instituting such a major new preventative method could be mitigated by developing a portable, installable, modular system instead of one integral to the construction of the vessel. Such a system would have to be designed, tested, and distributed before any effect could be noted. These limitations raise the concept of efficacy in post-introduction control of *D. trenchii* invasions. Post-introduction control would obviously occur after transport and surviving *D. trenchii* populations were exposed to their destination environment. Surveillance and detection would play key roles in this rapid response, as preventative action would no longer apply. Emphasis would need to be placed on the timeliness of response actions, as too slow a response could result in the establishment of invasive populations. Microbial control in a relatively open environment such as a coral reef ecosystem is a difficult proposition at best, as coral reefs are highly dependent on a functioning microbiome for survival and growth of foundational species like reef-building corals. Employing biocidal methods is likely to have unintended collateral consequences, especially in reef ecosystems that are already inhabited by congeners. Perhaps the best course of management action once *D. trenchii* has been introduced is to quarantine the affected area and establish buffer zones in adjacent reef systems. Hoey et al. (2016) stressed that quarantine should be employed quickly to prevent secondary invasions. Monitoring protocols would then need to focus on affected areas and adjacent invasion pathways to provide warning of potential secondary invasions. However, the subject organism for the framework developed in Hoey et al. (2016), *A. planci*, has a macroscopic component to its life-cycle, making it an easier target for non-quarantine eradication measures. In the invasion cases of microbial organisms like *D. trenchii*, little research has addressed effective methods for eradicating invasives. Simply removing individuals or colonies of hosts that harbor the invasive would be insufficient, as *D. trenchii* can exist as a free-living microbe in the water column or sediment. The dearth of proven methodologies

for removal combined with the limitations of quarantine procedures emphasizes the case for preventative management strategies to halt *D. trenchii* introductions before true invasions can begin.

5.5.5 Framework Step 4: Tracking Progress of Employed Methodologies Through Monitoring

The next key to effective management was mentioned in the discussion of quarantine procedures above. Hoey et al. (2016) cited effective monitoring protocols as key to marine pest species management, and distinguished monitoring from surveillance by the presence of a defined threshold to which monitoring samples are compared over time. When squaring this definition with Blackburn et al.'s (2011) unified framework for biological invasions, it appears that the distinction between surveillance and monitoring is found when examining either the invasion stage or management action. Detection methods designed for the post-introduction through the spread stages would fall under the category of monitoring, as there is a defined invader presence in these stages that does not occur prior. When comparing Hoey et al.'s definitions to the management aspect of Blackburn et al.'s framework, surveillance would correspond most closely with prevention, while monitoring would take place more often during eradication activities for the same reason. This differentiation between surveillance and monitoring implies that monitoring practices would be separate from surveillance in management frameworks.

Here, it is proposed that monitoring programs for *D. trenchii* be linked to surveillance programs. While the surveillance programs would focus effort into the transport vectors and introduction environments that are of greatest concern, monitoring programs would utilize similar detection methods in different geographical areas. Identified invasion pathways and areas of secondary invasion potential would be targets for monitoring programs. During non-invasion conditions, monitoring programs would maintain low-frequency detection schedules in these areas to establish baseline ecosystem dynamics. However, once introduction criteria are met, the monitoring frequencies would increase dramatically as part of a rapid response plan. Currents between reef systems would be sampled more often, and sediments in these areas tested for dispersing *D. trenchii* propagules. Host species on virgin reefs in dispersal pathways would be biopsied more often to determine the makeup of symbiont communities and their shifting dynamics. Species richness surveys would occur more often in these areas as well, with special interest given to any disturbance events such as coral bleaching or disease that may open them up

to invasion. The purpose of monitoring programs in the proposed framework would be the identification of secondary invasions and the construction of a knowledge base regarding behaviors of *D. trenchii* populations in the invaded environment. Identification of secondary invasions would help to establish the effectiveness of the quarantine/buffer system of mitigation, and the construction of the knowledge base would be useful for determining the conditions most and least favorable to *D. trenchii* invasions. Research into conditions may help to prevent further invasions in similar reef ecosystems. Monitoring, like surveillance, is also a potential area for public involvement.

5.5.6 Framework Step 5: Integrating the Public into Managing Biological Invasions

Public involvement through community education and preparedness is both an essential aspect of pest management (Hoey, et al., 2016) and a potential resource that can be used to advance management objectives. In many cases, environmental observations from members of communities near marine ecosystems have provided key signals of incipient or in-progress bioinvasions (Olenin, et al., 2011). Training in taxonomy, basic ecology, and invasion biology can mobilize concerned individuals into citizen scientists who can be used as technicians for surveillance and monitoring studies. Such opportunities for observation have implications across the various stages of the unified framework for biological invasions (Blackburn, et al., 2011). Environmental observation by the public could confirm individual stages, such as establishment of a population, or indicate transitions between invasion phases

Local SCUBA organizations could participate in citizen-science training programs. They would be targeted as assets because of the attractiveness of reef ecosystems to SCUBA divers and the friendly attitude that many divers have towards conservation of these ecosystems. The training programs would first describe the problems associated with a *D. trenchii* invasion, the importance of surveillance/monitoring, and the important role the public can play in combating these invasions. The curriculum would also contain modules centered on basic scientific survey techniques, data recording, reef ecology, and disturbance. Building a background knowledge base on reef ecology and disturbance would allow attendees to be useful by not only collecting data in as structured setting but also interpreting environmental cues in the field. The coursework would culminate in management-orchestrated data collection trips—essentially surveillance or monitoring expeditions—and the volunteer data collectors would work alongside professional

technicians and researchers. In addition to bolstering available people-power for detection programs, these trips would stimulate public interest in marine science as a whole. However, this citizen science education program would have to cost less than hiring professional data collection technicians. Additionally, public interest in the curriculum would have to first be assessed to determine if it is broad enough to merit creation. If not, additional funds would need to be allocated to foster public interest, but this may diminish cost-effectiveness of the solution as a whole. Maintaining cost effectiveness in volunteer programs is important when attempting to extract benefit, which is key to fiscal prudence in managing bioinvasions.

5.5.7 Framework Step 6: Allocation of Funding for a Management Protocol

The final key identified by Hoey et al. (2016) for effective pest management is proper appropriation and use of funding. The financial cost of biological invasions is calculated by assessing two overarching groups: 1) economic output costs include revenue lost from impact of the invasive species on the environment due to, e.g., drops in farming output, fishing yields, or tourism declines, and 2) mitigation costs incurred through any management efforts meant to curtail these impacts, e.g., surveillance/monitoring programs, control mechanisms, and ecosystem remediation (Mack et al., 2000). Mitigation costs can be prohibitively expensive, as invasions often are caught after establishment, and the ecosystem needs repair. Restoration of ecosystems is almost always more expensive than prevention, and it is the negative association of invasion management costs with the high price of environmental restoration that can make funding for invasion management difficult to find. This following should consider the various costs associated with these strategies and recommend the percentage of funds allocated to each. A percentage breakdown should provide options about how the funding should be allocated once it is applied to the broad strategy categories. The categories should closely follow the keys to pest management laid out in Hoey et al. (2016). Finally, the temporal component should be addressed, as management of invasions very often requires proper timing.

- Knowledge Base (15%): Construction and maintenance would include initial funding for trained staff to review extant studies, perform statistical analyses on extant data, and to synthesize new data from readily available sources. Additional funding for the design and application of new studies would be needed, as these new studies will almost certainly require materials, personnel, and establishment of their own knowledge base. These costs

should, if at all possible, not be incurred by the management body responsible for the invasion management, as published data could be used to further academic research careers. Partnerships between the responsible management body and local academic institutions should be fostered to encourage grant acquisition to study the invasion environment, potential invasive species, and transport mechanism. Grant acquisition would diversify funding for establishing the knowledge base and could have useful crossover implications for Surveillance, Monitoring, and Detection programs.

- **Surveillance, Monitoring, and Detection (25%):** The overarching strategy for this framework is based on proactive measures to ward against potential or incipient invasions. Surveillance and monitoring are key to this proactive approach, and initial surveillance needs to occur before an invasion is anticipated. This category was allocated the second largest funding percentage due to the temporal and spatial scale of its requirements. The design of surveillance/monitoring protocols should account for cost; however, their application will require the bulk of funding. Environmental surveys require substantial equipment, with most of it being relatively unspecialized (e.g., measurement materials, boats, cameras). Funding allocated for detection methods needs to account for the ocean surface area that must be surveyed. Additionally, these large areas will need to be examined repeatedly and regularly to determine the nature of populations in these environments. The costs inherent to large-scale detection methods will compound over time. Laboratory costs (for genetic sequencing of detection samples) will need to be assessed and weighed against the cost of purchasing equipment and qualified operators. Mitigating surveillance, monitoring, and detection costs is imperative wherever possible.
- **Invasion Prevention Methods (30%):** In keeping with a proactive approach to managing bioinvasions, management response methods focusing on prevention of invasive species introductions should comprise the majority of employed methods. Preventing invasions will minimize environmental restoration costs, which are almost always higher than preventative costs. The primary strategy for preventing invasions should focus on designing, constructing, and implementing an effective method for ballast-water biosecurity aboard international maritime trade vessels. The proposed three-part ballast tank sanitization system requires several subsections of funding. Design and construction of a portable UV sterilizer + mechanical filter loop combination requires engineering

personnel and development costs to ensure it is usable on multiple types of maritime transport vessels. Research and drafting will be required for standardizing a mid-ocean ballast exchange protocol. Finally, to ensure compliance, funds will likely need to be allocated for political lobbying in order to pass legislation that forces the adoption of the prevention system.

- **Post-Introduction Response Methods (15%):** These do not fall under the umbrella of preventative methods. However, they are important to consider should prevention fail. The establishment of marine quarantines and buffer zones require enforcement, for which funds must be allocated in a timely manner. In addition to enforcement, surveillance and monitoring, programs will need to track the post-introduction progress of the invasion. Funding from this category should be used to supplement those programs. Also, given the dearth of methodology for managing marine microbial invasions post-introduction, a small amount of this funding (~10% of the total in this category) should be used to commission review and research on the topic. A small staff could establish and grow this knowledge base and integrate data obtained by the surveillance and monitoring programs to provide new perspectives on this topic.
- **Public Education and Participation Programs (15%):** Numerous authors have stated the importance of public participation in invasion management programs. Utilizing the public as an asset can reduce costs in the other categories, which is reason enough to allocate funding for this category. First, research must determine the breadth of public knowledge about the specific biological invasion. Also, public interest in the subject must be gauged and encouraged. Once this has occurred and a cost/benefit analysis has determined the amount of effort/funding needed to effectively utilize the public, then design of an engagement strategy can begin. The example mentioned earlier was a course-based citizen science curriculum targeted towards recreational scuba divers. Course design, focus group testing, advertisement, and implementation costs will need to be quantified and paid. Incentives may need to be offered to drum up interest or support for citizen involvement. A number of hitherto unmentioned costs may become apparent once these programs are established, so percentages allocated may need to be adjusted.

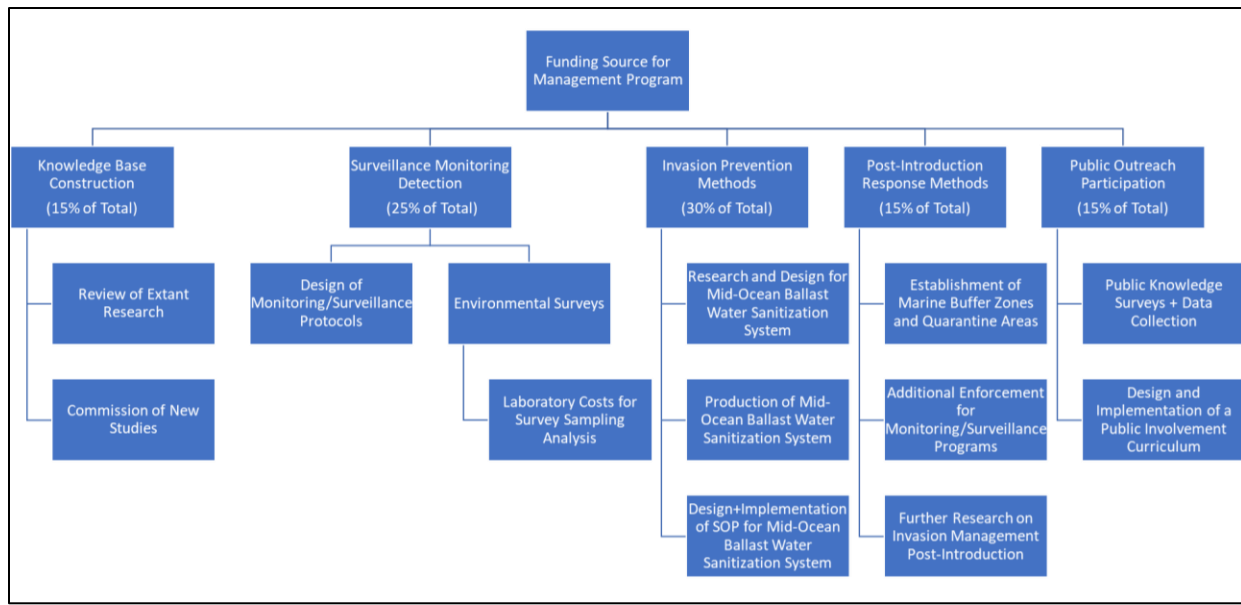


Figure 17: Funding Breakdown for the Proposed Biological Invasion Management Framework.

Key to the effective management of this potential biological invasion will be the appropriate timing of funding allocation and release. Surveillance/Monitoring, Post-Introduction Responses, and Public Education and Participation will need easy access to funding if and when an introduction event becomes an established biological invasion. The structure of this funding model should be streamlined to allow such access without administrative delay or temporally excessive approval processes. Emergency funding may therefore need to be allocated and set aside and access to emergency accounts made available to key management decision makers. Concurrently, management must secure funding with as few temporal limitations as possible. Consistency over time is key to studying biological invasions, and monitoring programs cannot be subject to the “Issue-Attention Cycle” (Downs 1972). Budgets for detection of invasions need to be kept as consistent as possible, with possible fluctuations accounted for during effort-intensive situations such as disturbance events.

This proposed framework uses several modern studies as conceptual bases but modifies them for consideration of the subject organism, the dinoflagellate *D. trenchii*. Additional considerations arise from the transport vectors and ecology of the potentially invaded environment. The result is a comprehensive management plan that integrates important aspects of management into a unified framework for biological invasions. It proposes a number of suggestions based on peer-reviewed scientific research and designs a strategy for observing and responding to invasions of coral reef ecosystems by *D. trenchii*.

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