**The ecology of sponges at Palmyra Atoll: variability,
introduced species and their potential direct and
indirect impacts**

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*Te Whare Wānanga
o te Ūpoko o te Ika a Māui*

“It is that range of biodiversity that we must care for—the whole thing—rather than just one or two stars.”

(David Attenborough)

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Abstract

Anthropogenic pressures, direct and indirect, have left no coral reef untouched. Those that remain in a near-pristine condition are remote islands and atolls removed from the majority of direct impacts, but even these are still subjected to the pressures of global climate change to which they are demonstrating a higher resilience than those which are already severely compromised. These near-pristine systems should be protected, managed and studied to better understand how they function and hopefully ensure the future of coral reefs. Unfortunately a number of the remote atolls and islands in the Pacific were modified or used by the US military during WWII, which altered these systems in unknown ways and threatened their surrounding reefs. Palmyra Atoll in the Central Pacific for example had its lagoons dredged and blocked to create more landmass for building, along with the creation of a channel through the reef to allow boat access into the lagoons making them susceptible to introduced species. Fortunately the surrounding reefs at Palmyra are still in a near-pristine condition with high densities of scleractinian corals, however the lagoon fauna is now predominantly sponges. Sponges in high enough densities can have considerable impacts on semi-enclosed bodies of water through their high filtering capabilities and could even threaten native species through competition if they were to extend onto non-lagoon reefs. Therefore, the broad aim of this thesis was to understand the ecology of the sponges in the lagoons at Palmyra and determine their potential impacts on the atoll directly and indirectly. To answer these questions I collected sponge assemblage data across the lagoons at both shallow and deep depths and sampled those species found on the reefs, which were surprisingly entirely different from the lagoon species. I then modelled a suite of environmental predictors to ascertain whether environmental conditions might be maintaining the sponges in the lagoon. To further examine whether the sponges were capable of extending onto the reef I also looked at larval recruitment patterns and assessed the temporal stability with semi-permanent quadrats. The initial surveys revealed the presence of at least two introduced species: *Haliclona caerulea* (Hechtel 1965) and *Gelliodes fibrosa* (Wilson 1925). Molecular tools were then employed to confirm the identification and attempt to ascertain the introduction pathway of *H. caerulea*. Finally, to assess the potential impact of the sponges on the water column I calculated the filtration rates of all the morphologies in the lagoon and extrapolated to

the time required to clear all the available water in the lagoon as well as the removal rates of dissolved organic carbon and oxygen.

Overall the lagoons appear to have undergone a phase-shift from a coral to sponge dominated system. However, the direct threat of the sponges extending onto the adjacent near-pristine reef currently seems negligible as they appear to be relatively “stable” (sponge mortality and recruitment are in equilibrium) and maintained in the lagoons by the environmental conditions, despite larval production. Sponge diversity changed over depths but the total number of species was consistent with other atoll systems despite the military modifications, with the most prominent sponge being a Hawaiian endemic species: *Iotrochota protea* (de Laubenfels 1950). The introduction of *H. caerulea*, a Caribbean sponge is thought to have occurred to Palmyra via Hawai‘i; however, the molecular data also revealed further cryptic speciation at both the species and order levels, suggesting greater species diversity at Palmyra than previously believed. Finally, the indirect impacts of the sponges on the water column also appear to be small and with limited future risks to the reef organisms, as recruitment and mortality are currently in equilibrium and therefore unlikely to increase dramatically in percentage cover.

Globally sponges can play important functional roles in semi-enclosed bodies of water and in summary, despite the sponges being the most dominant fauna on the hard substrate in the lagoons, they appear to pose little threat to the atoll and the adjacent reefs either directly or indirectly despite the confirmed introduction of non-endemic species. However, it must be noted that the time since the modification (70 years) is not that long in an evolutionary sense, so the sponges may still have the potential to extend onto the reef, particularly if there are any dramatic changes to the environmental conditions on the outer reefs. Therefore, to detect whether the sponges are extending onto the reef I propose, as a future management tool, the use of *I. protea* as an indicator species. The use of multidisciplinary approaches to answer important ecological questions with respect to the potential for sponges to have negative impacts on the non-lagoon reefs proved to be essential in understanding whether the modifications to the lagoons and the subsequent dominance of sponges could be threatening one of the last few remaining near-pristine reef systems in the world.

List of abbreviations

AICc = corrected Akaike's information criterion

bp = base pairs

BI = Bayesian inference

BLAST = basic local alignment search tool

BSA = bovine serum albumin

CAP = canonical analysis of principal coordinates

CO1 = cytochrome oxidase subunit 1

CPCe = coral point count with excel extensions

DOC = dissolved organic carbon

dbRDA = distance-based redundancy analysis

DistLM = distance-based multivariate analysis for a linear model

DMSO = dimethyl sulfoxide

ENSO = El Niño-southern oscillation

G = gamma rates

GTR = general time reversible model

ITS = internal transcribed spacer

MCMC = Markov chain Monte Carlo

ML = maximum likelihood

mtDNA = mitochondrial deoxyribonucleic acid

nDNA = nuclear deoxyribonucleic acid

NJ = neighbour joining

nMDS or **MDS** = non-metric multidimensional scaling

NNI = nearest-neighbour interchange

NOAA = National Oceanic and Atmospheric Administration

PCA = principle component analysis

PCOM = percentage organic matter

PERMANOVA = permutational analysis of variance

POC = particulate organic carbon

POM = particulate organic matter

PRIA's = Pacific Remote Island Areas

NIS = non-indigenous species

rDNA = ribosomal deoxyribonucleic acid

RNA = ribonucleic acid

SD = standard deviation

TIM = transitional model

TOC = total organic carbon

TNC = The Nature Conservancy

TPM = total particulate matter

TVM = transversion model

USFWS = United States Fish and Wildlife Service

WWII = World War Two

Contributions and Publications

This thesis is compiled as a series of published, submitted or in prep manuscripts under the guidance of my primary supervisor Dr James Bell who provided editorial comments and advice on methodologies and analysis. Therefore there is an aspect of repetition across the chapters, particularly with the introductions. All underwater SCUBA fieldwork was conducted with Dr Gareth Williams but the subsequent processing, lab work and writing were completed by the author with the following additional contributions from co-authors:

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Co-authors contribution:

Gareth J. Williams- statistical advice and editorial comments

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Co-authors contribution:

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General Introduction

1.1 Sponges: function, ecology and anatomy

Sponges (phylum Porifera) are the simplest and oldest multicellular organisms still extant today and are considered the evolutionary link between protozoans and metazoans, with cnidarians being their closest relatives (Müller 1995, Watkins & Beckenbach 1999, Muller et al. 2003). They are benthic sessile invertebrates which have been extant for approximately 580 million years, long before the notable ‘Cambrian explosion’, which occurred 540 MYA (Li et al. 1998, Budd 2008). Sponges can be found inhabiting marine (80% of species), freshwater and brackish environments (de Laubenfels 1936, 1947, Penney & Racek 1968, Hooper & van Soest 2002), in temperate, tropical and polar environments, from shallow water to the deep seas (Hoppe & Reichert 1987, Roberts & Davis 1996, Witte 1996, Bell & Barnes 2000a, McClintock et al. 2005).

Throughout this time and expansion, their body plan and mode of feeding has changed little, maintaining the principal organisational feature defining this phylum; an aquiferous system comprised of a network of water-conducting chambers and canals (Bergquist 1978, Brusca & Brusca 1990). The chambers and canals are lined with flagellated choanocyte (collar) cells, which create a flow of water into the ostia (pores) and over the microvilli and remove nutrients and food particles and expelling waste products out through the oscula/osculum.

Sponges can filter large volumes of water; up to 24,000 L kg⁻¹ of sponge (Vogel 1977). They primarily feed on ultraplankton (<20 µm) and picoplankton (<2 µm) (Reiswig 1971a, 1974, Pile et al. 1996, Ribes et al. 1999), as well as bacteria (1–10 µm) (Reiswig 1975, Frost 1978) and possibly viruses (0.03–0.1 µm) (Hadas et al. 2006). Picoplankton and bacteria are important sources of carbon and nutrients on coral reefs (Ribes et al. 2005) with roughly over 80% of the particles removed less than 10 µm in size (Yahel et al. 1998, Ribes et al. 2003). Retention rates of food particles by sponges are high, ranging from 58 to 99% (Pile et al. 1997, Lesser 2006, de Goeij et al. 2008), making them very important in linking primary and secondary production (Gili & Coma 1998).

The choanocyte cells filter and phagocytose food particles and metabolites from the passing water. Nutrients are then passed to the middle layer of the sponge; the mesohyl (mesenchyme). Archaeocyte cells (amoebocytes) then distribute the nutrients to other sponge cells as well as carry any waste products back to the choanocyte cells. Many of the cells within a sponge, including the archaeocytes and choanocytes are highly plastic and can differentiate into other, more specialized, cells such as spermatocytes and/or oocytes (Galtsoff 1923, 1925, Buscema et al. 1980, Bond 1992, Anakina & Drozdov 2001).

There are 3 sponge classes: Calcarea, Demosongiae, and Hexactinellida. The majority of these secrete supportive proteinaceous fibres; spongin, and/or mineral (calcareous or siliceous) skeletal structures known as spicules (Uriz et al. 2003). The skeletal structures of each class, along with genetic analysis, can be distinctive enough to separate species and allow the reconstruction of evolutionary relationships (Chombard et al. 1998, Schutze et al. 1999, Uriz & Carballo 2001). Overall, sponges are a highly diverse as well as ecologically and economically important marine invertebrate taxon, but are vastly and notoriously understudied (Hooper & van Soest 2002, Becerro 2008), despite the fact that on some reefs sponges can even exceed the species diversity and biomass of the scleractinian corals (Wulff 2001, Wulff 2011).

1.2 Coral reef atolls and sponges

Coral reef atolls are found only in tropical and sub tropical waters and consist of an annular reef encircling a lagoon system. Most, but not all also have areas of land above sea level. Coral reefs are one of the most productive and diverse ecosystems in the world (Grigg 1984, Crossland et al. 1991, Hughes et al. 2003). Sponges when present perform important functional roles in reef ecosystems (Wulff 2001, Bell 2008a) including erosion and consolidation of reefs (Rützler 1975, Wulff 1984), benthopelagic coupling (Lesser 2006), nutrient recycling (Diaz & Ward 1997), and facilitating primary production through symbiosis (Wilkinson 1983), as well as providing microhabitats, affecting of near-boundary flow systems (Gili & Coma 1998), and being food sources for other organisms (Wulff 1994) and important spatial competitors. Therefore, reduced sponge abundance could result in some of these functions not being performed and also lead to the acceleration of a reefs decline (Wulff 2001). A lot of notable work carried out on Caribbean reefs has shown that sponges can benefit a reef by binding corals to the substrate, which is particularly important when environmental

conditions would otherwise dislodge them (Wulff & Buss 1979), as well as shielding them from other reef excavating species (Goreau & Hartman 1966). Sponges can also aid in the regeneration of reefs via the stabilization of coral fragments allowing recruitment of coral larvae (Wulff 1984).

Coral reef atolls, unlike most coastal reefs, have central lagoons, which are a remnant of the oceanic volcano that formed them (Delesalle & Sournia 1992, Kench 1998). Lagoons are generally considered low-current systems, which act as sediment ‘sinks’ and therefore experience high levels of sedimentation (Ilan & Abelson 1995). Generally, excessive sedimentation is considered detrimental to coral reef ecosystems affecting both the structure and functioning by smothering the reef organisms and reducing light penetration (Rogers 1990). Perhaps surprisingly, sponges have been commonly documented inhabiting areas of high sedimentation (Ilan & Abelson 1995, Bell & Barnes 2000a, Bell 2004, Bell & Smith 2004, Carballo 2006). Hiscock et al. (1983) noted that mechanisms to minimise sediment clogging are beneficial for organisms in highly sedimented habitats and it has been observed that many mechanisms exist allowing sponges to survive in such conditions (Bell 2004), including water flow reversal (Simpson 1984), and the production of a mucus layer, which the sponge then sheds to remove any settled sediment (Barthel & Tendal 1993). Sponges also exhibit micro and macro morphological acclimation to survive habitat disparities (Palumbi 1984, 1986, Bell & Turner 2000, Bell et al. 2002). These mechanisms are especially important in low current systems, such as lagoons, as the high levels of sediment can clog the delicate filtering apparatus of sponges (Bell 2004).

1.3 Roles in atoll ecosystems: Palmyra Atoll

1.3.1 Study site

Palmyra Atoll is located 1,930 km south West of Hawai‘i (5°53’N, 162°05’W) and lies toward the northern end of the Line Islands in the north Central Pacific (**Fig 1.1**). Palmyra is a U.S. National Wildlife Refuge (USFWS) and part of the Pacific Remote Islands Marine National Monument (proclamation 8336) established in 2009. Together with neighbouring Kingman Reef, Palmyra represents a biodiversity hotspot in the Central Pacific (Maragos & Williams 2011b). The atoll is approximately 12 km long, comprised of 50 islets with 4 lagoons (Collen et al. 2009) and a surrounding near-pristine reef system (Sandin et al. 2008). Palmyra has remained essentially uninhabited throughout its history, with the exception of the U.S. naval presence during WWII, and

is now co-owned by The Nature Conservancy (TNC) and the United States Fish and Wildlife Service (USFWS). The only inhabited island is Cooper Island (**Fig 1.1**), which is also the location of the field station.

Figure 1.1 Location of Palmyra Atoll and its lagoons, within the Central Pacific. 1 = excavated entrance channel, 2 = the airstrip made of dredge spoil and coral rubble and 3 = the north-south causeway, specifically the cut allowing boat access between Center and East Lagoons.

Palmyra's lagoons are characterised by high sedimentation and low water flow with the substrate composed largely of fine/coarse sediment ($63\ \mu\text{m}$ – $2.8\ \text{mm}$) and boulders ($<0.5\ \text{m}^2$) (see **Chapter 2**) with low hard coral diversity and cover (Williams et al. 2008). The current flow moves from east to west (Gardner et al. 2011) at an average rate of $0.0102\ \text{m sec}^{-1}$ (± 0.0143) (see **Chapter 2**). Water also tidally comes in through the breaks in the islets, and flows in and out of the boat channel and Western flats (See Collen et al. 2009). The present conditions are likely due to the modifications made by the U.S. Navy to the atoll to construct a naval air station during WWII, which included extensive dredging of the lagoons to deepen them for boats, using the spoil to build-up and connect the islets, building large iron wharfs and docks and excavating a channel through the western reef terrace to allow boat access (Dawson 1959, Maragos 1993) (**Fig 1.1**). The military presence is still clearly visible today with iron structures in and around the lagoons. The north-south causeway has almost completely blocked

the flow of water between the lagoons, but between 1958 and 1979 it breached, and it now has cuts in several places returning some movement between the lagoons (Maragos 1993). Dawson (1959) noted that soon after the military modifications the lagoons were essentially dead with “no living coral worth mentioning”, and the east lagoon was filled with sediment, was stagnant and often smelt of hydrogen sulphide. To increase the flow of water across the lagoons in 2008, USFWS even outlined a proposal to remove a section of the causeway to relieve the sediment stress on the eastern backreefs (Williams et al. 2011c).

Palmyra Atoll is an unusual atoll system; the outer reefs are considered near ‘pristine’ due to minimal human impacts (Sandin et al. 2008), however the lagoon system has been vastly altered (Collen et al. 2009). Unlike other reefs e.g. in the Indo-Pacific or Caribbean, there are few sponges on the main reef at Palmyra (*authors pers. obs.*), however in the lagoons the sponges are the dominant fauna (Bell & Carballo 2008). This may potentially be as a result of the extensive alterations made to the atoll during the U.S Naval occupation during WWII. Alterations and modifications of environments are likely to change the structure and function of an ecosystem (Vitousek et al. 1997), as well as composition of the biotic community by making the habitat more susceptible to invasions by non-indigenous species (NIS) (Mooney & Cleland 2001).

1.4 Invasive species and their dispersal potential

Globally, invasive species result in substantial ecological and economic damage, with the estimated cost being US\$ 1.4 trillion a year (5% global GDP) (Pimentel et al. 2001, Pimentel et al. 2005), and once NIS have established they are almost impossible to remove (Kolar & Lodge 2001). The arrival of NIS is a global phenomenon with examples found in all ecosystems. NIS can be ecologically damaging to the host habitat by displacing indigenous species, and altering community structure and food webs, as well as other important biological processes, such as nutrient cycling and sedimentation (Molnar et al. 2008).

Although transportation of a species to an ecosystem outside of its natural range can potentially occur via natural dispersal mechanisms, there has been a notable increase in invasion events with the advent of global trade (Ruiz et al. 1997, Vitousek et al. 1997). Aquaculture, agriculture, trading and recreation, as well as other human activities have exacerbated the transportation of species across great biogeographic barriers, something which was historically unlikely, but is now an everyday occurrence

(Mooney & Cleland 2001). However, it is important to note that despite the high number of NIS transported everyday, only a small fraction of species manage to establish themselves permanently in new locations (Mack et al. 2000); nevertheless the impacts of these few successful species can still be considerable (Molnar et al. 2008). For example, the global expansion of the green shore crab (*Carcinus maenas*) from Europe to the United States of America, Canada, South Africa and Australia (Lafferty & Kuris 1996) has had major impacts. *C. maenas* is a voracious predator of many benthic marine invertebrates and can have a considerable impact on native intertidal fauna (Cohen et al. 1995) as well as devastating shellfish fisheries (Walton et al. 2002, Behrens Yamada & Gillespie 2008).

The variability in success rate of NIS establishment can often depend on the propagule pressure or ‘introduction effort’, a measure which is dependent on the number of release events (propagule number) and the number of individuals released per event (propagule size); as the number of either of these two factors increases so does the propagation pressure (Lockwood et al. 2005). The invasion process is complex and not predictable in its outcome. There are a range of scenarios resulting in successful invasions; from the introduction of one large population in a single event or multiple smaller incursions (Lockwood et al. 2005). Understanding the process of invasion is important from a management perspective as this information can vastly alter how manager might deal with NIS populations; whether to stem the source population or tackle the resultant invasive population.

Islands are particularly at risk from NIS and have a higher proportion of invaders than mainland biota (Mooney & Cleland 2001). It is thought that this may be because, in comparison to the mainland, species complexity and competition on islands is lower leaving niche gaps, which are more prone to invasion (Elton 1958). Paleobiological reconstructions (Vermeij 1991) of the direction of invasions show historically that a higher percentage of species from more speciose communities will establish in less speciose communities, than in the opposite direction. Therefore it has been hypothesised that highly speciose communities (mainland biota) are more resistant to invasion, because they have fewer niche gaps, and are more likely to successfully colonise lower speciose communities (island biota) (Lodge 1993).

In Hawai‘i, 20–40% of species across all taxa are thought to be non-indigenous (DeFelice et al. 2001, Molnar et al. 2008). Invasive marine and brackish invertebrates alone total nearly 7% of local fauna (DeFelice et al. 2001). The non-indigenous

invertebrates appear to mostly originate from the Indo-Pacific and Philippines, as well as the Western Atlantic and Caribbean (DeFelice et al. 2001). The key pathway of invasion of NIS to Hawai'i is shipping, with 68% of harmful species having been imported in this manner (Molnar et al. 2008).

There are many factors leading to a successful invasion of an NIS including the attributes of the donor region, the potential disperser species, and the route, means and conditions of transport (Vermeij 1996). The process of invasion can essentially be split into 3 successive phases: arrival (successful transportation to the recipient biota), establishment (development through local reproduction and recruitment), and integration (spreading and forging new ecological links with recipient biota and evolving to the new selective regime) (Vermeij 1996, Puth & Post 2005).

Non-indigenous marine species (NIMS) can be transported via commercial or even recreational boats, hull fouling, ballast water, sediments and sea chests (Carlton & Geller 1993, Coutts et al. 2003, Ruiz & Carlton 2003). Maritime traffic between ports has been reported as the primary mechanism for transporting larval NIMS around the world (Lafferty & Kuris 1996, Godwin 2003b, Levine & D'Antonio 2003). Oceanic vessels can be viewed as 'biological islands' providing settlement substrata, in protected recesses and enclosed bodies of water, for mobile and sessile organisms to inhabit (Wonham et al. 2000, Godwin 2003b). The majority of marine NIS arrive as larvae and as such are introduced to the recipient biota without many natural enemies (parasitic castrators, specialized predators and pathogens of adults), which might normally control their numbers in their native regions (Lafferty & Kuris 1996). Hull fouling is also an important vector transporting numerous organisms around the world including algae, sponges, cnidarians, annelids, molluscs, crustaceans, bryozoans and chordates (Godwin 2003b). These organisms tend to proliferate in sheltered areas of the hull e.g. sea chests, and areas where the antifouling paint is compromised e.g. weld seams (Coutts et al. 2003, Godwin 2003b).

A number of factors allow the introduction and proliferation of invasive species in a new environment including: a lack of natural enemies, high adaptability, new parasitic hosts available, exploitation of resources in novel ways, presence of artificial or disturbed habitats, and/or effective predatory skills or defences suited to the new environment (Bax et al. 2001, Pimentel et al. 2001, Callaway & Ridenour 2004). The NIMS can then direct more resources towards growth or reproduction (Bax et al. 2001) resulting in high NIMS population densities that can then effectively compete with the

native species or even impact the recipient ecosystem (Lafferty & Kuris 1996, Vitousek et al. 1997).

It is important to ascertain the process of invasion to understand and manage the affects that NIS can have on community assemblages (Webb et al. 2002). It is also important to understand the establishment and interaction of metapopulations of invasive species (Leibold et al. 2004). Metapopulations can establish as a result of continual re-introductions of non-indigenous individuals to an established NIS 'population' in a recipient biota (Grosholz & Ruiz 1996, Jansen & Vitalis 2007, Dlugosch & Parker 2008). From a management perspective it would be prudent to focus on isolated introductions before an NIS is able to adapt and integrate to the host biota and develop novel invasive behaviours such as predator avoidance or even phenotypic plasticity (Dlugosch & Parker 2008). These rapid adaptive behaviours can often develop on time scales of years to decades, and are often associated with the impacts of anthropogenic factors such as habitat degradation, fragmentation, over harvesting and the introduction of NIS (Stockwell et al. 2003, Stockwell & Ashley 2004).

Sponges have long been transported around the world; for example, *Mycale armata* (Thiele, 1903), which is now found in Hawai'i, originated in the Indo-Pacific (DeFelice et al. 2001) or *Crambe crambe* (Schmidt, 1862), which extended its range from the Mediterranean to the Micronesian islands via hull fouling, ballast water or natural rafts (Duran et al. 2004a). Generally, sponges are highly effective spatial competitors (Jackson & Buss 1975) and therefore can significantly impact the environment where they are abundant (Reiswig 1974, Coles 2007) particularly on oceanic islands and in lakes (Vermeij 1996), because in shallow locations high sponge biomass can potentially remove large quantities of nutrients and oxygen from the water column (Reiswig 1971b, 1974).

Sponges are unlikely to become invasive over large biogeographical barriers without human-mediated transportation because of their limited dispersal mechanisms and capabilities (Zea 2001, Maldonado 2006). Therefore they make an interesting model system to look at anthropogenic mediated dispersal. Sponges are hermaphrodites and can reproduce asexually via budding, gemmule formation and fragmentation (Fell 1974, Simpson 1984) or sexually via larval production. As is common with most sessile marine organisms, sponges are indirect developers with the embryos developing into larvae before metamorphosing into the adult form (Maldonado 2006). Sexual

reproductive modes in sponges include oviparity (Fromont & Bergquist 1994, Corriero et al. 1996) (external fertilization of the ova) and viviparity (Ilan 1995, Whalan et al. 2005) (internal fertilization). A number of marine invertebrates with a planktonic phase in their life cycle have been transported via ballast water (Carlton & Geller 1993). However dispersal distance via asexual reproduction is thought to be low in sponges (Bergquist et al. 1970, Ayling 1980, Wulff 1985, Wulff 1991) and their larvae only remain in the water column for a limited period (days at most) before settling on the sea floor and metamorphosing into their sessile adult form (Uriz et al. 1998, Maldonado 2006). Therefore the global transport of these species is unlikely to be via ocean currents but by fouling on ships, which can maintain the adult forms at sea for weeks or even months (Godwin 2003b). For example the sponge *Gelliodes fibrosa* (Wilson, 1925) (grey encrusting sponge), an established Hawaiian NIMS, was documented in 1992 to foul the hull of a floating dry dock brought to Pearl Harbour from Subic Bay in the Philippines, its country of origin (Godwin 2003b). Slow moving vessels e.g. barges, towed vessels or floating dry docks are particularly high risk to fouling organisms as they have long residence times in ports giving time for organisms to establish in the port away from the vessel (Godwin 2003b).

1.5 Sponges and genetics: a tool for understanding the process of invasion

To conserve and manage marine ecosystems effectively, a better understanding of the global demographic connectivity between populations of NIS and their distribution patterns is needed (Palumbi 2004, Hoshino et al. 2008, Wörheide et al. 2008). The genetic ‘tools’ currently available potentially allow us to determine the amount of genetic connectivity between and within populations, and with that knowledge we can improve our understanding of the ecological and evolutionary relationships of invasive species (Wörheide et al. 2005).

Sponge phylogenetics has become increasingly popular since the 1980’s (Wörheide et al. 2005, Hoshino et al. 2008) and with this enhanced knowledge comes the understanding that biogeographic studies should not be based solely on morphometric data (Wörheide et al. 2008). The plastic and cryptic nature of some species makes it difficult to clearly identify individual species and therefore distribution patterns of populations (Stockman & Bond 2007). Sponge species that previously appeared to be morphologically homogenous, when later analysed using molecular techniques have often been found to have high genetic divergence between groups and

even within ‘species’ (e.g. Solé-Cava & Thorpe 1986, Muricy et al. 1996, Klautau et al. 1999). For example, the sponge species, *Chondrilla nucula* (Schmidt, 1862), *Plakina trilopha* (Schulze, 1880) and *Suberites ficus* (Johnston, 1842) were initially identified as having wide distribution patterns with different colour morphotypes, but further genetic analysis indicated a high rate of cryptic divergence and speciation within each of these species. The difficulty associated with identifying species using morphological characters therefore led to an initial overestimation of distribution ranges and underestimation of biodiversity (e.g. Klautau et al. 1999, Xavier et al. 2010).

One of the difficulties of working with marine species is that the nature of the environment makes it difficult to delineate a species’ range due to the high dispersal potential associated with ocean current flow systems (Wörheide et al. 2008). Clear habitat fragmentation is particularly difficult to observe in marine environments, unlike freshwater and terrestrial ecosystems (Hoshino et al. 2008). The small size of pelagic larvae also makes it difficult to directly track their movement *in situ* (Graham & Sebens 1996). Molecular tools are therefore increasingly being employed to investigate species taxonomy and phylogeography. The results from molecular data have significantly aided in our understanding of sponge systematics and in reconstructing metazoan evolutionary relationships (Wörheide et al. 2008), as well as helping to understand the phylogeographic distributions of NIS (Le Roux & Wiczorek 2009). Molecular phylogeography uses genetic techniques to help trace the evolutionary history of populations. It aids in unravelling the genomic genealogical patterns developed due to historical genetic subdivisions (Avice 2000b). Dissecting the relative relatedness and age of alleles and placing them in a geographical context aids in examining the ongoing gene flow in a population (Hare 2001). Understanding this gene flow can provide clues to the ecological history that has led to a species current taxonomic status (Stockman & Bond 2007).

Anthropogenic dispersal of NIS often leads to only a fraction of the genetic variability being transferred from the host to recipient biota (Nei et al. 1975, Dlugosch & Parker 2008). This can lead to the development of founder events, such as vicariance, population bottlenecks and range fluctuations, a product of the reduced genetic diversity and these events are usually detectable with molecular tools (e.g. Tarjuelo et al. 2001, Duran et al. 2004a, Wörheide et al. 2008). Molecular analyses indicate the original source population as the most genetically divergent population, because when species are transported to a recipient biota only a fraction of the genetic diversity is

transferred (Tsutsui et al. 2000). From a management perspective, knowing the origins of an NIS is very important as this is the stage of initial dispersal from which the invasion starts and this is the area easiest to prevent further invasions by stemming the flow of genetic diversity to invasive populations (Puth & Post 2005).

1.6 Sponges and Palmyra Atoll

Palmyra Atoll may not be as heavily inhabited as the nearby Hawaiian archipelago, but as an altered ecosystem it was potentially very susceptible to invasion after the lagoon dredging, military inhabitation and maritime traffic during WWII. The main biota on the hard substrates in the lagoons is now sponges, including possible introduced species. Therefore, from a management perspective it is important to establish the level of diversity and abundance of the sponges (Coles & Bolick 2007) in the lagoon and their potential direct and indirect impacts on the atoll system.

The hypothesis that islands are more susceptible to invasions (Elton 1958, Mooney & Cleland 2001) is difficult to test (Yiming et al. 2006). Therefore future research should investigate island invasions to better understand the impact that invasive species can have on these low diversity communities (Lodge 1993), particularly those under protection with little experience of human intervention, such as marine reserves and remote islands/atolls like Palmyra (Bax et al. 2003). Despite its remoteness the history of Palmyra makes it vulnerable to the 3 phases of successful invasion.

The most likely source for invasive species to Palmyra is the nearest large port Hawai‘i, which has been host to NIS for more than 1000 years (Mooney & Cleland 2001), is an important vector pathway for NIS, and had notably higher introduction rates during the wartime period (Coles et al. 1999). Naval ships during this time were also the cause of other introductions to remote Pacific islands (e.g. Coles et al. 1999, Paulay et al. 2002). Ships were transporting equipment, goods and building materials to Palmyra from Hawai‘i in high intensity from 1940 to 1945 and amongst the ships were often slow moving barges, allowing for higher survival rates of fouling organisms (Coutts et al. 2003, Godwin 2003b). In addition to this, the ship owners at this time were most likely less well informed of the impacts of invasive species and therefore ballast water and ships hulls were most likely only cleaned when fouling impacted the ships performance (Townsin 2003, Duggan et al. 2005).

After the lagoons were extensively scoured, potentially little of the native biota remained leaving the establishment of NIS relatively unchallenged. The alterations may also have inadvertently created conditions more suitable for the NIS than the recipient biota (Vitousek et al. 1997). Many of the invasive marine invertebrate species of the Hawaiian Islands have been found to survive and proliferate in the high sediment conditions of harbours, presumably an environment they are better adjusted to than the adjacent reefs (Coles et al. 1997), or perhaps predation levels are higher on the reefs (Pawlik 1998). If native species densities remained low after the dredging, invasive species, namely the sponges, may have been able to proliferate within the lagoon system and adjust to the new selective regimes. Unfortunately, Palmyra was subjected to both environmental changes and introduced species, potentially leaving it susceptible to great biotic change (Ruiz & Carlton 2003). The impact on the lagoon environment and biota may consequently have lasting affects on the adjacent 'near pristine' reef as the water flows out across the reef systems (e.g. Sultan & Ahmad 1990), because the lagoon areas are very shallow in places and the high sponge biomass could potentially remove large quantities of nutrients and oxygen from the water column (Reiswig 1974).

It is therefore important to ascertain the process of invasion to understand and manage the impacts that NIS can potentially have on communities or even directly on the ecosystem (Lafferty & Kuris 1996, Vitousek 1997, Webb et al. 2002). Further research should examine the potential impacts that these changes have had on such an altered system and whether they will impact not only the lagoon system, but on the surrounding reef as well. Firstly, the species need to be identified, particularly any non-indigenous sponge species (NISS). The origins of these species need to be established, as well as the number of potential invasion events i.e. whether the NISS were transported to Palmyra during the war alone or whether they are continually being brought to the atoll via other vessels. This will help establish whether preventative measures need to be taken to avoid further invasions, by the same or different species. Temporal changes in sponge species assemblages and spatial distribution patterns also need to be observed to look at rates of change and potentially ascertain whether they have the ability to cover the lagoons and extend onto the reef or whether the assemblages are relatively stable and confined within the lagoon. The potential that NISS have to invade new locations must also be considered to determine the rate at which invasive species can inhabit new locations if there is available substrate. Also, the impacts that the sponges could have on the water column are important to assess, as

the lagoon water flows out over the adjacent near-pristine reef. This information can be used to understand the potential impact the sponge fauna within the lagoons may have on the atoll system at Palmyra. This research will also improve our understanding of the potential direct and indirect impacts that sponges in high densities (particularly introduced species) can have on the water column in other semi-enclosed systems such as other atolls, harbours and bays, and their direct threat of expanding beyond these introduction sites to more unaltered systems.

Overall the aims of this thesis were to a) identify the sponges at Palmyra, particularly any non-indigenous species b) establish whether the sponges are maintained within the lagoons or have the potential to extend onto the adjacent reef c) confirm the introduction of non-indigenous species to Palmyra and d) establish whether the sponges are impacting the water column in the lagoons sufficiently to affect the water which flows out over the adjacent near-pristine reef.

1.7 Objectives of the research

This research examined the ecology of sponge assemblages at Palmyra Atoll in the Central Pacific to determine the potential direct and indirect impacts of sponge assemblages on this semi-enclosed body of water. The specific aims of my thesis were to:

- a) Quantify the abundance and distribution patterns of sponges at Palmyra Atoll and determine the factors influencing these distribution patterns.
- b) Identify potential sponge species that have been introduced to Palmyra.
- c) Measure the growth and recruitment of sponge species to determine if there is any potential for sponges in the lagoon to colonise the adjacent reef.
- d) Measure the ecological impacts of sponges on the water column at the atoll.

Environmental drivers of sponge diversity, cover and assemblage composition in the lagoons at Palmyra Atoll, Central Pacific

2.1 Abstract

Heavy disturbance usually leads to changes in local-scale environmental conditions, which may have subsequent effects on the associated biota and leave communities with a reduced ability to resist invasive species. Palmyra Atoll in the Central Pacific was subject to major military modifications across its lagoon system and islets during WWII and now the dominant fauna on the remaining hard substrate are sponges. Given the limited dispersal capacity of sponges and isolation of Palmyra, it is likely that many of the sponge species currently found in the atoll lagoons are non-indigenous. In this study, I quantified the physical and biological factors explaining the variation in sponge distribution patterns across 11 sites to determine the potential for the sponges in the lagoon at Palmyra to invade the surrounding reef systems if non-lagoon environmental quality changes in the future. Significant differences in sponge assemblages were found between all but three sites. Overall, 40% and 96% of the variability in sponge assemblages and mean sponge cover was explained by the biotic and abiotic variables measured, respectively. The main factors explaining the variability in total sponge cover were turbidity, percentage of hard substrate, variability in water flow and percentage of coarse sand. Substrate angle, variability in chlorophyll-*a* and percentage of hard substrate were the top three variables explaining the variability in sponge assemblages, although no single factor explained more than 8% of the total variation. The findings suggest that the lagoon sponges at Palmyra are likely to be restricted to these environments where they are particularly associated with conditions resulting from the earlier heavy disturbance, and are therefore unlikely to spread to the outer reefs unless there is a dramatic decline in environmental quality. Therefore, any occurrence of lagoon-inhabiting sponges on outer atoll reef systems in the future may indicate a decline in environmental quality.

2.2 Introduction

Coral reefs are highly diverse ecosystems providing numerous ecological and economic benefits (Moberg & Folke 1999, Barbier et al. 2011). However, despite their importance, coral reefs are experiencing major anthropogenic pressure (Pandolfi et al. 2003, Bellwood et al. 2004, Hoegh-Guldberg 2011) to the extent that no remaining reefs can be considered pristine (Jackson 1997). Alterations and modifications to marine environments, such as coral reefs, change the structure and function of the receiving ecosystem by reducing diversity, lowering light levels and available substrate, and increasing sediment loading and nutrient pollution (Rogers 1990, Cranfield et al. 2004, Fabricius 2005). When such disturbances are extreme, complex ecosystems can be rapidly transformed to simple ones and become characterised by reduced diversity (Rapport & Whitford 1999). Furthermore, modifications and the removal of native fauna often make habitats more susceptible to colonisation by non-indigenous species (NIS) (Mooney & Cleland 2001), as well as inadvertently create conditions and provide substrate that are preferential to the NIS over the existing biota (Byers 2002, Tyrrell & Byers 2007).

There are a large number of atolls in the Central Pacific, most which have a long history of human colonisation, and which are typically characterised by degraded lagoon systems with low levels of water exchange and high levels of turbidity (Sandin et al. 2008). These environments appear to have gone through major changes compared to their original state and many such lagoons support a large number of non-indigenous species (**see Appendix I**). Given that many of these introductions are thought to have occurred in recent human history (past 50–100 years) because of shipping traffic, there is concern that these relatively recent introductions to lagoon systems as the point of incursion may lead to a later invasion of outer atoll reef systems. While such invasions have not yet been widely reported (**but see Appendix I**), it is possible that such species are still in an establishment phase. Therefore, it is important to understand the factors influencing the distribution patterns of species within degraded lagoons to determine their potential to invade outer reef systems.

Palmyra Atoll, situated 1,930 km south of the Hawaiian archipelago, is considered a near-pristine atoll system and represents a biodiversity hotspot in the Central Pacific (Maragos & Williams 2011a). However, although the outer reefs at Palmyra are characterized by a high cover of calcifying organisms and abundant predatory fish (Sandin et al. 2008), the prominent benthic fauna on the hard substrate in

the lagoons, where extensive habitat modifications occurred (Collen et al. 2009), are sponges. The lagoons at Palmyra were subject to a number of military modifications during WWII (Dawson 1959) including extensive dredging and construction that removed endemic fauna, including corals from the lagoons (Anon. 1947), and altered the environmental conditions by stemming the flow of water between lagoons (Dawson 1959, Maragos 1993, Collen et al. 2009). It has been proposed, because of its shape, that the pre-war lagoon at Palmyra might have been similar to Millennium Atoll's lagoons (pers. comms. Jim Maragos), which is dominated by *Acropora* spp., but also characterised by high crustose coralline algal cover, turfing algae and the giant clam *Tridacna maxima* (Barott et al. 2010). A large number of old *T. maxima* shells remain in Palmyra's lagoon supporting this suggestion. The military modifications at Palmyra therefore most likely resulted in a major decline in species diversity, and potentially made the lagoon system more vulnerable to NIS and led to the current environmental conditions, where sponges are the dominant benthic fauna on hard substrates.

Sponges are often understudied and overlooked despite performing a range of important functional roles in coral reef ecosystems, particularly resulting from their filtering activities, competitive ability and complex relationships with other organisms (Wulff 2001, Bell 2008a). Furthermore, where sponges occur in high enough densities they can have considerable impacts on benthic and pelagic ecosystems (e.g. Peterson et al. 2006). It is currently unknown how many of the sponge species in the lagoons at Palmyra are native to the atoll; however two non-indigenous sponge species have already been reported (see **Appendix I**). Given the generally limited dispersal potential of sponges (Maldonado & Young 1996) and the isolated nature of the atoll, it is likely that many of the sponges found in this disturbed environment are non-native to the Palmyra system.

Several features of the sponges at Palmyra are of particular concern from a conservation and management perspective, and are potentially problems for other atoll systems where sponges are an abundant component of lagoon fauna. Firstly, sponges are usually top spatial competitors and have the ability to overgrow other reef organisms (Bell 2008a). Secondly, the filtering activity of sponges has the potential to impact the water column (e.g. nutrient removal). Therefore changes to sponge abundance in the lagoon or an increase in the distribution range of sponges at Palmyra, has the potential to alter overall community structure and ecosystem functioning (Bell 2008a). However, recent surveys have found no common sponge species between the

lagoon and outer atoll reefs where sponges are rare suggesting abiotic and biotic factors might be restricting the sponges to the lagoons. Despite this, given that the establishment of the sponge assemblage is likely to be relatively recent (last 60 years since major military occupation) compared to the likely age of the atoll colonisation, this needs further investigation as sponges might still be in an establishment phase.

In general there is a paucity of studies that have examined sponge assemblage diversity and abundance in the Central Pacific (e.g. Bell & Carballo 2008 and Appendix II), with little known about the environmental parameters shaping the sponge assemblages in these coral reef ecosystems. Previous studies have shown that spatial variation in sponge distribution patterns and abundance depend on a number of environmental and biological factors, including depth (**see Appendix II**), water flow (Bell & Barnes 2003a), sedimentation (Carballo 2006, de Voogd & Cleary 2007), predation (Dunlap & Pawlik 1996), salinity (Roberts et al. 2006), nutrient levels (Wilkinson & Cheshire 1989), light levels (Cheshire & Wilkinson 1991), substrate type and angle (Bell & Barnes 2000a, Powell et al. 2010), turbidity (Zea 1994), and larval dispersal and recruitment (Maldonado & Young 1996).

In this study, Palmyra Atoll was used as a model atoll system to examine the factors explaining the variation in sponge abundance and distribution patterns to assess any potential for sponges to spread from the lagoon to the outer reefs systems in the future. The aims of this study were: 1) to identify the sponge species distribution patterns across the lagoons; 2) to determine the variables that best explain the variation in sponge assemblage patterns and overall sponge percentage cover in the lagoons; and 3) to explore the potential for the lagoon inhabiting sponges to extend on to the adjacent reef systems in the future.

2.3 Methods

2.3.1 Sponge assemblage surveys

Between June to November 2008 sponge assemblages were surveyed across the four lagoons at 11 sites (**Fig 2.1**) at depths between 5–8 m. The site selection criterion was based on achieving representative sampling across the lagoons. These observations focused on sponges found on the surface of the boulders rather than those underneath boulders (See Bell & Carballo 2008). Excavating (bioeroding) species were not included because accurate estimates of area cover were not possible. At each site, ten 1 m² quadrats (divided into twenty five 40 cm² sub-sections) were haphazardly placed on

boulder patches at each site. In total, 110 m² of substrate were surveyed across the lagoons at Palmyra. Within each quadrat sub-section, the percentage cover of each sponge species was estimated along with the area of available hard substrate. If a sponge extended beyond the quadrat only the area in the sub-section was estimated and if a sponge extended into an adjacent sub-section the area cover was estimated independently in each. Sponge cover was standardised against available hard substrate between quadrats to enable direct comparisons between sites by accounting for the variability in overall boulder densities:

$$= (c \times 4) \div (b \div 100) \div 100$$

where c is the cover of sponges (per species) (cm²), which was multiplied by 4 because each of the 25 sub-sections equates to 4% of the total quadrat and b is the average % of available substrate per quadrat. Then to get the average % area cover of sponge per available substrate the sum of both brackets was % by 100.

Figure 2.1 Location of the 11 survey sites within the 4 lagoons: Sand Island (Sa); Strawn Island (St); Ripple Wharf (RW); Paradise Island (P); The Dolphins (D); The Cut (C); South of the Cut (S.C); Quail Island (Q); Eastern Island (E); Central East (C.E); Turtle Pool (TP) across the 4 lagoons. The white arrows indicate locations of military modifications: 1 = excavated entrance channel, 2 = the airstrip made of dredge spoil and coral rubble and 3 = the north-south causeway, specifically the cut allowing boat access between the Centre and East Lagoons.

2.3.2 *Sponge collections and identifications*

Sponge samples were collected from all unidentified species inside and outside of the quadrats. Where possible, three samples of each species were collected and stored in >95% ethanol. All samples were identified by examining phenotypic characteristics, spicule preparations and sections.

2.3.3 *Biotic and abiotic variables*

A suite of variables was collected at each site at depths of 5–8 m from 2008 to 2010. All individual variables were collected simultaneously to ensure comparability across sites. Temperature (°C) data were collected using pendant HOBO[®] temperature data loggers (accuracy of ± 0.54 °C) (www.onsetcomp.com). Each logger was set to record once every 2 minutes between June–July 2010. RBR[®] XR-420 data loggers (www.rbr-global.com), recording once every minute between June–November 2008 and June–July 2010, measured salinity, turbidity (as a proxy for sediment load), chlorophyll-*a* and additional temperature data. Chlorophyll-*a* is used here as an approximation of food availability because it is a measure of the available phytoplankton in the water and generally correlates positively with bacteria levels (Cole et al. 1988), which are a major food source for sponges (Reiswig 1971b, 1975, Pile et al. 1996). Unfortunately, the remote nature of Palmyra Atoll made it impossible to collect water samples for complete food particle analysis (e.g. with flow cytometry).

Water flow was recorded once every 10 seconds using a Valeport[®] Model 106 Current Meter (www.valeport.co.uk) deployed between June–November 2008 and June–July 2010 for approximately two days at each site. Percentage organic matter (%) (PCOM) and total particulate matter ($\text{mg m}^{-2} \text{ d}^{-1}$) (TPM) in settled sediment were measured 2–5 times between June–November 2008 and 2009 using PVC sediment traps (See Bloesch 1994). The traps were 50 cm long with an internal diameter of 5.1 cm and placed 30 cm above the substrate. Upon collection, the trap contents were filtered through grade 114 wet strengthened Whatman[®] filter papers and put into a drying oven at 60°C to desiccate until they reached a constant weight. The dry weight and number of days the trap remained in the water were used to calculate TPM. The samples were then placed into a muffle furnace at 500 °C for 24 hours to obtain the ash-free dry weight. Weight loss on combustion was used to calculate the Particulate Organic Matter (POM) matter ($\text{mg m}^{-2} \text{ d}^{-1}$). PCOM was calculated as $\text{POM/TPM} \times 100$ (Craft et al. 1991).

Percentage hard and soft substrate (boulders and sand) were quantified using 10 x 1 m² photoquadrats taken at each site. Estimates of benthic cover were collected independently from the sponge cover surveys because boulders were targeted for this portion of the survey and would give a biased representation for each site. In order to obtain random sampling when characterising the habitats, a 30 m transect was laid at 5–8 m depth and a 1 m² quadrat placed on either side of the transect at 5 m intervals (n = 10) from 5 m to 25 m. By not placing the quadrats at either 0 m or 30 m, the likelihood of observer bias was removed when laying the transect tape. Substrate percentage cover values were calculated using Coral Point Count with excel extensions (CPCe) software (Kohler & Gill 2006). One hundred randomly assigned points were placed over each 1 m², equalling 1000 points per site and 11,000 points across the atoll, covering a total of 110 m².

Grain size composition of the sediment was examined by collecting *in situ* bulk sediment samples from the beginning and end of the quadrat transects. Each sample was then separated using standard brass sieves (USA Standard Testing Sieve- ASTM. E-11 specifications) into the fractions: rubble (>2.8 mm), coarse sand (500 µm–2.8 mm), medium sand (250–500 µm), fine sand (63–250 µm) and silt/clay (<63 µm) in accordance with the Wentworth scale (Folk 1974). The largest grain size class (>2.8 mm) was subsequently discarded because there is no upper size limit and it would therefore skew the results. The four remaining fractions were poured into and drained through pre-weighed grade 114 wet strengthened Whatman® filter papers and dried in an incubator for 2 days at 60 °C. Samples were allowed to cool to room temperature, weighed and then dried for additional 24 h periods until a constant weight was achieved. Proportions (%) for each size class were calculated as 100/total weight x class weight. The final variable included in the model was substrate slope /angle, which was categorical and sites were either classed as (1) sloping ≈ 40–50° or (2) horizontal ≈ 0°.

2.3.4 Statistical analyses

2.3.4.1 Diversity indices

Across sites, I characterised and compared sponge assemblages using a number of diversity indices: number of species (S), Hill's numbers N1 and N2 and the modified Hill's ratio (N21'). Hill's numbers N1 and N2 indicate the influence of rare and

dominant species, respectively, and Hill's ratio examines species equitability (evenness) without any dependency on the number of species (Rogers et al. 1999).

2.3.4.2 Multivariate analyses

All assemblage data, prior to the analyses, were dispersion weighted (based on 1000 permutations), which differentially weights species depending on their observed variability between samples (Clarke et al. 2006a) and is suitable for species prone to spatial clustering, such as sponges. Similarity matrices were based on zero-adjusted Bray-Curtis coefficients and accounted for denuded data (Clarke et al. 2006b), which were characteristic of a number of sites.

A permutational multivariate analysis of variance (PERMANOVA) (based on 9999 permutations) (Anderson 2001, Clarke et al. 2006b) and pairwise *post-hoc* tests were used to examine differences in sponge cover at two separate factor levels: lagoon (West, Center, East and Turtle pool) and site (all 11 sites) (**Fig 2.1**). To visualise the PERMANOVA results, non-metric multi-dimensional scaling (nMDS) and canonical analysis of principal coordinates (CAP) plots (using 9999 permutations), with allocation success values, were examined (Anderson & Willis 2003). Allocation success values (%) were calculated using the leave-one-out procedure of the CAP analysis (Anderson & Willis 2003) and indicated the relative distinctness between assemblages. Allocation values greater than 25% (for the 4 lagoons) and 9.09% (for the 11 sites) were considered to indicate more distinct assemblages than those expected by chance alone. Indicator species, i.e. those which most characterise a site (Dufrêne & Legendre 1997) and were responsible for the observed differences between assemblage patterns in the CAP plot, were examined by calculating Pearson's product-moment correlations of canonical ordinations axes with the original sponge percentage cover data (Anderson 2004). Those species identified as indicator species (defined as ≥ 0.5 in this study) were graphically displayed as vectors on a bi-plot.

Ranked species abundances were calculated by separately totalling sponge cover data per species and ranking them from the most to the least abundant, indicating how common or rare a sponge species ranked relative to all other sponges across the lagoons.

2.3.4.3 Environmental associations with sponge cover and assemblages

Analysis and modelling of the univariate and multivariate sponge data with the environmental, biological and categorical predictor variables were conducted using a distance-based linear model (DistLM) (McArdle & Anderson 2001). Three important species were also examined individually, two potentially introduced species and the most dominant sponge in the lagoons. *Haliclona caerulea* (Hechtel 1965) (also known as *Haliclona* (Gellius) *caerulea*, *Haliclona* (Soestella) *caerulea* or *Sigmatocia caerulea*) are two confirmed non-indigenous species introduced to Palmyra (see **Appendix I**) and Hawai'i (DeFelice et al. 2001) from the Caribbean and the Philippines, respectively. *Iotrochota protea* (de Laubenfels 1950) (purple staining sponge), the dominant species in the lagoons at Palmyra, is native to Hawai'i, but it is unknown whether it was found on Palmyra prior to military occupation. For further information on these highlighted species please see Chapter 5 and Appendices I and V. The DistLM routine conducts a multivariate multiple regression to quantitatively explain how much variation in the sponge assemblage data can be explained by the biotic and abiotic variables. Twenty-one environmental and biological variables were initially measured (**Table 2.1**), seven of which were one standard deviation (+1SD) values, which represent the variability of the factor at an individual site. To examine colineation between the variables, I examined draftsman plots and Principal Component Analysis (PCA) ordinations. Inter-correlation values > 0.75 (lower than the recommended 0.95 cut-off (Anderson et al. 2008) between two variables were considered for removal but were first examined further using PCA plots. Those factors removed from the model lay in the same direction and length as one or more of the other colineating variables in the PCA plots and were therefore redundant as variables. The following variables were removed from the final model: Temperature, chlorophyll-*a*, turbidity (+1SD), water flow, total particulate matter (TPM), TPM (+1SD), and medium and fine sediment, resulting in the inclusion of thirteen predictor variables into the DistLM model (see **Table 2.1**). The routine was run using the 'best' selection procedure, based on 9999 permutations of the residuals under the reduced model. The model selection criteria were determined using Akaike's information criterion (Akaike 1973) with a second-order bias correction applied (AICc) (Burnham & Anderson 2002). The most parsimonious model with the highest R^2 value and lowest AICc value was selected. In this study, each site (**Fig 2.1**), consisting of 10 x 1 m² quadrats, was considered an independent observation with independent variables collected for each of

the 11 locations. Distance-based redundancy analyses (dbRDA) were used to visualise and interpret the optimal models (McArdle & Anderson 2001). Sites are displayed by a group centroid: centroids closer together represent more similar sponge assemblages. The length and direction of each vector line indicates the strength and sign (+ve or -ve) of the relationship between the predictor variables and the sites. Sites in the opposite direction of the line are negative or lower values of the variable. All analyses were conducted using PRIMER v6 (Clarke & Gorley 2006) and the PERMANOVA+ add-on package (Anderson et al. 2008).

Table 2.1 Table of environmental (Env.), biological (Biol.) and categorical (Cate.) predictor variables and mean sponge cover for all species and target species used in the DistLM analysis. * = colineating variables not included in the final model. Values in bold denote the maximum or minimum mean values. Minimum numbers are not in bold if more than one site has the same value. n/a = not applicable, var. = variability (+ 1 SD) and Av. = availability.

Predictor	Code	Type	Units	Sa	St	RW	P	D	C	SC	Q	EI	CE	TP	Min	Max	Range	Mean
Temperature*	Temp	Env.	°C	28.57	28.71	28.63	28.50	28.11	28.79	28.95	28.98	28.93	29.10	27.51	27.51	29.10	1.58	28.62
Temperature var.	Temp var.	Env.	°C	0.59	0.66	0.32	0.18	0.71	0.33	0.27	0.39	0.24	0.36	0.35	0.18	0.71	0.53	0.40
Chlorophyll- <i>a</i> *	Chl- <i>a</i>	Env.	µg l ⁻¹	0.19	0.20	0.42	0.71	0.18	0.41	0.14	0.30	0.78	0.33	0.26	0.14	0.78	0.65	0.36
Chlorophyll- <i>a</i> var.	Chl- <i>a</i> var.	Env.	µg l ⁻¹	0.11	0.14	0.24	0.22	0.13	0.24	0.18	0.11	0.33	0.11	0.13	0.11	0.33	0.22	0.18
Turbidity	Turb	Env.	STU	1.06	1.03	0.99	1.61	0.97	1.50	1.04	0.68	1.01	1.09	5.97	0.68	5.97	5.29	1.54
Turbidity var.	Turb var.	Env.	STU	0.32	0.33	0.33	1.00	0.56	0.26	0.31	0.30	0.50	0.31	2.05	0.26	2.05	1.79	0.57
Salinity	Sal	Env.	psu	34.80	34.60	34.61	34.21	34.45	34.49	34.10	34.06	34.24	34.30	34.62	34.06	34.80	0.74	34.41
Salinity var.	Sal var.	Env.	psu	0.23	0.24	0.14	0.06	0.22	0.25	0.14	0.18	0.06	0.33	0.15	0.06	0.33	0.27	0.18
Water flow*	Flow	Env.	m sec ⁻¹	0.0408	0.0096	0.0024	0.0028	0.0060	0.0367	0.0034	0.0039	0.0003	0.0038	0.0024	0.0003	0.0408	0.04	0.01
Water flow var.	Flow var.	Env.	m sec ⁻¹	0.0422	0.0185	0.0099	0.0133	0.0138	0.0363	0.0124	0.0145	0.0031	0.0133	0.0128	0.0031	0.0422	0.04	0.02
Total particulate matter*	TPM	Env.	g m ⁻² day ⁻¹	32.81	54.40	21.19	42.70	81.28	60.36	49.95	65.55	105.22	53.07	157.2	21.19	157.2	135.97	65.79
Total particulate matter var.*	TPM var.	Env.	g m ⁻² day ⁻¹	5.52	6.78	5.66	17.99	17.93	11.33	9.16	20.34	73.36	5.09	121.63	5.09	121.63	116.54	26.80
Percentage organic matter	PCOM	Env.	% in sediment	12.97	11.14	13.72	15.25	11.45	13.81	14.51	12.16	11.44	14.02	11.23	11.14	15.25	4.11	12.88
Percentage organic matter var.	PCOM var.	Env.	% in sediment	1.27	5.92	3.31	2.56	4.14	5.68	3.13	2.76	2.93	7.38	4.41	1.27	7.38	6.11	3.95
Av. of coarse sand	C. sand	Env.	proportion (%)	41.37	35.30	21.09	29.01	11.10	33.40	22.13	22.63	36.49	33.38	25.20	11.10	41.37	30.27	28.28
Av. of medium sand*	M. sand	Env.	proportion (%)	24.42	24.72	17.70	28.08	20.58	22.02	27.10	12.76	23.29	35.50	11.01	11.01	35.50	24.49	22.47
Av. of fine sand*	F. sand	Env.	proportion (%)	30.50	28.94	30.25	35.20	43.97	35.02	40.93	35.89	26.94	29.82	36.35	26.94	43.97	17.03	33.98
Av. of silt	Silt	Env.	proportion (%)	3.71	11.03	30.95	7.71	24.34	9.55	9.84	28.72	13.28	1.29	27.44	1.29	30.95	29.66	15.26
Av. of hard substrate	Hard sub.	Env.	proportion (%)	18.6	44.1	12	37.3	25.4	30.9	27.4	33	35.8	31.1	35	12.00	44.10	32.10	30.05
Av. of soft substrate	Soft sub.	Env.	proportion (%)	73.80	53.10	43.70	51.80	61.70	55.60	57.90	59.90	57.90	60.90	56.10	43.70	73.80	30.10	57.49
Angle	Slope	Cate.	sloped vs. horizontal	2	2	2	2	1	2	2	2	2	1	2	n/a	n/a	n/a	n/a
Mean cover																		
All species	n/a	n/a	%	30.83	31.96	17.73	26.58	23.08	30.53	24.99	33.10	30.39	31.67	15.77	15.77	33.10	17.33	26.97
<i>Haliclona caerulea</i>	n/a	n/a	%	0.12	1.88	1.01	0.23	1.44	0.00	0.02	0.01	0.00	0.55	1.69	0.00	1.88	1.88	0.63
<i>Gelliodes fibrosa</i>	n/a	n/a	%	0.00	0.27	0.06	1.15	0.00	0.62	1.98	0.00	0.00	0.00	0.00	0.00	1.98	1.98	0.37
<i>Iotrochota protea</i>	n/a	n/a	%	18.90	20.61	7.25	17.37	4.63	7.35	11.75	17.29	3.92	3.97	8.47	3.92	20.61	16.69	11.05

2.4 Results

A total of 36 sponge species were recorded across the lagoons at Palmyra Atoll (**Table 2.2; Appendix III**). Twenty-four of these were collected during the quadrat surveys in 2008 and the remainder were rare species and observed outside of the quadrats, found deeper than 8 m or were excluded because they were excavating species. On the non-lagoon reefs, we found 15 sponge species, therefore across the atoll in total, we found 51 species and importantly no species were found in both the lagoons and on the outer reefs. Overall there were 16 genera, 13 families and 7 orders of sponges observed in the lagoons and 9 genera, 11 families and 8 orders on the reefs.

Excluding excavating species, four morphological groups were represented in the lagoon: encrusting, cushion, massive and digitate. The Ripple Wharf in the West Lagoon had the lowest number of species ($S=8$) and the Cut in the Center Lagoon and Quail Island in East Lagoon the highest ($S=17$), with an overall average of 15 (± 2.6) sponge species per site (**Fig 2.2b**). Both Hill numbers $N1$ and $N2$ indicated similar patterns across sites with the highest measure of diversity and evenness (**Fig 2.2c**) found at the Dolphins ($N1$ and $N2$) and the Ripple Wharf ($N21'$) (**Fig 2.2d**), respectively, and the lowest at Strawn Island for all indices.

Table 2.2 Species found within the lagoons and on the reefs at Palmyra Atoll, see Appendix III for photographs. A-X samples are those species found within the quadrats. AA-AL are those species found outside the quadrats or are excavating species in the lagoons and BA-BO are those species found on the reefs. ^c = calcareous sponge. For further information on the ecological and morphological characteristics of the sponges in this list please refer to Appendix IV.

ID List	Genus	Family	Suborder	Order	Reference
A <i>cf. Aplysilla rosea</i>	<i>Aplysilla</i>	Darwinellidae	n/a	Dendroceratida	Barrois, 1876
B <i>Dysidea</i> sp.1	<i>Dysidea</i>	Dysideidae	n/a	Dictyoceratida	Johnston, 1842
C <i>Dysidea</i> sp.2 <i>cf. etherea</i>	<i>Dysidea</i>	Dysideidae	n/a	Dictyoceratida	Johnston, 1842
D <i>Spirastrella</i> sp.	<i>Spirastrella</i>	Spirastrellidae	n/a	Hadromerida	Schmidt, 1868
E <i>Scopalina</i> sp.	<i>Scopalina</i>	Dictyonellidae	n/a	Halichondrida	Schmidt, 1862
F <i>Halichondria</i> sp.1	<i>Halichondria</i>	Halichondriidae	n/a	Halichondrida	Fleming, 1828
G <i>Halichondria</i> sp.2	<i>Halichondria</i>	Halichondriidae	n/a	Halichondrida	Fleming, 1828
H <i>Hymeniacidon</i> sp.1	<i>Hymeniacidon</i>	Halichondriidae	n/a	Halichondrida	Bowerbank, 1858
I <i>Halichondriidae</i> sp.1	unknown	Halichondriidae	n/a	Halichondrida	Gray, 1867
J <i>Halichondriidae</i> sp.2	unknown	Halichondriidae	n/a	Halichondrida	Gray, 1867
K <i>Chalinula</i> sp.	<i>Chalinula</i>	Chalinidae	Haplosclerina	Haplosclerida	Schmidt, 1868
L <i>Haliclona</i> (Gellius) <i>caerulea</i>	<i>Haliclona</i>	Chalinidae	Haplosclerina	Haplosclerida	Hechtel, 1965
M <i>Haliclona</i> sp.1	<i>Haliclona</i>	Chalinidae	Haplosclerina	Haplosclerida	Grant, 1836
N <i>Challyspongiidae</i> sp.	unknown	Challyspongiidae	Haplosclerina	Haplosclerida	de Laubenfels, 1936
O <i>Gelliodes fibrosa</i>	<i>Gelliodes</i>	Niphatidae	Haplosclerina	Haplosclerida	Wilson, 1925
P <i>Niphatidae</i> sp.1	unknown	Niphatidae	Haplosclerina	Haplosclerida	Van Soest, 1980
Q <i>Haplosclerida</i> sp.	unknown	unknown	unknown	Haplosclerida	Topsent, 1928
R <i>Acarnus</i> sp.1	<i>Acarnus</i>	Acarnidae	Microcionina	Poecilosclerida	Gray, 1867
S <i>Acarnus</i> sp.2	<i>Acarnus</i>	Acarnidae	Microcionina	Poecilosclerida	Gray, 1867
T <i>Chondropsidae</i> sp.	unknown	Chondropsidae	Myxillina	Poecilosclerida	Carter, 1886
U <i>Iotrochota protea</i>	<i>Iotrochota</i>	Iotrochotidae	Myxillina	Poecilosclerida	Ridley, 1884
V <i>Iotrochotidae</i> sp.	unknown	Iotrochotidae	Myxillina	Poecilosclerida	Dendy, 1922
W <i>Clathria</i> sp.	<i>Clathria</i>	Microcionidae	Microcionina	Poecilosclerida	Schmidt, 1862
X <i>Hexadella</i> sp.	<i>Hexadella</i>	Lanthellidae	n/a	Verongida	Topsent, 1896
AA <i>Darwinellidae</i> sp.	unknown	unknown	Darwinellidae	Dendroceratida	Merejkowsky, 1879
AB <i>Cliona</i> sp.1	<i>Cliona</i>	Clionidae	n/a	Hadromerida	Grant, 1826
AC <i>Cliona</i> sp.2	<i>Cliona</i>	Clionidae	n/a	Hadromerida	Grant, 1826
AD <i>Suberitidae</i> sp.1	unknown	Suberitidae	n/a	Hadromerida	Schmidt, 1870
AE <i>Suberitidae</i> sp.2	unknown	Suberitidae	n/a	Hadromerida	Schmidt, 1870
AF <i>Hymeniacidon</i> sp.	<i>Hymeniacidon</i>	Halichondriidae	n/a	Halichondrida	Bowerbank, 1858
AG <i>Halichondriidae</i> sp.3	unknown	Halichondriidae	n/a	Halichondrida	Bowerbank, 1858
AH <i>Haliclona</i> sp.2	<i>Haliclona</i>	Chalinidae	Haplosclerina	Haplosclerida	Grant, 1836
AI <i>Niphatidae</i> sp.2	unknown	Niphatidae	Haplosclerina	Haplosclerida	Van Soest, 1980
AJ <i>Microcionidae</i> sp.	unknown	Microcionidae	Microcionina	Poecilosclerida	Carter, 1875
AK <i>Myxillidae</i> sp.	unknown	Myxillidae	Myxillina	Poecilosclerida	Dendy, 1922
AL <i>Poecilosclerida</i> sp.	unknown	unknown	unknown	Poecilosclerida	Topsent, 1928
BA <i>Astrophorida</i> sp.	unknown	unknown	unknown	Astrophorida	Sollas, 1887
BB <i>Jaspis</i> sp.	<i>Jaspis</i>	Ancorinidae	n/a	Astrophorida	Gray, 1867
BC <i>Halisarca</i> sp.	<i>Halisarca</i>	Halisarcidae	n/a	Chondrosida	Johnston, 1842
BD <i>Clathrina</i> sp. ^c	<i>Clathrina</i>	Clathrinidae	n/a	Clathrinida	Gray, 1867
BE <i>Hyatella</i> sp.	<i>Hyatella</i>	Spongiidae	n/a	Dictyoceratida	Lamarck, 1814
BF <i>Cliona</i> sp.3	<i>Cliona</i>	Clionidae	n/a	Hadromerida	Grant, 1826
BG <i>Tethya</i> sp.	<i>Tethya</i>	Tethyidae	n/a	Hadromerida	Lamarck, 1815
BH <i>Callyspongia</i> sp.	<i>Callyspongia</i>	Callyspongiidae	Haplosclerina	Haplosclerida	Duchassaing & Michelotti, 1864
BI <i>cf. Chalinidae</i> sp.	unknown	Chalinidae	Haplosclerina	Haplosclerida	Gray, 1867
BJ <i>Haliclona</i> sp. 4	<i>Haliclona</i>	Chalinidae	Haplosclerina	Haplosclerida	Grant, 1836
BK <i>Haliclona</i> sp.3	<i>Haliclona</i>	Chalinidae	Haplosclerina	Haplosclerida	Grant, 1836
BL <i>Niphatidae</i> sp.	unknown	Niphatidae	Haplosclerina	Haplosclerida	Van Soest, 1980
BM <i>Plakinidae</i> sp.	unknown	Plakinidae	n/a	Homosclerophorida	Schulze, 1880
BN <i>Crella</i> (<i>Crella</i>) sp.1	<i>Crella</i>	Crellidae	Myxillina	Poecilosclerida	Gray, 1867
BO <i>Crella</i> (<i>Crella</i>) sp.2	<i>Crella</i>	Crellidae	Myxillina	Poecilosclerida	Gray, 1867

Figure 2.2 Measures of overall % cover, number of species, Hill's numbers N1, N2 and N21' across all sites (means $\pm$ SD). Lagoons are indicated as blue = West, yellow = Center, green = East and red = Turtle Pool.

Mean sponge cover varied among sites across the lagoons (**Fig 2.3a**) and ranged from 15.8% ($\pm$ 5.7) at Turtle Pool, in the far East Lagoon, to 33.1% ($\pm$ 12.4) at Quail Island, in West Lagoon (**Fig 2.1**), with an overall mean of 27.0% ($\pm$ 11.6). Significant differences in sponge cover occurred among sites (Pseudo- $F_{10, 109} = 6.6015$, $p < 0.001$). Further pairwise tests revealed that all sites were significantly different from each other except in West Lagoon between Strawn and Paradise Islands ($t = 1.25$, $p = 0.1403$), and Sand and Paradise Islands ($t = 1.36$, $p = 0.0933$). Significant differences were also found between all four lagoons (Pseudo- $F_{3, 109} = 8.3042$, $p < 0.001$). The CAP output also identified significant differences in sponge assemblages between sites and lagoons (**Fig 2.3 and Table 2.3**). Each site and lagoon had higher allocation success values than would be expected by chance alone.

Figure 2.3 Canonical analysis of principal coordinates (CAP) bi-plot ordination showing the indicator sponge species across sites and lagoons. Sand Island (Sa); Strawn Island (St); Ripple Wharf (RW); Paradise Island (P); The Dolphins (D); The Cut (C); South of the Cut (S.C); Quail Island (Q); Eastern Island (E); Central East (C.E); Turtle Pool (TP) across lagoons West (W); Center (C); East (E) and Far East (F.E). Based upon a zero-adjusted Bray-Curtis similarity matrix with a dispersion weighting pre-treatment on sponge area cover data, which are pre-adjusted to the amount of available substrate. The analyses were based on 9999 random permutations. A Pearson's correlation coefficient of ≥ 0.5 was used to identify these vectors.

Table 2.3 Results from CAP analyses examining sponge assemblage variability in cover between sites and lagoons. Sa, Sand Island; St, Strawn Island; Rw, Ripple Wharf; P, Paradise Island; D, the dolphins; C, the cut; S.C, south of the cut; Q, Quail Island; E.I, Eastern Island; C.E, Center East; TP, Turtle pool; W, West Lagoon; C, Center Lagoon; E, East Lagoon; TP, Turtle pool. Where m is the number of principal coordinate (PCO) axes used in the analyses, % Var the percentage of the total variation explained by the first m PCO axes, δ^2 the squared canonical correlation and allocation success values the percentage of samples correctly allocated into their corresponding groups using the first m PCO axes from the model.

Factor	m	% Var	δ^2	P
Site	7	79.1	0.78	0.0001
Lagoon	9	89.3	0.71	0.0001

Allocation success											
Site	Sa	St	RW	P	D	C	S.C	Q	E.I	C.E	TP
	50	50	90	40	80	30	60	50	60	40	50

Lagoon	W	C	E	F.E
	86	60	63	70

2.4.1 Ranked and indicator species

Of the 24 species identified within the survey quadrats three were characteristic of different sites (**Fig 2.3**): *Iotrochota protea* (U) for the majority of the West Lagoon sites, *Dysidea* sp. 1 (B) (Johnston, 1842) for The Dolphins and Chondropsidae sp. (T) (Carter, 1886) for Center and East Lagoons. The ranked abundance data (**Fig 2.4**) indicated the dominance of a few common species and a large number of ‘rare’ species in the Palmyra system resulting in low evenness. The two species with the highest cover across all lagoons were *Iotrochota protea* and Chondropsidae sp. (**Fig 2.3**). *Iotrochota protea* had the highest percentage mean cover for all lagoons, except East Lagoon (**Fig 2.4**), with the greatest abundance ($14.17\% \pm 8.2$) in West Lagoon.

Figure 2.4 Ranked abundances of sponge species in the lagoons at Palmyra. Bars are stacked and indicate the mean average per lagoon, but not the total mean across all lagoons where W/blue = West, C/yellow = Center, E/green = East and F.E/red = Far East. (★) marks the indicator species in **Figure 2.3**. The letters in brackets refer to the ID codes in **Table 2.2**.

2.4.2 Changes in the abundance of *Haliclona caerulea*, *Gelliodes fibrosa* and *Iotrochota protea*

The abundance of these three species was all correlated with different environmental variables. Salinity levels had a positive relationship with *Haliclona caerulea* (blue Caribbean sponge), explaining 25.2% of the variability in its abundance (see **Table 2.4**). *Haliclona caerulea* was most abundant in West Lagoon at Strawn Island, The Dolphins and Turtle Pool where salinity levels were >36.45 psu (**Table 2.1**). At those sites where *Gelliodes fibrosa* was recorded, cover was positively correlated with Percentage Organic Matter (PCOM), which explained 29.0% of the

variability. The second highest PCOM value and highest *G. fibrosa* cover was at South Cut with 15.3% and 1.98%, respectively. The Ripple Wharf and Strawn had the lowest cover of *G. fibrosa* (0.06 and 0.27%, respectively) along with the lowest levels of PCOM (13.7 and 11.1%, respectively). The abundance of *Iotrochota protea*, the species with the highest cover across the majority of the lagoons, was correlated with substrate angle and variability in chlorophyll-*a*, which explained 34.0% and 34.2% of the variation, respectively. Sloped sites, with the exception of Eastern Island, had the highest *I. protea* cover ranging from 7.3% to 20.6%. Central East, The Dolphins (horizontal sites) and Eastern Island had the lowest cover (3.97, 4.63 and 3.92%, respectively). Variability in chlorophyll-*a* had a weak negative relationship with *I. protea* abundance, with the lowest cover and highest standard deviation in chlorophyll-*a* levels (1.30% and 0.33 $\mu\text{g l}^{-1}$) both at the Eastern Island site.

Table 2.4 Results of the distance-based permutational multiple regression analysis (DistLM) for overall mean % cover, sponge species assemblage and the introduced and potentially introduced species. Only the optimal variables of spatial change in sponge assemblages are shown along with the proportion of variability they explain. Model selection was based on Akaike's Information Criterion with a second-order bias correction applied (AICc), with the total variation explained (r^2) by each best-fit model shown (% total) based on 9999 random permutations of the raw data. Variables were normalized and fitted in a step-wise manner.

Model	AICc	Predictor	Pseudo-F	p	%Var
Mean % cover	41.26	Turb	5.71	0.0499	38.83
		Hard_cov	10.02	0.0168	34.02
		Flow_1SD	11.87	0.0104	17.08
		C. Sand	8.26	0.0183	5.83
		Total			95.76
Species assemblages	793.14	Angle	8.72	0.0001	7.47
		Chl- <i>a</i> _1SD	7.79	0.0001	6.28
		Hard_cov	6.07	0.0001	4.67
		Turb	5.70	0.0001	4.20
		Silt	5.60	0.0001	3.40
		Sal_1SD	5.00	0.0001	3.31
		Soft_cov	4.60	0.0001	3.28
		PCOM_1SD	4.76	0.0001	3.27
		Flow_1SD	3.57	0.0003	2.31
		C. Sand	2.87	0.0020	1.82
		Total			40.01
<i>Haliclona caerulea</i>	89.58	Sal	2.27	0.1211	25.21
<i>Gelliodes fibrosa</i>	88.43	PCOM	2.88	0.0757	28.97
<i>Iotrochota protea</i>	69.22	Angle	4.64	0.0541	34.01
		Chl- <i>a</i> _1SD	8.59	0.0125	34.17
		Total			68.18

2.4.3 Changes in overall sponge cover

Out of 13 variables (**Table 2.1**), 95.8% of the variation in overall sponge cover between sites was explained by four factors: turbidity (38.8%), percentage hard substrate (34.0%), flow rate variability (1SD) (17.1%) and percentage cover of coarse sand (5.8%) (**see Table 2.4**). Turbidity had an overall negative relationship with sponge area cover, with Turtle Pool having the highest turbidity ($5.97 \text{ STU} \pm 2.05$) and lowest sponge cover (15.8%) (**Table 2.1**). Percentage hard substrate was lowest at the Ripple Wharf (12.0%) and highest at Strawn Island (44.1%). Turtle Pool had the fourth highest hard substrate cover (35.0%), but the lowest area cover of sponges (15.8%). Variability in water flow was highest at Sand Island ($0.0422 \text{ cm}^2 \text{ sec}^{-1}$) and The Cut ($0.0363 \text{ cm}^2 \text{ sec}^{-1}$) and lowest at Eastern Island ($0.0031 \text{ cm}^2 \text{ sec}^{-1}$) with the remaining sites between 0.0099 and $0.0185 \text{ cm}^2 \text{ sec}^{-1}$. Percent of coarse grain sand was positively related to percent sponge cover with the highest values at Sand Island (41.4%) and the lowest at The Dolphins (11.1%).

2.4.4 Changes in sponge assemblages

Variability in sponge assemblages among sites (**Fig 2.5**) primarily correlated with substrate angle, chlorophyll-*a* variability and the percentage of hard substrate, explaining 7.5%, 6.3% and 4.7% of the variability, respectively (**see Table 2.4**). The total amount of variability explained by all significant environmental and biological factors was 40.0%, with the top three explaining approximately half (18.4%) of the variability. Substrate angle was negatively associated with The Dolphins (one of the two horizontal sites). Variability in chlorophyll-*a* concentration was positively associated with Central East, Eastern Island and The Cut. Percentage hard cover was positively associated at South Cut and Turtle Pool and negatively correlated at Sand Island and the Ripple Wharf.

Figure 2.5 Distance-based redundancy analysis (dbRDA) visualising the similarity in sponge assemblages and the correlated change in environmental conditions among the 11 lagoon sites (**Fig 2.1**) for mean percentage area cover (a) and sponge assemblages (b).

2.5 Discussion

Many Central Pacific atolls have suffered extensive degradation of their lagoons as a result of human disturbance (Sandin et al. 2008). The habitats in the altered lagoon system at Palmyra Atoll in the Central Pacific are very different to the surrounding near-pristine coral reefs (**Fig 2.6a**). At Palmyra Atoll sponges dominate the hard substratum lagoon environments, which is unlikely to have been the case prior to the

disturbance during WWII. I found that turbidity, percentage hard substrate, variability in water flow rate and percentage cover of coarse sand together explained a total of 95.76% of the variability in overall sponge cover between sites. In addition, although I found that 40% of the variability in sponge assemblage structure was explained by 10 variables, no single variable explained more than 8% of the variation. Of the 24 species observed in the quadrats, three characterised different site groups (**Fig 2.3**) and three of the most important species, *Haliclona caerulea*, *Gelliodes fibrosa* and *Iotrochota protea*, were each correlated with different variables when modelled individually. My results suggest that the sponge species within the lagoons are strongly associated with environmental and biological characteristics associated with the current lagoon environment and are unlikely to spread to the outer reefs unless there are future declines in reef environmental quality.

Figure 2.6 Underwater photos of Palmyra reef, lagoon and sponges. A) Current day conditions in the lagoon (left) versus the reef (right). B) Sponges and sediment; different strategies to deal with sedimentation. From left to right: *Iotrochota protea* (U), no sediment on oscula or veins; *Dysidea* sp.2 (C), sediment accumulates primarily on the protruding spikes, raised oscula; *Spirastrella* sp. (E), extended oscula. All letters in brackets relate to the species ID in **Table 2.2**.

2.5.1 Sponge species at Palmyra Atoll

There is a paucity of ecological research on sponge assemblages from Pacific islands, particularly in the Central Pacific region. Previous studies have shown that the

number of species reported from Pacific islands ranges between 8 and 60 (de Laubenfels 1951, 1954, Bergquist 1965, Chiriboga et al. 2011, van Soest et al. 2011). To date, 36 sponge species have been found within the lagoons at Palmyra and at least 15 more on the reefs (**Table 2.2; Appendix III**), totalling 51 species units identified thus far, which is therefore similar to other Pacific islands, but much lower than other tropical areas such as Indonesia and Australia where similar sampling effort and area to the present study has detected more than 100 species (see Bell 2007).

The number of species per site at Palmyra was similar across the lagoons with the exception of the man-made Ripple Wharf in West Lagoon, which had the lowest number of species. This is probably due to the high percentage of anthropogenic debris resulting in low available hard substrate for sponges to inhabit; also some sponge species may not settle on artificial substrate because these surfaces are smoother (Knott et al. 2004). West Lagoon at Palmyra has been proposed as the location for most of the non-indigenous species introductions (**see Appendix I**), however this area generally had low diversity with only a few abundant species at Strawn Island and Paradise Island. *Iotrochota protea* accounted, on average, for half of the total sponge cover at all of the sites in West Lagoon. The Dolphins was the only exception, which was distinctly different to all other sites because it had a greater number of rare species and was actually characterised by one of the rarer species: *Dysidea* sp.1 (Johnston, 1842). It is possible that the high number of rare species at The Dolphins is because it was the location of a WWII boat dock resulting in high exposure to introduced species via fouling and ballast water, but many of these species have been unable to spread extensively to other areas in the lagoon.

2.5.2 Distributions of likely NIS sponge species in the lagoons

It is unknown how many of the sponge species found in the surveys are native to Palmyra. Across all lagoons the native Hawaiian species, *I. protea*, had the highest cover and was found at all sites. Little is known about *I. protea* except that it is found on sandy or rubble substrates in sheltered bays (Hoover 2006). *I. protea* is likely a NIS since like all sponge species, it has very poor dispersal ability and it seems unlikely that natural colonisation would have occurred. If it is a NIS, it is of concern as it covers approximately 13% of the boulders at the majority of sampling sites, which on average is half of the total sponge cover. However, considering *I. protea* is generally associated with low current conditions (Hoover 2006) this species is probably prevented from

dispersing to the outer reef sites because of the environmental conditions associated with higher water flow rates in these environments.

The environmental parameters driving sponge distribution patterns in the lagoons at Palmyra differed between some sponge species. Cover of *I. protea* was negatively correlated with variability in chlorophyll-*a* and positively correlated with inclined surfaces over horizontal ones. A negative correlation with variability in chlorophyll-*a* indicates that those sites with higher sponge cover are likely to have more stable food sources (i.e. less variation). Substrate inclination has also long been identified as influencing the distribution of sponges because of the different physical characteristics such as sedimentation, light, and water flow correlated with an inclined versus horizontal surfaces (Wilkinson & Evans 1989, Bell & Barnes 2000b, Bell & Smith 2004). However, despite the ability of *I. protea* to inhabit all sites in the lagoon, cover was in general higher on inclined sites compared to horizontal ones where sediment settlement will be lower.

Haliclona caerulea is believed to be an introduced species to Palmyra since it is native to the Caribbean (Hechtel 1965; Appendix I). Its abundance was positively correlated with salinity, and its cover was found to be higher in the West Lagoon and Turtle Pool (in the Far East Lagoon). At Palmyra the salinity levels are tidally driven and ranged by 0.74 psu overall with the highest values found at both ends of the atoll (West and Far East) where proximity to the surrounding seawater is the greatest (**Fig 2.1**) and freshwater run-off is less influential on the salinity. A decrease in salinity has the potential to reduce growth and reproductive activity of sponges. For example, Roberts et al. (2006) found a reduction in salinity by 2 psu negatively impacted a temperate reef sponge species. However, it is unknown what salinity levels (high or low) are likely to affect the growth or reproduction of *H. caerulea* and whether salinity differences prevents this species establishing on the outer reefs.

Gelliodes fibrosa is another known non-indigenous sponge species to Palmyra (see **Appendix I**) and its abundance was positively correlated with the percentage of organic matter (PCOM) in the sediment. A large number of the encrusting species at Palmyra, including *G. fibrosa* have a fine sediment layer on their surfaces (**Fig 2.6b**), but not on their osculum and, depending on the species, their surface veins. Concentrations of particulate and dissolved organic matter in sediments are higher than in the water column, so the lagoon sponges, and possible symbionts, have the potential to use this as a nutritional resource (Ilan & Abelson 1995). Back reef values of PCOM

(Williams et al. 2011c) are 4–5 times lower than in the lagoon, so if *G. fibrosa* distributions are determined by food availability related to PCOM levels, they are probably too low on the back reef to support this species.

2.5.3 Drivers of sponge cover and assemblage patterns

The composition of the sponge assemblages in the lagoons at Palmyra was significantly associated with 10 biotic and abiotic variables. However, none of the variables accounted for more than 8% of the variability and therefore individually are likely to have relatively low ecological significance, with the top 3 variables only explaining 18.4% of the total variation. The top two variables in this model were the same as the two variables for *I. protea*, perhaps suggesting that this species is having a large influence on the overall sponge assemblages across the lagoons (despite dispersion weighting).

The top four variables explaining overall sponge cover were turbidity, percentage hard substrate, variability in water flow and percentage coarse sediment, which explained 95.76% of the variability between sites. The current environmental conditions in the lagoons at Palmyra are that of low water flow and high sedimentation with the benthos characterised by fine/coarse sediment and boulders. Furthermore, substrate availability is low in the lagoon; boulders ($<0.5 \text{ m}^2$) are common but larger areas of hard surface are rare. Not surprisingly therefore, I found that percentage hard substrate had a weak positive correlation with sponge cover.

Water flow was also positively correlated with sponge cover among sites, with Sand Island in the West Lagoon and the Cut in the Center Lagoon, situated nearest to access channels, having the highest water flow levels along with the greatest percentage cover of sponge. Water flow rate is important for facultatively-active suspension feeders, like sponges, because passage into and through the sponge can be passively induced as a result of the Bernoulli principle (Vogel 1977), reducing the energy required for pumping. In addition, higher water flow will supply more food to the sponge per unit time compared to low flow. I also found a negative relationship between turbidity and sponge cover, which is most likely explained by the potential for high sediment levels to clog the delicate filtering apparatus of sponges (Bell 2004), as well as smother available hard substrate for larval settlement (Rogers 1990). Turtle Pool, in the far east lagoon, had the highest turbidity levels and lowest sponge cover, whilst Quail Island had higher than average cover and had the lowest turbidity levels. In

contrast to my results, Bell and Smith (2004) found that percentage cover of sponges was greater on reefs experiencing higher sedimentation levels, although their study compared two coastal coral reef locations, which more than likely experience greater water flow and lower sediment loading than the most turbid site in the lagoons at Palmyra, Turtle Pool. Turtle Pool had nearly 3 times higher turbidity levels than the next most turbid site (Paradise Island, in the West Lagoon); therefore, there is a relatively large range of turbidity levels for which I have no sponge assemblage data (2–5 STU). The high turbidity levels at Turtle Pool do, however, provide an insight into how sponge cover might be affected by large increases in sedimentation, for example if the north-south military causeway (**Fig 2.2**) was removed as discussed by Williams et al. (2011c). If turbidity increases substantially in the lagoons there is a possibility that not only coral species will be affected, but the sponges as well.

The distribution of reef organisms can be greatly affected by increases in sedimentation because of the impact on larval settlement and survival (Hodgson 1990, Connell et al. 1997). Research on the factors controlling sponge larval settlement and recruitment is limited. However, water flow can, for some species, be positively associated with juvenile survivorship and adult abundance (Maldonado & Young 1996) and if strong enough remove larval selection at settlement (Woodin 1986, Butman 1987). The mean water flow rate in the lagoons at depths of 5–8 m was 0.01 m sec^{-1} (± 0.01) and sponge larval swimming speeds have been recorded as between 0.001 – 0.01 m sec^{-1} (e.g. Bergquist et al. 1970, Maldonado & Young 1996). Therefore, theoretically during slow flowing tides, the sponge larvae in the lagoons at Palmyra could swim against the prevailing westerly water flow to settle in more suitable habitats. Interestingly, I recorded both *H. caerulea* and *I. protea* at Turtle Pool, which is located furthest away from the West Lagoon, the suggested location for all the introduced species (see **Appendix I**). Therefore, because these species have managed to extend to the opposite side of the atoll over several km, against the prevailing westward water flow (Gardner et al. 2011), it appears unlikely that the sponges will extend onto the reef unless there is a decline in environmental quality there. This hypothesis is further supported by the difference between lagoon and reef species lists, with no species common to both environments.

Other factors might also be preventing the sponges from within the lagoon establishing on the outer reefs. Specifically, fish predation has been shown to influence sponge distributions in the Caribbean (Pawlik 1998, Wulff 2000). However, a

preliminary fish predation experiment at Palmyra by the author, where seven lagoon sponge species were transplanted onto five back reef sites on multiple occasions, were not consumed and is therefore it unlikely that predation is preventing colonisation of the outer reefs by sponges at Palmyra. For further information on this trial experiment see Appendix VI.

2.5.4 Conclusions

Sponge diversity at Palmyra is comparable to other Pacific islands and atolls; however, it is still unknown how many species are native to the lagoons. Species diversity, when examining Hill's N1, was on average the lowest in West Lagoon, indicating that few species dominate these habitats. An exception to this was a site known as The Dolphins, which was one of the main boat docks during WWII and had the highest species diversity atoll-wide. For all the models examined (overall sponge cover, sponge assemblages and individual notable species) the strongest environmental relationships were found for sedimentation/turbidity and food/habitat availability. The current distribution and abundance patterns of sponges, particularly for *I. protea* (the most abundant species in the lagoons) suggests that unless there is a dramatic decline in environmental quality on the reefs at Palmyra, particularly increased sedimentation and turbidity, the sponges will most likely remain confined to the lagoons. This information may prove useful for monitoring declines in environmental quality for Palmyra and other atoll systems, as the appearance of lagoon sponge species on outer reefs could act as an early warning of environmental degradation.

Temporal variability and recruitment of sponges in an altered atoll lagoon; assessing their potential to extend on to an adjacent near-pristine coral reef

3.1 Abstract

Coral reefs support a high diversity of life making them ecologically and economically rich ecosystems; however, no reef has been left unaffected by human activities. Although some of the last remaining near-pristine reefs are in the Central Pacific, unfortunately a number of these atoll lagoons and islets were modified by the US military during WWII. Surprisingly, despite the quality of the reefs at these modified atolls, little work has examined their altered lagoons and the potential of current species, particularly non-indigenous species (NIS), to extend onto the non-lagoon reefs. This study examines the temporal variability and recruitment of the current dominant fauna (sponges) of the once (pre-WWII) coral inhabited lagoons at Palmyra Atoll to assess whether the sponges, including NIS, have the potential to extend from the altered lagoon to the adjacent near-pristine reefs. Semi-permanent sampling stations and ceramic panels were used to examine temporal variability in the sponge assemblage and larval recruitment, respectively. Over 3 years I recorded sponge densities, cover and species diversity, but only found significant inter-annual variation in species diversity. The recruitment panels, observed over 2 years, indicated that the number of recruits found on panels increased over time. The ability of the sponges to recruit to newly available substrate and the relative stability of the established sponge assemblage suggests that recruitment and mortality are in equilibrium. This study found that the sponges are unlikely to be limited to the lagoons due to recruiting capabilities and therefore could have already extended onto the reef. I also explore the possibility that the lagoon has undergone a phase-shift from a coral to sponge dominated ecosystem due to the military modifications.

3.2 Introduction

Coral reefs globally are ecologically and economically important (Moberg & Folke 1999); however, they remain under threat from direct and indirect anthropogenic pressures (Hughes et al. 2003, Pandolfi et al. 2003, Bellwood et al. 2004, Hoegh-Guldberg 2011). Remote and uninhabited atolls support some of the last remaining near-pristine coral reefs and therefore further research into the impacts of threats to these environments is important. Pressures on uninhabited atolls include construction, fishing, poaching, shipwrecks, coral bleaching, disease outbreaks, and non-indigenous introductions (See Maragos et al. 1996, Williams et al. 2010, Williams et al. 2011a; Appendix I).

Atoll systems, unlike most coastal reefs, have central lagoons; remnants of the oceanic volcano that formed them and depending on the structure of the islets the lagoons often connect with the adjacent coral reefs (Delesalle & Sournia 1992, Kench 1998). Therefore, the processes and organisms inhabiting an atoll lagoon may impact the adjoining reef through introducing new non-indigenous species or through water movement loaded with higher levels of dissolved inorganic and organic carbon and nitrogen, bacterioplankton, phytoplankton and sediment, as a result of anthropogenic pressures such as nutrient inputs and construction and natural biological processes (Pastorok & Bilyard 1985, Charpy-Roubaud et al. 1990, Torrton et al. 1997, Dufour & Berland 1999, Suzuki & Kawahata 2003).

Lagoons like those of Palmyra, Johnston, Midway and Wake Atolls in the Central Pacific still support a rich diversity of marine life, but were heavily altered to varying degrees during WWII due to US military occupation and construction (Maragos et al. 2008, Rooney et al. 2008). A variety of methods were used to establish military bases at each atoll, including lagoon dredging to build up the islets along with creating shipping access channels through the reef if necessary (Dawson 1959, Maragos 1993). Alterations like dredging dramatically reduce habitat complexity and species composition, diversity and richness (See Thrush & Dayton 2002), resulting in phase-shifts (Done 1992) and often making systems more susceptible to non-indigenous species (NIS) (Mooney & Cleland 2001, Byers 2002, Tyrrell & Byers 2007). It is therefore likely that the introduction of NIS to these Pacific atolls occurred relatively recently (<70–80 years ago) because of the dredged condition of the lagoons and the increased shipping activity associated with the WWII military actions (see **Appendix I**). However, due to the lack of species lists prior to the alterations, it is unknown how

many of the current species are native and whether any of the non-indigenous lagoon species could extend onto the adjacent reefs.

Environmental conditions at these atolls may be maintaining the introduced species in the lagoons (**see Chapter 2**) or perhaps the species are still in the establishment phase and have not yet had enough time to extend onto the reefs. While introductions of NIS have been reported for Pacific atoll lagoons such as Johnston and Palmyra (Coles et al. 2001; Appendix I), their temporal variability and potential to recruit (the introduction of new individuals to a population) to newly available substrate is mostly unknown.

The dominant fauna on the remaining hard substrate in the lagoons at Palmyra Atoll are sponges (**Chapter 2**). Sponges perform a large number of functional roles in reef systems (See Wulff 2001, Bell 2008a) including acting as top spatial competitors (de Voogd et al. 2004), being an important link in benthic-pelagic coupling (Lesser 2006), nutrient recycling (Diaz & Ward 1997) and as a food source for other organisms (Duffy 1992, Wulff 1994). Therefore, it is important to ascertain whether the sponges inhabiting the lagoon at Palmyra have the potential to extend onto the reef, particularly as the non-indigenous species, *Haliclona caerulea* and *Gelliodes fibrosa*, have already been observed here, along with a native Hawaiian species; *Iotrochota protea*, which is the dominant species in the lagoons (**see Chapter 2 and Appendix I**). Currently, the sponges extend across all the lagoons at Palmyra covering 27% ($\pm 6\%$) of the available hard substrate down to 8 m (**Chapter 2**) and can potentially filter all of the circulating water in the lagoons every 3–6 days (**Chapter 4**). Therefore, if sponge cover increases they could have an even greater impact on the lagoon and reef fauna both directly and indirectly (**see Chapter 4**). Predominantly sponge growth increases cover, while sponge density (abundance) increases through the production of larvae to create new sponges and asexually via fragmentation, budding and gemmule formation (Ayling 1980). Research globally has revealed variability in temporal changes in sponge cover and density assemblages. For example, in the Mediterranean and Atlantic, studies have reported fairly stable sponge assemblages (Fowler & Laffoley 1993, Garrabou & Zabala 2001), while in the Caribbean, Hughes (1996) found that abundances were fairly stable but assemblage composition varied over time. However, more recent research in the Atlantic, East and West Pacific and Indo-Pacific (Bell et al. 2006, Wulff 2006, Carballo et al. 2008, Berman 2012) has revealed more significant temporal changes in the sponge assemblages, including seasonal variation with periods of sponge growth

and shrinkage (Garrabou & Zabala 2001, Bell 2008b, Carballo et al. 2008). However, to date, no studies have examined temporal variation in sponge assemblages in the Central Pacific.

Temporal variation in the majority of sponge assemblages will also be affected by larval dispersal and fragmentation because it allows these sessile invertebrates to colonize new locations (Maldonado 2006) and is therefore the main mode by which the sponges in Palmyra's lagoon could extend onto the adjacent reef. However, sponge larvae only remain in the water column for a few minutes to a few days before settling on the sea floor and metamorphosing into their sessile adult form, therefore limiting their potential to increase their spatial distribution (Uriz et al. 1998, Maldonado 2006). Therefore movement of the sponges at Palmyra through growth or reproduction could be so limited that they have not yet progressed on to the reef, but have the potential to in the future. Alternatively if the sponges can recruit to new surfaces over short periods (<1 year) and grow rapidly, then they already have the potential to extend onto the reef, but have not due to other constraints such as environmental conditions, and are therefore unlikely to do so in the future.

Distinguishing between growth and fragmentation of established sponges and new recruits is often difficult in natural environments. I therefore elected to study the temporal variability of existing sponge assemblages (annually and seasonally) and settlement panels to study the potential of sponges to successfully recruit to newly available substrate. The aims of this study were to: a) gain a greater understanding of the potential for the sponges to recruit to newly available substrate over a short period (<1 year) and b) determine whether the sponge assemblages change (species composition and/or percentage cover) over time and between seasons in order to c) determine whether the sponges are likely to extend from the lagoon onto the adjacent near-pristine reef.

3.3 Methods

3.3.1 Evidence to suggest the condition of Palmyra's lagoons before WWII

Due to its shape, it has been suggested that the lagoons at Palmyra, before the WWII modifications, could have been analogous to that at Millennium Atoll (pers. comms. Jim Maragos), which is dominated by *Acropora* spp. and characterised by high crustose coralline algal cover, turfing algae and giant clams (*Tridacna maxima*) (Barott et al. 2010). Supporting this hypothesis, a large number of old *T. maxima* shells remain

in the Palmyra lagoons today (authors pers. obs.). Also reports of the pre-military condition of the atoll state the presence of coral reefs in the lagoon (Woodbury et al. 1946, Anon. 1947) with the serviceable runway on Cooper Island (**Fig 3.1**) made up of the corals removed from the lagoon (Anon. 1947).

Figure 3.1 Location of the recruitment panels in the lagoons at Palmyra Atoll: Strawn Island (St), The Dolphins (D) and Quail (Q) and semi-permanent quadrats (D). The arrows indicate the location of military modifications; 1 = excavated entrance channel, 2 = the airstrip made of dredge spoil and coral rubble and 3 = the north-south causeway, specifically the cut allowing boat access between the Center and East Lagoons. CI = Cooper Island.

3.3.2 Settlement panels

In a preliminary trial, five ceramic settlement panels were placed at The Dolphins and three by Strawn Island in October 2008 (**Fig 3.1**). After observing the successful settlement of sponges, in July 2009 twelve new panels were placed at 6–8 m at The Dolphins, thirteen by Strawn Island and fourteen by Quail Island, and observed in October 2009 and July 2010. Each settlement panel was 12 x 12 cm with a 1 cm hole in the centre. The panel was placed over a stainless steel nail hammered into the hard substrate (secured with marine epoxy). A cable tie was placed around the top of the nail to secure the panel but was cut-off and replaced with each observation. This loose fixing allowed for the area under the panel to remain accessible to larvae. The top and underside surface of the panels and the substrate underneath were photographed, with

close-up photographs taken to aid species identification. The sponge density was recorded on each side of panels, which was estimated from the photographs; sponges on the edges of the panel were not recorded. Where possible, the panel was placed in areas devoid of sponges, however this was not always possible. Therefore those species found initially under the panels were removed from the total recruits counted as they were considered as overgrowth species. Sponge identification is difficult and even more so for small newly recruited individuals on settlement panels, so those species that I could not identify to genus were grouped in an ‘other’ group.

3.3.3 *Semi-permanent quadrats*

Three sets of 5 x 1 m² semi-permanent quadrat stations were established at 6–8 m depth at The Dolphins in July 2008 (total area of 15 m²) (**Fig 3.1**). The quadrat stations were semi-permanent so any trace of them could be removed at the end of the study. The three stations were measured five times over three years in July 2008, October 2008, July 2009, October 2009 and July 2010. They were marked by metre long stainless steel rods hammered into the soft sediment. The rods delineated the 2 corners of where the quadrat should be placed with the bottom corner of one quadrat identifying the top of the next quadrat. The quadrat was 1 m², subdivided into 25 20 x 20 cm sub-sections, and placed over the rods to estimate the sponge cover and density of each species and estimate the available hard substrate. If a sponge extended beyond the quadrat, only the area in the sub-section was included, and if a sponge extended into an adjacent sub-section the area cover was estimated independently in each. The cover and density data were subsequently adjusted to the percentage of available substrate to enable direct comparisons between sites by accounting for the variability in overall boulder densities. The observations for this study focused on species on the upper surface of the boulders as opposed to those found underneath boulders (see Bell & Carballo 2008). Bioeroding species were not included because of difficulties making accurate estimates of area cover. Species were identified by examining phenotypic characteristics, spicule preparations and sections (**Table 3.1**).

Table 3.1 List of species found in the semi-permanent quadrats (Q) and on the recruitment panels (P) as marked by ticks. The ID letter refers to those given in Chapter 2.

ID	Q	P	List	Genus	Family	Suborder	Order	Reference
A	✓	✓	<i>cf. Aplysilla rosea</i>	<i>Aplysilla</i>	Darwinellidae	n/a	Dendroceratida	Barrois, 1876
B	✓		<i>Dysidea</i> sp.1	<i>Dysidea</i> sp.1	Dysideidae	n/a	Dictyoceratida	Johnston, 1842
C	✓	✓	<i>Dysidea</i> sp.2 cf. <i>etherea</i>	<i>Dysidea</i> sp.2	Dysideidae	n/a	Dictyoceratida	Johnston, 1842
E	✓	✓	<i>Scopalina</i> sp.	<i>Scopalina</i> sp.	Dictyonellidae	n/a	Halichondrida	Schmidt, 1862
F	✓		<i>Halichondria</i> sp.1	<i>Halichondria</i> sp.1	Halichondriidae	n/a	Halichondrida	Fleming, 1828
G	✓		<i>Halichondria</i> sp.2	<i>Halichondria</i> sp.2	Halichondriidae	n/a	Halichondrida	Fleming, 1828
H	✓		<i>Hymeniadidon</i> sp.1	<i>Hymeniadidon</i> sp.1	Halichondriidae	n/a	Halichondrida	Bowerbank, 1858
I	✓		Halichondriidae sp.1	unknown	Halichondriidae	n/a	Halichondrida	Gray, 1867
J	✓		Halichondriidae sp.2	unknown	Halichondriidae	n/a	Halichondrida	Bowerbank, 1858
K	✓		<i>Chalinula</i> sp.	<i>Chalinula</i> sp.	Chalinidae	Haplosclerina	Haplosclerida	Schmidt, 1868
L	✓	✓	<i>Haliclona (Gellius) caerulea</i>	<i>Haliclona</i>	Chalinidae	Haplosclerina	Haplosclerida	Hechtel, 1965
M	✓		<i>Haliclona</i> sp.1	<i>Haliclona</i> sp.1	Chalinidae	Haplosclerina	Haplosclerida	Grant, 1836
N	✓		Challyspongiidae sp.	unknown	Challyspongiidae	Haplosclerina	Haplosclerida	de Laubenfels, 1936
O	✓	✓	<i>Gelliodes fibrosa</i>	<i>Gelliodes</i>	Niphatidae	Haplosclerina	Haplosclerida	Wilson, 1925
R	✓		<i>Acarus</i> sp.1	<i>Acarus</i> sp.1	Acaridae	Microcionina	Poecilosclerida	Gray, 1867
S	✓		<i>Acarus</i> sp.2	<i>Acarus</i> sp.2	Acaridae	Microcionina	Poecilosclerida	Gray, 1867
T	✓	✓	Chondropsidae sp.	unknown	Chondropsidae	Myxillina	Poecilosclerida	Carter, 1886
U	✓	✓	<i>Iotrochota protea</i>	<i>Iotrochota</i>	Iotrochotidae	Myxillina	Poecilosclerida	Ridley, 1884
W	✓	✓	<i>Clathria</i> sp.	<i>Clathria</i> sp.	Microcionidae	Microcionina	Poecilosclerida	Schmidt, 1862
X	✓		<i>Hexadella</i> sp.	<i>Hexadella</i> sp.	Ianthellidae	n/a	Verongida	Topsent, 1896
AA	✓	✓	<i>Darwinellidae</i> sp.	unknown	unknown	Darwinellidae	Dendroceratida	Merejkowsky, 1879
AD	✓		Suberitidae sp.1	unknown	Suberitidae	n/a	Hadromerida	Schmidt, 1870
AG	✓	✓	Halichondriidae sp.3	unknown	Halichondriidae	n/a	Halichondrida	Bowerbank, 1858
AL		✓	Poecilosclerida sp.	unknown	unknown	unknown	Poecilosclerida	Topsent, 1928

3.3.4 Statistical analyses

All multivariate temporal assemblage data were dispersion weighted (based on 1000 permutations), which is suitable for species prone to spatial clustering, such as sponges because it differentially weights species depending on their observed variability between samples (Clarke et al. 2006a), while the univariate total sponge cover was $\log(X+1)$ transformed. Similarity matrices were based on zero-adjusted Bray-Curtis coefficients to account for denuded data (Clarke et al. 2006b), which was characteristic of a number of the quadrats and panels. Permutational multivariate analysis of variance (PERMANOVA) (based on 9999 permutations) (Anderson 2001, Clarke et al. 2006b) and pairwise *post-hoc* tests were used to examine differences in sponge assemblages and total cover. The PERMANOVA design for the semi-permanent quadrats examined sponge densities and cover at 2 fixed factor levels: ‘year’ (2008, 2009 and 2010) and ‘season’ (July and October). The settlement panel analysis examined sponge densities with 3 fixed factors: ‘year’ (2009 and 2010), ‘side’ (top and underside) and ‘site’ (Dolphins, Strawn and Quail) nested in year (**Table 3.2**). The effect of all factors and their interactions were tested by a permutation of the residuals under a reduced model (max. 9999) with Type III (partial) sums of squares. Canonical Analysis of Principal components (CAP) were used to visualise the PERMANOVA results (**Fig 3.2**).

Table 3.2 Results from the permutational multivariate analysis of variance (PERMANOVA) for total cover (univariate), assemblages (multivariate), individual species (univariate) and diversity (Hills N1) (semi-permanent quadrats only) for the semi-permanent quadrat sponge densities (a) and cover (b) over time (seasons and years) and recruitment panel densities (c) between sites, years and sides (top or underside) with p values followed by Pseudo-*F* values in brackets. Figures in bold indicate significant differences. ^a Term has one or more empty cells.

	Factor	df	Total	Assemblage	<i>H. caerulea</i>	<i>G. fibrosa</i>	<i>I. protea</i>	Hills N1
(a)	Season	1	0.1293 (2.36)	0.0908 (1.77)	0.9295 (0.03)	0.4346 (0.62)	0.9225 (0.04)	0.2637 (1.26)
	Year	2	0.1661 (1.85)	0.0332 (1.89)	0.1698 (1.80)	0.2486 (1.39)	0.9542 (0.09)	0.0166 (4.29)
	Year x Season ^a	1	0.5414 (0.38)	0.5806 (0.80)	0.7917 (0.11)	0.7639 (0.13)	0.9934 (0.00)	0.4711 (0.56)
	Residuals	70						
	Total	74						
(b)	Season	1	0.4353 (0.62)	0.6426 (0.70)	0.5317 (0.41)	0.7426 (0.14)	0.9905 (0.01)	
	Year	2	0.9919 (0.024)	0.2197 (1.29)	0.2055 (1.62)	0.505 (0.70)	0.9491 (0.09)	
	Year x Season ^a	1	0.3965 (0.75)	0.6771 (0.67)	0.1829 (1.83)	0.7866 (0.10)	0.8908 (0.06)	
	Residuals	70						
	Total	74						
(c)	Year	1	0.0001 (37.66)	0.0001 (10.53)	0.6910 (0.18)	0.0162 (4.75)	0.0003 (6.53)	
	Side	1	0.0001 (237.27)	0.0001 (34.22)	0.0001 (29.84)	0.0009 (8.41)	0.3138 (1.11)	
	Site (Year)	4	0.2498 (1.37)	0.0447 (1.67)	0.2306 (1.40)	0.0843 (1.99)	0.0032 (4.01)	
	Year x Side	1	0.0001 (16.59)	0.0001 (7.87)	0.6938 (0.18)	0.0174 (4.75)	0.3090 (1.11)	
	Site (Year) x Side	4	0.1245 (1.82)	0.2021 (1.26)	0.2310 (1.40)	0.0906 (1.99)	0.0341 (2.33)	
	Residuals	140						
	Total	151

Figure 3.2 Canonical analysis of principal components (CAP) ordination visualising sponge assemblage differences over years showing non-significant differences in the semi-permanent quadrat a) densities and b) cover, respectively and significant differences ($p < 0.001$) in c) sponge densities on recruitment panels based upon a zero-adjusted Bray-Curtis similarity matrix with a dispersion weighting pre-treatment.

The abundance data of three important species (univariate data) as identified in Chapter 2 were also $\log(X+1)$ transformed and analysed using the same PERMANOVA designs as described above. These species were *Haliclona caerulea* (also known as *Haliclona (Gellius) caerulea*, *Haliclona (Soestella) caerulea* or *Sigmatocia caerulea*) and *Gelliodes fibrosa*, which are two confirmed non-indigenous species introduced to Palmyra (see **Appendix I**) and Hawai'i (DeFelice et al. 2001) from the Caribbean and the Philippines, respectively, and *Iotrochota protea* (de Laubenfels 1950), which is the dominant species in the lagoons at Palmyra and native to Hawai'i. It is unknown, however, whether this latter species was found on Palmyra prior to the WWII modifications.

Changes in species diversity (an index based on richness and evenness) were examined in the semi-permanent quadrats using the same PERMANOVA designs as described above, using the Hills N1 index (exponential of the Shannon Wiener function), which is sensitive to changes in rare species and therefore appropriate to examine introductions or losses of new species to the quadrats over time. All analyses were conducted using PRIMER v6 (Clarke & Gorley 2006) and the PERMANOVA+ add-on package (Anderson et al. 2008).

3.4 Results

3.4.1 Sponge recruitment variability

In total, 11 species were identified on the settlement panels (**Table 3.1**) in addition to the 'other' group. All but one (*Poecilosclerida* sp.) of the identified species on the panels were also found in the quadrats (**Table 3.1**). Total sponge abundance, per panel, on average increased from 2 (± 5) individuals to 6 (± 8) between 2009 and 2010 and per site in total increased by 137% from 59 (± 35) individuals in 2009 to 140 (± 25) in 2010. Interspecific recruitment was highly variable (**Fig 3.3a**) with sponge assemblages becoming more dissimilar from each other over time (**Fig 3.2**). Sponge settlement was also predominantly to the underside of the panels with an average of 0.3 (± 0.9) individuals on the top and 7.6 (± 7.8) on the underside (**Fig 3.3**).

Figure 3.3 Mean ($\pm$ SD) sponge abundance (no. of individuals) across sites, panel side and year for all species (a), non-indigenous species (b and c), and the dominant species (d) in the lagoons at Palmyra.

Total sponge abundance (number of individual recruits) did not differ between sites, but differed between years and panel side (**Fig 3.3**), with a significant interaction between these two factors (**Table 3.2**) as sponge density increased significantly between years predominantly on the underside of the tiles. Significant differences in sponge assemblages were found between all factors (site, year and side) with a significant ($p < 0.05$) interaction between side and year. Further pairwise analysis revealed that Quail (East Lagoon) had significantly different assemblages to The Dolphins (West Lagoon) ($t = 1.52$, $p = 0.035$).

Recruitment levels differed between species (**Fig 3.3**), with *Iotrochota protea* recruits only establishing to the underside of the panels in 2009 and increasing over time, while recruitment to the top of the panels only occurred in 2010. Recruitment events were obvious on the panels as individual patches were often not close to each other, which would be the case if they formed from fragmentation. Overall, *I. protea* had significantly higher ($p < 0.05$) abundance in 2010 versus 2009 and overall significantly ($p < 0.05$) higher densities at Strawn versus The Dolphins ($t = 2.27$, $p = 0.017$); however there was also a significant ($p < 0.05$) interaction between site and side (**Table 3.2**). An interaction between sites and sides here is indicative of sponge recruitment levels differing between panel sides at the different sites. *Haliclona caerulea* and *Gelliodes fibrosa* densities were significantly ($p < 0.05$) higher on the underside of the panel versus the top, with *G. fibrosa* only recruiting to panels at two sites (The Dolphins and Strawn Island) (**Fig 3.3**). *Haliclona caerulea* and *G. fibrosa* did not recruit to the top of the panels but had mean densities of $0.8 (\pm 1.7)$ and $0.1 (\pm 0.5)$, respectively, on the underside of each panel. *Gelliodes fibrosa* densities also increased significantly between 2009 and 2010, but significantly ($p < 0.05$) interacted with site.

3.4.2 Temporal variability in sponge assemblages

In total, 23 species were identified in the quadrats at the 3 stations over the 3 years (**Table 3.1**). Mean total sponge cover was $19.1\% (\pm 9.7)$ while densities were $99.0 (\pm 41.9)$. Sponge assemblages did not differ significantly between years or seasons (**Fig 3.2 and Table 3.2**), but species diversity (**Fig 3.4 and Table 3.2**) was significantly lower in 2010 (3.7) compared with 2008 (4.6) ($t = 2.56$, $p = 0.013$) and 2009 (4.8) ($t = 2.45$, $p = 0.016$). In addition, no significant differences were found between the cover and densities of the non-indigenous and dominant species (important notable species)

over time, despite an apparent decline in cover in *Haliclona caerulea* and *Gelliodes fibrosa* between July 2009 and July 2010.

Figure 3.4 Mean ($\pm$ SD) sponge species diversity in the quadrats over 3 years; based on the Hills N1 index (exponential of the Shannon Wiener function).

3.5 Discussion

Remote and uninhabited atolls and islands support some of the last remaining near-pristine reefs in the world (Knowlton & Jackson 2008) but a number of the Pacific islands that support some of these ecosystems were altered by the US military during WWII (Maragos 1993). Palmyra Atoll is an example of a military modified near-pristine system where currently in the lagoons sponges, not corals, dominate the hard substrate (see **Chapter 2 and Appendix I**). One of the concerns regarding the sponges in the lagoons is their potential to extend to the adjacent near-pristine reef. I found that the sponges were capable of recruiting to newly available substrate but there were no significant differences in sponge cover or densities (number of individual sponges) on boulders over time, indicating that mortality and recruitment of sponges are in equilibrium. Therefore the sponges have the potential to extend onto the reef via larval dispersal. The sponge assemblages and important notable species, excluding *Iotrochota protea*, were also significantly more abundant on the underside of the panels indicating that the majority of sponge species recruit to cryptic locations and then growth allows them to colonise the tops of boulders as they establish.

3.5.1 Sponge recruitment

Sponge abundance on the panels increased and assemblages changed significantly over time and between locations, which is consistent with earlier studies (Uriz et al. 1998, Fairfull & Harriott 1999, Vermeij 2006). This indicates that the sponges are reproducing and/or fragmenting and recruiting in the lagoons and are therefore capable of extending onto the reef. When examining the important species, *I. protea* and *Gelliodes fibrosa*, their abundance increased significantly over time whilst *Haliclona caerulea* did not. *Haliclona caerulea* in the Caribbean releases larvae between July and September (peaking in August-September) (Maldonado & Young 1996). Therefore when the tiles were examined for the 2nd time in July 2010, at the beginning of the reproductive season (1st time in October 2009), it is unlikely that even if the larvae settled on the panels they would have grown large enough to be visible. Unfortunately nothing is known of the larval release times for *I. protea* and *G. fibrosa*; however, it is possible that they spawn at different times (Bergquist & Sinclair 1973, Zea 1993) to *H. caerulea* resulting in the different growth patterns observed over the time of the study. They obviously release larvae in the summer as they were observed in the 1st collection (October 2009) but may have a different peak spawning time to *H. caerulea*, perhaps in the winter months between the sampling periods, or they may spawn all year round, although further recruitment studies are required to establish this fact. This temporal interspecific variability in recruitment patterns is noted across all the species identified on the panels (**Fig 3.4a**), so it appears likely that there may be seasonal differences in larval release times for all sponges (Bergquist & Sinclair 1973, Zea 1993, Mariani et al. 2005) including those found in the lagoon at Palmyra.

Spatial variability was also observed for *I. protea*, which had significantly lower densities at The Dolphins compared to Strawn Island. Chapter 2 indicated that across the lagoons *I. protea* covered approximately 20% of the available hard substrate at Strawn Island and only 5% at The Dolphins. Therefore the lower number of conspecifics at The Dolphins most likely led to the reduced recruitment to the panels here because the recruitment stock for larvae is smaller compared to Strawn Island (Uriz et al. 1998). A significant interaction between site and side may belie this result; however it appears that the levels of successful recruitment of *I. protea* at Quail Island were similar on both sides of the panels over time (**Fig 3.3d**) but not at Strawn Island or The Dolphins. This suggests that under different conditions *I. protea* may or may not settle more in cryptic locations.

Recruitment to the underside of the panels was also significantly greater than to the tops, with the exception of *I. protea*. In 2010 there was however, an increase in recruits the top surface of the tiles, due perhaps to the build up of a biofilm here, which is preferentially settled on by sponge larvae when compared to smooth surfaces (Kjerfve 1986, Whalan et al. 2008). Settlement to the underside of surfaces is however more common in sessile invertebrates because the larvae are predominantly negatively phototactic (Hurlbut 1992, Maldonado 2006). Cryptic surfaces also provide protection from predators (Pawlik 1998), reduced sedimentation (Maida et al. 1994), UV radiation (Jokiel 1980) and algal biomass (Vermeij 2006). I propose that the majority of those species recorded on both the underside of the panels and in the quadrats (species found on top of boulders) initially settle in these cryptic locations and then grow or fragment to the top of the boulders (Pawlik 1998).

3.5.2 Temporal variation in sponge assemblages

This study found no inter- or intra-annual variation in assemblages or total cover over three years, supporting some earlier studies (Fowler & Laffoley 1993, Garrabou & Zabala 2001). However, species diversity did change over time and sponges successfully recruited to panels, suggesting that mortality and recruitment of sponges in the lagoons are in equilibrium. This equilibrium of sponge assemblages suggests that the sponge populations are self-sustaining and maintained in the lagoons.

Variability in sponge assemblages over time have been attributed to both environmental and biological parameters (Bell et al. 2006, Carballo et al. 2008, Berman 2012). Therefore, the non-significant temporal changes in the sponge assemblages may be due to the lack of environmental and seasonal variation in the lagoons (see Gardner et al. 2011). It is unknown why there would be a drop in sponge diversity in 2010 compared to the other years and it may be due to natural biological variation. Alternatively, the reduction in sponge diversity may be in response to an El Niño-Southern Oscillation (ENSO) event which reached Palmyra in late 2009 and averagely raised water temperatures by 2 °C resulting in a mild coral bleaching event across the atoll (Williams et al. 2010). This rise in water temperature may therefore have subsequently affected the sponge diversity in the lagoon; however, little is known of the thermal tolerance or ecological effects of ENSO events on sponges but they may be important in shaping their assemblages (Carballo 2006). Annual seasonal temperature changes can result in a reduction in sponge body size and the formation of silica and

spongin skeletons (Simpson 1978, Francis 1984, Bavestrello et al. 1993) with warmer temperatures reducing silica uptake and spiculogenesis (Hartman 1958, Stone 1970, Hooper 1991). Therefore, the different thermal tolerances of sponges may have resulted in different diversities in 2010 compared to the other years. The temperature change however, was not sufficiently high enough to alter the sponge assemblages, which suggests a possible reduction in the number of species and/or abundance of the species rather than a shift in assemblages over time.

3.5.3 Evidence of a phase-shift in the lagoon

The overall equilibrium between sponge recruitment and mortality over time suggests that a) the sponges are capable of recruiting to boulders on the reef and b) they are self-sustaining in the lagoon, possibly indicating that the lagoon has experienced a phase-shift away from the original coral reef community, as purported in pre-military modification reports (Woodbury et al. 1946, Anon. 1947). A phase shift occurs when there has been a sustained change in the environment due to pulse perturbations, which were transient but large in impact and scale. These perturbations result in the removal of the original species, which largely maintained the system, and are replaced with new individuals, which are self-sustaining and maintained in the newly altered ecosystem (Petraitis & Dudgeon 2004).

Establishing that the lagoons at Palmyra have undergone a phase shift may require further experimental research to confirm this hypothesis, such as coral transplantations from the reef to the lagoon. A phase-shift assumes a new single state (e.g. environmental change or predator loss) different from that of the original, which is unlikely to change compared to an ‘alternate stable state’, which implies that the at least two assemblages are changeable and both can occur in the same environmental conditions but just not at the same time (Done 1992, Edmunds & Carpenter 2001, Petraitis & Dudgeon 2004). However, from the evidence so far it appears likely that the lagoons at Palmyra did experience a phase shift from a coral to sponge dominated system based on the history of modifications and the current evidence of the sponge assemblages and the limited presence of coral species in the lagoons (Williams et al. 2008). The alterations to the lagoons at Palmyra were made over an 18–24 month period nearly 70 years ago (Dawson 1959) and the changes altered the lagoon sufficiently that it has not returned to an ecosystem represented by corals and is still

currently characterised by low water flow, high sedimentation and boulders ($<0.5 \text{ m}^2$), and the sponges appear self-sustaining in this modified environment.

Unlike phase shifts from coral to algal dominated systems, which are often, but not always, controlled by top-down dynamics (e.g. overfishing) (Hughes et al. 2007), shifts to sponge dominance appear to be associated primarily with bottom-up effects (e.g. mass coral bleaching, disease and water quality) (Holmes 2000, Ward-Paige et al. 2005); they are also attributable to top-down controls (spongivory) (Hill 1998).

This is the first study to observe a coral to sponge phase shift in response to substantial human influence in the form of a top-down impact, which removed the reef forming corals from the lagoon. However, bottom-up effects may be preventing the return of the corals to the lagoon because of the altered environmental conditions resulting from the military modification (Dawson 1959, Maragos 1993). Although it is difficult to establish what the conditions were like before the naval base was built the water flow was altered dramatically (Collen et al. 2009) which may have altered the water clarity, nutrient levels and sedimentation, possibly making it unsuitable for coral larval settlement and growth and more suitable for sponges (Rose & Risk 1985, Ward-Paige et al. 2005). The sponges may also be directly impeding the return of corals through competition for space (de Voogd et al. 2004) and recruit overgrowth (Vermeij 2006); however if the environmental conditions are not suitable for coral growth, the direct affect of sponges may be negligible. Also, as sponges have the potential to remove vast quantities of particulate matter and nutrients from the water (Reiswig 1974, Ribes et al. 1999) they may in fact be having a positive impact on the water quality (Peterson et al. 2006) in the lagoon.

Currently, without further testing, the evidence for a phase shift in the lagoons at Palmyra is compiled from the history of the modifications and the results from this study indicating that the sponges appear to be self-sustaining, supporting the notion that the lagoons have shifted from a coral to sponge dominated fauna on the hard substrate. It also appears likely that the corals will not return to the lagoon in the near future because in the last 70 years they have not yet successfully reseeded the lagoon despite the adjacent near-pristine reef acting as a source of viable coral larvae. However, it also seems unlikely that the sponges will extend on to the adjacent reefs and will remain in this altered ecosystem.

Filtering capability of sponges in an altered atoll lagoon and their potential impact on an adjacent near pristine reef.

4.1 Abstract

Sponges process large volumes of water to extract nutrients and food. Therefore, when their densities are high they can have a considerable influence on food nutrient availability for other organisms. At Palmyra Atoll, the reefs are near-pristine, but the lagoons were heavily modified during WWII and now sponges are the dominant fauna on the remaining hard substrate. Tidal-driven water from the lagoon, which has been filtered by the sponges, flows out over the adjacent reef. The aim was to assess the turnover rate of the lagoon water by the sponge assemblages, and determine their influence on oxygen and dissolved organic carbon (DOC) in the water. In 2010, the pumping rates (volume flux per unit area) of six representative species inhabiting the lagoon were measured. The six species were then broadly placed into one of two morphological groups: encrusting and massive. This information was combined with data from the literature on sponge nutrient consumption rates to estimate the effects that sponges might be having on the water column. Excurrent flow speed from the sponges was measured *in situ* using fluorescein dye, an underwater camera system and image analysis software. No significant difference was found in volume flux per unit volume of sponge between massive and encrusting morphotypes at $0.06 \pm 0.01 \text{ cm}^3 \text{ s}^{-1} \text{ cm}^3$. Across the lagoons the sponges filter approximately $5.2 \text{ m}^3 \text{ H}_2\text{O sec}^{-1}$ or $389,892 \text{ m}^3 \text{ H}_2\text{O day}^{-1}$, and have the potential to filter all of the moving water every 3 to 6 days. Extrapolations based on the filtering rates of water revealed that sponges in the lagoons consume up to 19% of the DOC and 8% of the oxygen in the lagoon and therefore appear to be a sink for DOC, but do not remove considerable amounts of oxygen likely to cause concern for other species. I conclude that in altered atoll lagoons like Palmyra, where sponges are in high densities, they may have a considerable impact on the water column but the effects on reef organisms is likely to be small.

4.2 Introduction

Coral reefs are one of the most economically important and ecologically diverse but threatened ecosystems in the world (Moberg & Folke 1999, Knowlton 2001, Bellwood et al. 2004, Hoegh-Guldberg et al. 2007) and remote and uninhabited Pacific atolls and islands are amongst the last remaining quasi-pristine reef systems. However, despite their current lack of human habitation remote reefs are still at risk from external perturbations such as coral bleaching events, disease outbreaks, fishing, poaching and ship wrecks (see Maragos et al. 1996, Work et al. 2008, Williams et al. 2010, Williams et al. 2011a, Kelly et al. 2012). A number of these atolls and islands were also modified during WWII by the US military (Stoddart 1968, Maragos 1993), removing native species and habitat, and introducing non-indigenous species particularly to the islets and lagoons (Flint 1992, Eldredge 1994; Appendix I). Due to the connection that atoll lagoons often have with their adjacent reefs it is important to understand the impacts that any introduced species might have on the reef, either directly through competition with the corals by extending out of the lagoon or indirectly by influencing the water column, which subsequently flows out onto the reef.

Water quality is important to coral reef health (Fabricius 2005, De'ath & Fabricius 2010). Poor water quality increases mortality rates of corals through smothering and erosion, alters the chemical composition of the seawater, results in hypoxia (oxygen depletion) and encourages microbial and algal growth (Rogers 1990, Fabricius et al. 2005, Kline et al. 2006, Dinsdale et al. 2008). Nutrients such as nitrate, phosphate and ammonia are routinely measured as components of water quality; however, these may not directly result in substantial coral mortality, though hypoxia and high levels of dissolved organic carbon (DOC) can be fatal to corals (Simpson et al. 1993, Kuntz et al. 2005, Kline et al. 2006). DOC is the largest reservoir of carbon in the oceans and makes up the greater portion of total organic carbon (TOC), with the remaining fraction made up of particulate organic carbon (POC) (Martin & Fitzwater 1992, Benner 2002). Microbes are large consumers of DOC; therefore, high concentrations of this carbon source can increase bacterioplankton production resulting in localised hypoxia and coral death (Kuntz et al. 2005, Kline et al. 2006, Smith et al. 2006). High DOC can also disrupt the balance between the coral host and its associated microbiota (archaea, bacteria and viruses) in the coral mucus layer (Kline et al. 2006) resulting in increased susceptibility to coral disease or mortality (Segel & Ducklow

1982, Knowlton & Rohwer 2003). DOC is derived from primary producers, such as algae and mangroves, and is a major food source for suspension feeders (Khailov & Burlakova 1969, Roditi et al. 2000, Yahel et al. 2003, Dittmar et al. 2006, de Goeij et al. 2008).

Benthic suspension feeders, including sponges, are important and successful components of coral reef systems, primarily because of their ability to process large quantities of water and remove organic and inorganic matter and nutrients from the water column (Gili & Coma 1998). The high filtration rate of benthic suspension feeders means that when densities of individuals are high, substantial impacts on an ecosystem and its inhabitants can occur. This is particularly evident in partially enclosed bodies of water such as bays and lagoons, where benthic suspension feeders can dramatically reduce nuisance phytoplankton blooms and eutrophication through water filtration (Cloern 1982, Officer et al. 1982, Hily 1991, Gili & Coma 1998). The suspension feeders can also have this dramatic impact on these semi-enclosed locations because the time the water is in the lagoon or bay (residence time) is longer than the time taken by the suspension feeders to filter all of the water (clearance time), therefore giving them the opportunity to remove nutrients and particles before the water exits (Grall & Chauvaud 2002).

Research into the regulation of water quality by benthic fauna has primarily focused on molluscs, which have the potential to directly regulate primary production and indirectly secondary production because of their ability to filter and assimilate organic particles in the water column (Cloern 1982, Officer et al. 1982, Kimmerer et al. 1994). Sponges, however, also have strong interactions with the water column (Bell 2008a), filtering large volumes of water of up to 24,000 l kg⁻¹ of sponge day⁻¹ (Vogel 1977), and removing nutrients (e.g. oxygen) and food particles. Sponges primarily feed on DOC (< 0.45 µm) (de Goeij et al. 2008), ultraplankton (< 20 µm) and picoplankton (< 2 µm) (Reiswig 1971a, 1974, Pile et al. 1996, Ribes et al. 1999), as well as bacteria (1–10 µm) (Reiswig 1975, Frost 1978) and possibly viruses (0.03–0.1 µm) (Hadas et al. 2006). Retention rates of food particles by sponges are high, ranging from 58 to 99% (Pile et al. 1997, Lesser 2006, de Goeij et al. 2008), making them very important in linking primary and secondary production (Gili & Coma 1998).

In high densities, sponges can interact with the water column by depleting oxygen levels (Richter et al. 2001), playing key roles in nitrogen, phosphorus (Ribes et al. 2005, Jiménez & Ribes 2007) and silicon cycling (Maldonado et al. 2005), and

consuming large quantities of carbon with 90% of TOC removed in the form of DOC (Yahel et al. 2003, Lesser 2006, de Goeij et al. 2008). However, despite the large impact that sponges can have on ecosystems, studies into the water-clearing role of sponge assemblages on a system are limited, with the majority focussing on individual species (e.g. Savarese et al. 1997, Milanese et al. 2003, Longo et al. 2010). Peterson (2006) did, however, find that the decline in sponge assemblages in Florida Bay was most likely the cause of the subsequent phytoplankton and cyanobacteria blooms. Also Perea- Blázquez et al. (2012) found that sponge assemblages in New Zealand play an important role in water column interactions due to their high rates of particulate organic carbon (POC) removal. Therefore, overall sponge-assemblage removal rates of DOC in semi-enclosed systems could be considerable if the density of individuals is high.

Palmyra Atoll, located in the north Central Pacific, is a tropical system with near-pristine reefs surrounding partially enclosed and modified lagoons (Dawson 1959). Interestingly, while the outer-reefs are mostly devoid of large macro-invertebrates (Williams et al. in review), sponges are the dominant fauna in the lagoons covering 27% ($\pm$ 6%) of the available hard substrate (**see Chapter 2**). Given their abundance, sponges have the potential to exert a considerable effect on the water column in the lagoons through removal of DOC and oxygen. Therefore, given the altered condition of the lagoon, one potential concern at Palmyra is the potential influence of the lagoon outflow onto the adjacent near-pristine reef. Sponges could be reducing oxygen levels but helping to reduce the DOC in the water that flows over the reef, which could potentially cause coral disease and mortality.

In this chapter, I estimate total sponge densities and the filtration rates for all morphologies to determine the turnover rate of the lagoon water by the entire sponge assemblage. Then based on my calculations and data from the literature, I estimate the potential influence that the sponges are having on the DOC and oxygen levels in the lagoon water, which subsequently flows over the adjacent reef.

4.3 Methods

4.3.1 Sponge percentage cover surveys

Sponge cover surveys were completed in 2008 on SCUBA at ten sites across 3 of the lagoons (**Fig 4.1**) at depths between 5–8 m. See **Chapter 2** for further explanation of the sponge survey methods.

Figure 4.1 Location of the 10 survey sites within the 3 lagoons: Sand Island (Sa); Strawn Island (St); Ripple Wharf (RW); Paradise Island (P); The Dolphins (D); The Cut (C); South of the Cut (S.C); Quail Island (Q); Eastern Island (E) and Central East (C.E). The black arrows indicate locations of military modifications; 1 = excavated entrance channel, 2 = the north-south causeway, specifically the cut allowing boat access between the Center and East Lagoons.

4.3.2 *Sponge species and morphologies*

All measurements of sponge filtration rates were taken at sites in West and East lagoon (05° 53.089 N 162° 05.074 W and 05° 53.037 N 162° 04.079 W) at 5 to 8 m depth. The rates were measured for six species representing the four sponge morphologies in the lagoon (**Fig 4.2**): *Clathria* sp. (Schmidt, 1862) (thinly encrusting, < 2 mm); *Spirastrella* sp. (Schmidt, 1868) and *Chondropsidae* sp. (Carter 1886) (thickly encrusting, < 5 mm); *Dysidea* sp. 2 (Johnston, 1842) and *Haliclona* (*Sigmatocia*) *caerulea* (Hechtel, 1965) (massive); and *Halichondria* sp.1 sp. (Fleming, 1828) (digitate). Three to four individuals were sampled per species with three to four oscula recorded per individual, giving a total of 74 oscula measured across all sponges. The six sampled species represented all morphologies in the lagoon and therefore all species, making it possible to extrapolate to the total volume flux by all sponges across the lagoons.

Figure 4.2 Photos of the 6 species filtration rates were calculated for *in situ* in the West and East lagoons. a) *Clathria* sp. (thinly encrusting); b) Chondropsidae sp. and c) *Spirastrella* sp. (thickly encrusting); d) *Dysidea* sp. (with dye stream) and e) *Haliclona* (*Sigmadocia*) *caerulea* (massive) and f) *Halichondria* sp.1 sp. (digitate).

Permutational multivariate analyses of variance (PERMANOVA) (Anderson 2001, Clarke & Gorley 2006) was used to examine differences between the volume flux per unit area of sponge cover ($\text{cm}^3 \text{ sec}^{-1} \text{ m}^{-2}$) of the four morphological groups to determine whether any morphologies could be combined. Analyses (both the global test and subsequent pairwise comparisons) were based on 9999 random permutations of the raw data and zero-adjusted Bray-Curtis similarity matrices, and completed using PRIMER v6 (Clarke & Gorley 2006) and the PERMANOVA+ add-on (Anderson et al. 2008). No pre-weighting was applied to the raw data because the values were based on

sum values over multiple species. On the basis of this analysis, the four morphological groups were reduced down to two as the PERMANOVA analysis revealed significant differences between the encrusting species and the massive/digitate species but not within the two groups (**Table 4.1**). The thin and thick encrusting species are termed hereafter as ‘encrusting’ and the massive and digitate species as ‘massive’. The two morphological groupings were used for all subsequent calculations.

Table 4.1 Pairwise *post-hoc* test results indicating significant differences between encrusting and massive/digitate species. a) *Clathria* sp. (thin encrusting); b) *Spirastrella* sp. and c) Chondropsidae sp. (thick encrusting); d) *Dysidea* sp. and e) *Haliclona caerulea* (massive) and f) *Halichondria sp.1* sp. (digitate).

	Thin (a)	Thick (b)	Thick (c)	Massive (d)	Massive (e)
Thin (a)					
Thick (b)	0.2199				
Thick (c)	0.6128	0.5327			
Massive (d)	0.0001	0.0003	0.0003		
Massive (e)	0.0052	0.0336	0.0339	0.0945	
Digitate (f)	0.0001	0.0001	0.0001	0.7643	0.0652

4.3.3 Filtration measurements

Sponge pumping rates were recorded *in situ* on SCUBA using a solution of fluorescein dye and seawater (2.5 mg: 100 ml) to visualise the excurrent flow velocity (**Fig 4.2d**). Fluorescein dye is a nontoxic fluorescent dye and is commonly used as a tracer to indicate water movement patterns. The dye was released next to the sponge using a 50 ml syringe and filmed leaving the osculum. To improve visibility of the fluorescein dye and minimise the surrounding water flow, each sponge was carefully placed on a shelf in a dark box (with the front and top removed) (**Fig 4.2a**). Next to the sponge, attached to the back of the box, was a scale bar, which was used to record the height of the exhalent flow of dye during video analyses. All selected sponges were between 3 and 25 cm in length and on small moveable boulders, which could be transported to the box without touching the samples. It was important to avoid direct contact and other disturbances before and during filming because jarring/touching sponges can momentarily stop them from pumping for 20–50 seconds (Lawn et al. 1981) to several minutes (Leys et al. 1999). I noted in particular that *Spirastrella* sp. was prone to closing its oscula for an indeterminate amount of time (longer than a

single dive) if disturbed too much. Therefore, all oscula were counted, and measured and the length and width of the sponge were recorded after filming.

All sponges, before filming, were first tested for active pumping by releasing dye near the sponge. If a sponge was not filtering it was replaced and if this occurred for more than three individuals then surveying was postponed, as it was assumed that the sponges were no longer actively filtering water. All dives were conducted in the morning to early afternoon to reduce the potential variability in sponge pumping rates with time of the day. Reiswig (1971a) noted species-specific patterns in filtering cessation times from diurnal to periodic to continuous pumping. I noted a midday reduction of sponge pumping across all species examined as described by Annandale (1907), who presumed that this was because of the increase in temperature associated with this time of day. The cessation of pumping due to an increase in temperature was also found by Leys et al. (1999). To examine temperature change at the experimental site, a pendant HOBO[®] (accuracy $\pm 0.54^{\circ}\text{C}$) (www.onsetcomp.com) was located on a stainless steel pole at 5 m and set to record once every 2 minutes for 15 days (the duration of the research trip). It was observed that the sponges would stop pumping around 14:00 when the water temperature was 29.6°C (± 0.4) and would not drop to below this temperature until around 16:00. Therefore, the calculations were based on the sponges pumping for 21 hours a day.

4.3.4 Filtration calculations

Pumping velocity was calculated by measuring the time it took the dye plume to travel 2 cm once it left the target osculum. All measurements of sponge area cover and osculum diameter were made using ImageJ. Tracker[®] v4.05 (Brown 2004) video analysis software was used to determine the number of frames taken for the dye to travel 2 cm to then calculate the flow speed (cm sec^{-1}) (**Table 4.2**). For both morphological groups (encrusting and massive), the volume flux per unit area of sponge (F) ($\text{m}^3 \text{sec}^{-1} \text{m}^{-2}$) was calculated as

$$F = dls$$

where d is the number of oscula per unit area, l is the oscula area (m^2) and s is the flow speed (m/sec). The sponge pumping rates used in the extrapolation calculations were based on the volume flux per unit area of sponge cover ($\text{cm}^3 \text{sec}^{-1} \text{m}^{-2}$) rather than volume flux per unit volume of sponge ($\text{cm}^3 \text{sec}^{-1} \text{m}^{-3}$). This is because all the surveys estimated sponge area cover (top-down view) not volume. However, to make volume

flux calculations comparable with the literature, I converted volume flux per unit area to per unit volume (**Table 4.2**). To calculate the sponge volumes the sponges were assumed to be cylindrical in shape with an estimated average thickness for each species; volume flux was then calculated per cm³ of sponge.

Table 4.2 Species mean and standard deviation values used to calculate the volume flux per unit area and per unit volume of sponge (cm³). Where n = number of individuals, VF = volume flux.

Species name	n	Morphology	Number of oscula	Oscular area (cm ²)	Sponge surface area (cm ²)	Flow speed (cm s ⁻¹)	VF (cm ³ s ⁻¹)	VF/ unit area of sponge (cm ³ s ⁻¹ cm ²)	VF/ unit volume of sponge (cm ³ s ⁻¹ cm ³)
Clathria sp.	4	Thin enc.	19 (± 7)	0.08 (± 0.04)	147.90 (± 47.3)	4.58 (± 1.07)	0.34 (± 0.16)	0.04 (± 0.02)	0.09 (± 0.03)
Spirastrella sp.	4	Thick enc.	7 (± 3)	0.09 (± 0.04)	130.90 (± 102.7)	5.58 (± 1.54)	0.52 (± 0.28)	0.05 (± 0.08)	0.05 (± 0.01)
Chondropsidae sp.	3	Thick enc.	7 (± 2)	0.14 (± 0.12)	114.20 (± 49.3)	5.06 (± 1.46)	0.81 (± 0.94)	0.05 (± 0.04)	0.05 (± 0.03)
Dysidea sp.	4	Massive	24 (± 11)	0.1 (± 0.08)	59.20 (± 40.4)	5.66 (± 1.56)	0.53 (± 0.42)	0.21 (± 0.11)	0.07 (± 0.02)
Haliclona caerulea	4	Massive	17 (± 8)	0.14 (± 0.13)	124.20 (± 147.3)	5.33 (± 1.71)	0.84 (± 0.93)	0.24 (± 0.27)	0.06 (± 0.05)
Halichondria sp.	4	Digitate	14 (± 8)	0.08 (± 0.06)	19.10 (± 13.8)	4.87 (± 1.60)	0.37 (± 0.33)	0.27 (± 0.24)	0.05 (± 0.04)

Due to the large uncertainty associated with many of the variables in calculating the total flux across the lagoons, propagation of errors was used to enumerate the overall error of the lagoon flux based on the individual errors associated with each measured or inferred variable (Lo 2005, Wu et al. 2006). Given the equation for F , the equation for the standard deviation of volume flux is given as the sum of partial derivatives (∂) of F with respect to its constituent components (**Table 4.3**):

$$\sigma_F^2 = \left(\frac{\partial F}{\partial d} \right)^2 \sigma_d^2 + \left(\frac{\partial F}{\partial s} \right)^2 \sigma_s^2 + \left(\frac{\partial F}{\partial l} \right)^2 \sigma_l^2$$

Therefore the error on the volume flux was calculated according to:

$$\sigma_F^2 = (ls)^2 \sigma_d^2 + (dl)^2 \sigma_s^2 + (ds)^2 \sigma_l^2$$

Where a standard deviation could not be calculated, but appropriate, e.g. sponge area an error of 20% of the mean was assumed.

Table 4.3 Pairwise *post-hoc* test results indicating site differences in a) percentage encrusting species cover b) percentage massive species cover and c) percentage available hard substrate. Those sites with no significant differences for all variables were grouped together for subsequent analyses. Sites names: Sand Island (Sa); Strawn Island (St); Ripple Wharf (RW); Paradise Island (P); The Dolphins (D); The Cut (C); South of the Cut (S.C); Quail Island (Q); Eastern Island (E) and Central East (C.E).

Encrusting		Sa	St	Rw	P	D	C	S.C	Q	E.I
	St	0.896								
	Rw	0.019	0.001							
	P	0.629	0.466	0.002						
	D	0.061	0.014	0.606	0.042					
	C	0.743	0.905	0.001	0.315	0.006				
	S.C	0.599	0.399	0.012	0.895	0.089	0.258			
	Q	0.759	0.907	0.001	0.305	0.008	0.979	0.246		
	E.I	0.628	0.821	0.000	0.271	0.002	0.955	0.210	0.858	
	C.E	0.550	0.745	0.000	0.274	0.004	0.856	0.212	0.727	0.977
Massive		Sa	St	Rw	P	D	C	S.C	Q	E.I
	St	0.951								
	Rw	0.144	0.093							
	P	0.232	0.299	0.002						
	D	0.216	0.128	0.470	0.006					
	C	0.137	0.165	0.000	0.604	0.001				
	S.C	0.010	0.011	0.000	0.184	0.001	0.084			
	Q	0.942	0.943	0.061	0.401	0.108	0.271	0.015		
	E.I	0.025	0.027	0.000	0.390	0.001	0.252	0.911	0.046	
	C.E	0.443	0.471	0.303	0.028	0.215	0.007	0.000	0.303	0.001
Hard substrate		Sa	St	RW	P	D	C	S.C	Q	E.I
	St	0.000								
	RW	0.028	0.000							
	P	0.003	0.060	0.000						
	D	0.655	0.000	0.003	0.007					
	C	0.112	0.001	0.000	0.045	0.195				
	S.C	0.555	0.013	0.009	0.098	0.788	0.583			
	Q	0.130	0.079	0.002	0.444	0.212	0.701	0.457		
	E.I	0.042	0.579	0.001	0.738	0.065	0.215	0.154	0.435	
	C.E	0.067	0.311	0.001	0.964	0.101	0.361	0.244	0.627	0.755

4.3.5 Estimating lagoon area and volume

The number of site groups (**Fig 4.3**), based on the original sponge survey sites (**Fig 4.1**), was reduced to make the estimation of sponge and boulder cover across the lagoon simpler and more accurate. PERMANOVA analyses determined that the 10 survey sites could be reduced down to 6 (**Fig 4.3, Table 4.3**) based on non-significant differences in all of the 3 factors: percentage cover of encrusting sponges, percentage cover of massive sponges and percentage available hard substrate. The area occupied by sponges (m²) for each group (1–6) (**Fig 4.3**) was determined by the vicinity of the

group to the original sponge survey sites (Sa, St, Rw etc) (**Fig 4.1**) with estimates extended across the lagoons to areas of similar characteristics (**Fig 4.3**). Average available hard substrate was calculated from habitat surveys conducted in 2010 (see **Chapter 2**). The encrusting and massive sponge covers for each site were calculated as covering a proportion of the available hard substrate; for example, site 1 (**Fig 4.3**) had a total area of 100,741 m² and 23% (23170 m²) of it was hard substrate and 26% of this hard substrate was covered by encrusting sponges (6023 m²) and 4% (926 m²) by massive species.

Figure 4.3 The site values and groupings based on the PERMANOVA analyses from Table 5.3 (similarity in percentage cover of encrusting and massive species and available hard substrate). Sites names: Sand Island (Sa); Strawn Island (St); Ripple Wharf (RW); Paradise Island (P); The Dolphins (D); The Cut (C); South of the Cut (S.C); Quail Island (Q); Eastern Island (E) and Central East (C.E).

The maximum depth of the lagoons was 52 m but sponge cover estimates were made from 2 to 20 m because sponges were rarely seen shallower than 2 m (and most sites had drop-offs from the lagoon flats), and boulder and sponge densities were sparse beyond 20–25 m (author's pers. obs.). Sponges can inhabit locations below thermoclines and in low oxygen conditions, but at reduced abundances (Bell & Barnes 2000c, a) , so it was assumed that most of the sponge assemblage was in these

shallower depths since oxygen levels are low in the deeper parts of the lagoon (Gardner et al. 2011). Based on the measurements of the proportion of available habitat and percent cover of encrusting and massive species at each of these sites the overall volume flux, F^T is given by

$$F^T = F^E \left(\sum_{s=1}^{s=6} A^s H^s E^s \right) + F^M \left(\sum_{s=1}^{s=6} A^s H^s M^s \right)$$

where F^E and F^M are the volume flux for encrusting and massive species respectively, A is the area of each site, H is the % available habitat, E and M are the % cover of encrusting and massive species respectively and superscript s denotes the site label.

The depth ranges used in the water volume calculation were 0 m to: 20 m, 30 m and ≈ 50 m. Water volume down to 20 m was examined because it is the depth limit known to be colonised by sponges and 52 m was the maximum depth in the lagoons. Thirty meters was included as a middle depth because across the lagoons at Palmyra, vertical water mixing below 30 m is reduced, with low to zero dissolved oxygen, increased turbidity and relatively stable temperature, salinity and density below this depth (Gardner et al. 2011). Therefore, it is possible that the sponges have access to this water, but below 30 m water movement is reduced. The lagoon has a maximum tidal range of 1.18 m therefore the volume was calculated as the median of this (0.64 m) with an error of 10% included. All area cover and volume estimates were calculated using ArcGIS (**Fig 4.4**). Given the lagoon volume, the clearance time (t) due to sponge pumping was

$$t = \frac{V}{F^T}$$

where V is the volume of the lagoon (m^3), F is the flux per unit area of sponge ($\text{cm}^3/\text{sec}/\text{m}^2$) and T is the area of lagoon habitable by sponges (m^2).

Figure 4.4 Bathymetric maps indicating a) the depth gradient and b) the depth contours for the lagoons at Palmyra, Central Pacific. Area and volume estimates for GIS analysis were based on the outlines (of the 3 largest lagoons) as indicated in this figure.

Several assumptions were made when calculating overall turnover rate of the lagoon water: 1) for an individual sponge all oscula are the same size and have the same density as those measured for flow speed; 2) other species with similar morphologies filter water at the same rate; 3) hydrodynamic conditions in the lagoon restrict water movement to the top 30 m; 4) all sponges filter for 21 hours a day, due to the high temperatures around midday; 5) available hard substrate and sponge cover can be extrapolated out to the entire lagoon down to 20 m.

4.3.6 Oxygen and dissolved organic carbon estimates

While sponges can be heterotrophic or phototrophic, and can derive up to 50% of their nutrition from phototrophic associations (Wilkinson 1983), it was assumed that the sponges in the lagoons were heterotrophic feeders because of the low light conditions. Sponges found on reefs, where phototrophic species typically inhabit, generally experience high light and low organic nutrient levels (Wilkinson 1983) unlike the conditions in the lagoons at Palmyra (McCauley et al. 2012; Chapter 2).

Total available dissolved oxygen in the lagoon was taken from Gardner et al. (2011) and estimated for water down to 30 m; below this depth water mixing is much reduced. Oxygen saturation was averaged at 70% ($\pm 15\%$) because levels went from $\sim 100\%$ down to 50–60% at 30 m. Sponge oxygen consumption rates were taken from de Goeij et al. (2008) at $6.7 (\pm 1.9) \text{ mmol m}^{-2} \text{ h}^{-1}$. Based on these consumption rates the overall oxygen removal by sponges (δ^{O_2}) across Palmyra's whole lagoon is:

$$\delta^{O_2} = R^{O_2} \left(\sum_{s=1}^{s=6} A^s H^s (E^s + M^s) \right)$$

where R^{O_2} is the oxygen consumption rate. Unfortunately, because I was examining area cover of sponges and not volume, the values found in other studies were not comparable because of problems with converting the units. Percentage removal of oxygen by the sponges was estimated for a 21 hour day and calculated based on the volume of water down to 30 m and the sponge cover down to 20 m.

It was not possible to measure dissolved organic carbon (DOC) directly for logistical reasons, so estimates were based on the literature with the same lagoon criteria and calculations as the oxygen levels. Ambient DOC levels were taken from Pagès et al. (1997) and based on the most similar atoll (Haraiki Atoll, Tuamotu archipelago) to Palmyra in size and morphometric characteristics, such as permeability to the surrounding waters (Pagès et al. 1997, Torrèton et al. 2002). Also, mean chlorophyll-*a* levels at Haraiki Atoll are similar at $0.33 \mu\text{g l}^{-1} \pm 0.05$ (Torrèton et al. 2002) compared to Palmyra: $0.36 \mu\text{g l}^{-1} \pm 0.21$ (**see Chapter 2**) and chlorophyll-*a* levels are positively associated to DOC levels (Naumann et al.). The average DOC levels for the lagoon were estimated as $0.83 (\pm 0.07) \text{ mg C l}^{-1}$ (Pagès et al. 1997), which is similar to the average for the other atolls at $0.96 (\pm 0.20) \text{ mg C l}^{-1}$ (Pagès et al. 1997, Torrèton et al. 2002). DOC removal rates by sponges range between 80 and 1800 $\text{mg C m}^{-2} \text{ sponge d}^{-1}$ (Pile 1996, Pile et al. 1997, Gili & Coma 1998). Due to the range in DOC removal rates, I calculated an upper and lower bound for the DOC removed

across the whole lagoon based around DOC removal rates of 80 and 1800 mg C m⁻² sponge d⁻¹ according to

$$\delta^{DOC} = R^{DOC} \left(\sum_{s=1}^{s=6} A^s H^s (E^s + M^s) \right)$$

where R^{DOC} is the DOC removal rate per unit area of sponge.

4.3.7 Lagoon residence time

Delesalle and Sournia (1992) found a linear relationship between lagoon residence time and phytoplankton biomass (based on chlorophyll-*a* levels). Residence time here is defined as “the average time during which a molecule or unit volume of water stays in a lagoon before being flushed out”. Average chlorophyll-*a* levels were taken from Chapter 2 and residence time was calculated using the regression equation in Delesalle and Sournia (1992).

4.4 Results

4.4.1 Lagoon characteristics

The lagoons at mid-tide hold approximately 0.029 km³ of water down to 20 m, 0.043 km³ down to 30 m and 0.066 km³ in total. Total substrate area (sand and boulders) of the lagoons between 2–20 m was 2.42 km² and 3.55 km² in total (**Table 4.4**). The mean number of days ($\pm$ SD) taken for all the sponges in the lagoon (between 2 and 20 m) to filter the water down to 20 m was 2.8 ± 1.1 and down to 30 m 4.1 ± 1.6 , and it would take 6.4 ± 2.4 days for them to filter all the water in the lagoon.

Table 4.4 Approximate lagoon area cover (sand and boulders) (km²) and volume (km³). Measurements made using ArcGIS. *Actual maximum depth across all lagoons is 52m. In bold are overall total values for the lagoons.

Lagoon	Area (km ²)			Volume (km ³)		
	2-20m	2-30m	2-50m*	0-20m	0-30m	0-50m*
West	1.001	1.412	1.904	0.011	0.022	0.041
Center	0.446	0.554	0.664	0.004	0.006	0.011
East	0.975	0.985	0.985	0.014	0.015	0.015
Total	2.423	2.951	3.552	0.029	0.043	0.066

4.4.2 Sponge surveys

The six species sampled were reduced to two groups (encrusting and massive) due to significant differences in volume flux per unit area of sponge cover ($\text{cm}^3 \text{sec}^{-1} \text{m}^{-2}$) for different groups of sponges (Pseudo- $F_{5, 73} = 7.6628$, $p < 0.001$) (**Table 4.1**). Of the 29 species recorded in the survey quadrats, 79% were encrusting species and the remainder were massive/digitate species. Encrusting morphologies dominate the lagoons with an average cover of hard substrate of 21.2% (± 7.0) compared to massive species with an average cover of 4.1% (± 1.9) (**Fig 4.3**).

Significant differences were found between sites for encrusting species cover (Pseudo- $F_{9, 99} = 3.1367$, $p < 0.05$), massive species cover (Pseudo- $F_{9, 99} = 3.7091$, $p < 0.05$) and hard substrate availability (Pseudo- $F_{9, 99} = 5.0289$, $p < 0.001$). Based on the similarities in these three variables, the 10 sites were reduced down to 6 site groups (**Table 4.3**).

4.4.3 Filtration rates

There was no significant difference in the volume flux per cm^3 (unit volume) of sponge between encrusting and massive sponges (Pseudo- $F_{1, 73} = 4.9913$, $p = 0.9834$), with an average rate of $0.06 \pm 0.01 \text{ cm}^3 \text{s}^{-1} \text{cm}^3$ for both morphotypes. For individual species values see **Table 4.2**. However, the volume flux per unit area occupied of encrusting and massive sponge was $4.9 \pm 1.2 \times 10^{-4} \text{ cm}^3 \text{sec}^{-1} \text{m}^{-2}$ and $22.9 \pm 4.5 \times 10^{-4} \text{ cm}^3 \text{sec}^{-1} \text{m}^{-2}$, respectively. The total volume flux of all the sponges between 2–20 m in the lagoon was $137 \pm 50 \text{ cm}^3 \text{sec}^{-1}$, which equates to 5.2 m^3 of water being pumped by all of the sponges in the lagoon every second or $389,892 \text{ m}^3 \text{H}_2\text{O day}^{-1}$ (21 hours pumping).

4.4.4 Residence time, nutrients and DOC

The mean water residence time in the lagoons at Palmyra was estimated as 22 days (± 13). The percentage of oxygen removed by all the sponges in the lagoons per day was estimated at 0.36% (± 0.1) of the total available oxygen down to 30 m assuming 70% saturation. Assuming that the water stays on average 22 days in the lagoon, the sponges could remove 8% (± 5) of the oxygen from the water over that time before leaving the lagoon. The average oxygen concentration across the lagoons down to 30 m (based on $70\% \pm 15\%$ saturation) was $5.60 (\pm 1.2) \text{ mg O}_2 \text{l}^{-1}$. After 1 day (21 h), assuming no water movement, the sponges would reduce the oxygen concentration to $5.58 \text{ mg O}_2 \text{l}^{-1}$ and after 22 days to $5.2 (\pm 0.8) \text{ mg O}_2 \text{l}^{-1}$.

Removal estimates revealed that the sponges might filter 13,365–300,713 g C d⁻¹ equal to 0.04–0.8% of the total DOC every day, and assuming the residence time of the water is 22 days, the sponges may remove 0.8–18.7% of the DOC in the water column in the lagoons during this time.

4.5 Discussion

Sponges perform essential functional roles in marine ecosystems, and in high densities can have a considerable influence on the water column due to their highly efficient filtering capabilities (Peterson et al. 2006). This study aimed to calculate the turnover time of the lagoon water at Palmyra Atoll in the Central Pacific by all of the resident sponges and to use these values to estimate the potential influence that they have on the DOC and oxygen levels in the lagoons. Appreciating the influence that the sponges have on the water column in the lagoon is important because in semi-enclosed areas suspension feeders can exert a considerable influence on the water column, and have even been shown to mitigate nuisance phytoplankton blooms and eutrophication events (Officer et al. 1982, Hily 1991, Peterson et al. 2006). At Palmyra, understanding the influence of the lagoon sponge assemblage on the water quality in the lagoons is particularly important because it flows out and over a quasi-pristine reef the likes of which are in decline globally (Knowlton & Jackson 2008). It was found that the sponges filter all of the water in the lagoons every 3–6 days, have a negligible impact on the oxygen levels but are a potential sink for DOC.

4.5.1 Filtration rates

The average filtration rate per unit volume of sponge for the six species was 0.06 ($\pm$ 0.01) cm³ s⁻¹ cm⁻³, which is comparable to other freshwater and marine species that are reported to filter between 0.006 and 0.583 cm³ s⁻¹ cm⁻³ (Reiswig 1974, Frost 1980, Savarese et al. 1997, Osinga et al. 2001, Peterson et al. 2006). This study however, found far less variability between species as reported in other studies (e.g. Reiswig 1974, Peterson et al. 2006), which may be because all observations were carried out *in situ*, which reduces the stress on the sponge, a response which varies among species (Reiswig 1971a). Variability between species may also be low because morphological variation in the lagoon is low (no species grows higher than 25 cm) with no large morphologies like chimneys or vases. However, it is difficult to make direct

comparisons between filtration rates for different species because of the variation in method used (Bell 2008a).

The sponge assemblages in the lagoons (per m²) at Palmyra filtered 8–13 cm³ (or ml) H₂O sec⁻¹. Across the 3 lagoons the sponges down to 20 m filtered approximately 5.2 m³ H₂O sec⁻¹ and had the potential to filter all of the moving water (<30 m depth) every 3–6 days. Of the six species recorded in this study, we found significant differences in the volume flux per unit area of sponge (cm²) between massive and encrusting species but no difference in the volume flux per unit volume of sponge (cm³). Most studies estimate filtration rates to examine and compare the physiological attributes of different species using units of total dry weight, organic dry weight and caloric content (e.g. Reiswig 1981, Ribes et al. 1999, Yahel et al. 2003, Peterson et al. 2006). This study based calculated extrapolations on area-based estimates (cm²) of sponge filtration rates. Filtration rates based on area cover of sponges are more practical for ecological purposes because scaling up of values to estimate the impact of sponge assemblages is simpler. Also, because most of the sponges at Palmyra are encrusting species, estimating volumes accurately is difficult. Therefore, where massive species are in higher densities than encrusting ones; the amount of water filtered will be larger because they occupy the same area but the volume of the sponge is greater.

4.5.2 Carbon removal

The influence of sponge assemblages on ecosystems and their inhabitants has been relatively understudied compared to other sessile suspension feeders, such as molluscs. However, sponges have been shown to ameliorate phytoplankton blooms in the Florida Keys (Peterson et al. 2006) and remove considerable amounts of particulate organic carbon (POC) (the other portion making up TOC) in temperate waters (Perea-Blázquez et al. 2012). Also, in reef framework cavities sponge assemblages can influence phytoplankton, picoplankton and nutrient levels (Richter et al. 2001). Sponges in crevices and caves are considered sinks for DOC (de Goeij et al. 2008) but their role at larger scales has not been examined. DOC removal on a daily basis in the lagoons at Palmyra appears to be negligible, however assuming an average residency time of the lagoons of 22 days, the sponges could be removing up to 19% of the DOC in the water column, thus making the lagoonal sponges an important sink for DOC.

Although sponge recruitment and mortality were in equilibrium over the three year time period at Palmyra (**see Chapter 3**), it is unknown how stable the assemblages are over greater time periods. Theoretically, if sponge area cover increased to 50, 75 or even 100% on the available hard substrate (currently 27%), the sponges could remove up to 32, 47 and 63%, respectively, of the available DOC, assuming a residency time of 22 days. Therefore, the sponges have great potential for removing considerable amounts of DOC, which is of particular interest to Palmyra because between four of the reefs in the Northern Line Islands (two of which are inhabited), the highest DOC levels were found on the reefs at Palmyra (Dinsdale et al. 2008). The source is unknown but could be due to upwelling, terrestrial run-off or the high proportion of autotrophic bacteria in the water column (Dinsdale et al. 2008). Blooms of plankton, as a source of DOC, have also been observed annually in Palmyra's lagoons around the end of June each year (authors pers. obs. from 2008 to 2010) until after October, with a subsequent drop in horizontal water visibility from 5–8 m to 1–3 m. Therefore, the lagoon seems a plausible source of the high DOC values on Palmyra's reefs and if the sponges were ever removed, phytoplankton, DOC and bacterioplankton values could increase on the reefs. Knowing more about the trophic interactions and carbon flow across the atoll could therefore be important for its future management because the lagoon could be playing a key functional role in the carbon cycle of the atoll.

4.5.3 Oxygen removal

Whilst local oxygen depletion has been reported in conjunction with high sponge cover in consumption by sponges is low with only 3.3–10% of the oxygen pumped through the body being utilized (Jorgensen et al. 1986, Koopmans et al. 2010). I estimated that the sponges remove 0.36% (± 0.1) $\text{O}_2 \text{ day}^{-1}$ and it would take 308 days (± 66) to filter all of the oxygen out of the lagoon assuming no influx of oxygenated water, a relatively small fraction considering the hypoxia limits of organisms. Lethal and sublethal limits of hypoxia depend on the organism in question but are on average 4.6 and 5.0 $\text{mg O}_2 \text{ l}^{-1}$ for all marine organisms, respectively; however sublethal limits for cnidarians are far lower at 1.4 $\text{mg O}_2 \text{ l}^{-1}$ (Vaquer-Sunyer & Duarte 2008). Tolerance levels between motile and sessile organisms are also significantly different; sessile fauna are more tolerant than motile ones, indicating an adaptation to lower oxygen conditions because of their lack of motility, so a coral's tolerance to hypoxia is generally much higher compared to fish (Vaquer-Sunyer & Duarte 2008).

The sponges in Palmyra's lagoons have the potential to remove $0.02 \text{ mg O}_2 \text{ l}^{-1} \text{ d}^{-1}$ and $0.44 \text{ mg O}_2 \text{ l}^{-1}$ over 22 days. Oxygen concentrations across the lagoons above 30 m are variable (see Gardner et al. 2011), but on average even after 22 days, assuming no water movement, all the sponges do not remove enough oxygen to reach sublethal limits for the majority of organisms. Therefore, I conclude that the likelihood of the sponges reducing oxygen levels in the water column to sublethal levels for corals is unlikely.

4.5.4 Conclusions

The lagoons at Palmyra were heavily modified by the US military during WWII resulting in reduced water flow. However, despite this, over the last 60 years sponges (including introduced species) now occupy almost a third of the hard substrate in the lagoons. At Palmyra the sponges filter the equivalent of all of the water in the lagoons (<30 m) every 3–6 days at a rate of $0.06 (\pm 0.01) \text{ cm}^3 \text{ s}^{-1} \text{ cm}^{-3}$ regardless of morphology, consuming up to 19% of the DOC and 8% of the oxygen in the lagoon water. Therefore, overall the sponges in the lagoons appear to be a sink for DOC, but do not remove considerable amounts oxygen suggesting that they may be playing a positive interactive role on the water column in the lagoons and currently do not appear to pose a threat to the adjacent reef via water column interactions.

Origins of an invasive sponge to Palmyra Atoll (*Haliclona caerulea*), (order: Haplosclerida) are obscured by cryptic speciation

5.1 Abstract

Globally, few coral reef atolls remain near-pristine, including and a number in the Central Pacific, which have altered lagoons making them vulnerable to species introductions. Introduced species can have major ecological impacts on recipient biota and ecosystems, so it is important to determine their introduction pathway to implement management strategies. Many species, however, have cryptic species making identification difficult. Cryptic speciation in particular is widespread across the phylum Porifera, a problem which is not easily resolved with morphological characteristics. This is particularly apparent in the widespread Haplosclerida order due to their limited and plastic micromorphological features. Therefore the best approach to confirm the introduction of Haplosclerida species across oceans is to use molecular markers. This study attempted to examine the reported introduction of *Haliclona caerulea* from the Caribbean to Palmyra Atoll via Hawai'i, but cryptic speciation complicated the findings. Based on morphologically identified samples, *H. caerulea* was collected from seven sites. One nuclear (18s rDNA) and three mitochondrial (CO1, the barcoding CO1 extension (ext) and *rnl* rDNA) regions were sequenced and analysed using neighbour joining, maximum likelihood, and Bayesian inference. To determine between and within species level differences among subclades, sequence divergence values were ascertained based on the number of variable nucleotides in a sequence. Traditionally, spicules are used to identify species, so I also examined, at the site level, spicules lengths from Hawaiian and Palmyra samples. The micromorphological measurements failed to differentiate between groups; however, both mtDNA and nDNA phylogenetic trees supported similar topologies resolving two main clades from different orders: Halichondrida and Haplosclerida. Within the Haplosclerida clade, all individual gene regions (except the CO1 ext) showed no nucleotide differences between samples from all geographical regions. However, the concatenated mtDNA tree resolved twelve subclades corresponding to geographic locations, with the CO1 ext yielding most of the variability between the samples. Low sequence divergence values (0.68%) between two of the Haplosclerida subclades indicated that the same species is found at Palmyra,

Hawai‘i and the Caribbean, which supports the hypothesis that *H. caerulea* was introduced to Palmyra from the Caribbean. However, the pattern of fine-scale cryptic speciation complicates the introduction hypothesis, as it is not clear how many cryptic species there might be within those specimens considered to be *H. caerulea*. This study illustrates how understanding the scale of within species as opposed to between species level genetic variation is critical for determining the origins of introduced organisms.

5.2 Introduction

Cryptic species, which are morphologically indistinguishable from each other, have long been an problem for taxonomists and biologists across all biomes making estimates of biodiversity, and native and introduced species ranges difficult to measure (Bickford et al. 2007). The identification of a species is a basic, yet fundamental part of conservation and management (Ladner & Palumbi 2012) but is complicated by the lack of distinct morphological traits in some groups. This problem is particularly apparent in members of the phylum Porifera, which are notoriously difficult to identify in the field due to their limited yet plastic morphologies in response to environmental variability (Wulff 2001). Poriferans are also ecologically important (Bell 2008a), and invasive species can pose significant threats to ecosystems (Coles 2007) as they are efficient spatial competitors (Bell & Barnes 2003b, Wulff 2006) and can directly impact water column characteristics (Fu et al. 2006, Peterson et al. 2006; Chapter 6, Fu et al. 2007) through the removal of organic and inorganic particles, and nutrients (Gili & Coma 1998). Therefore, it is important to identify introduced sponge species before any management plans can be implemented.

In marine, brackish and freshwaters worldwide there are 7,500 confirmed sponge species with an estimated 15,000 in total (Hooper & van Soest 2002, van Soest et al. 2012). Some species long believed to be cosmopolitan, widely spread or introduced across several oceans have been revealed by molecular markers to be complexes of cryptic species (e.g. Klautau et al. 1999, Xavier et al. 2010). The issue of cryptic species identification is not only a taxonomic conundrum, because determining whether a species is introduced will affect management actions (Concepcion et al. 2010). This is particularly troublesome when it comes to sponges which are phenotypically plastic and difficult to identify in the field (Wulff 2001).

Morphological characteristics such as spicules and spongin architecture have classically been the primary focus in sponge taxonomy to identify species (Hooper &

van Soest 2002). However, morphological plasticity and the low number of phenotypic characteristics in sponges make consistent and accurate identification problematic (Wulff 2001). Therefore, genetic markers have become useful tools to identify divergent cryptic species and have revealed that the distribution ranges of many so-called “cosmopolitan species” have often been overestimated because they are not simply a single species (Boury-Esnault et al. 1992, Klautau et al. 1999, Hoshino et al. 2008). Confirming the identity of an introduced species is therefore the first step to studying its phylogeographic distribution and introduction pathway.

The class Demospongiae encompasses 85% of the known sponge species making it the largest, most diverse group in the phylum Porifera. The order Haplosclerida (Topsent 1928) is considered the most diverse group in this class and includes all freshwater species and a large number of shallow water marine sponges (van Soest & Hooper 2002), although the monophyly of this class is still contentious (Redmond et al. 2011). The range of spicule types in the Haplosclerida is very limited (megascleres are either oxeas or strongyloxeas and the microscleres, if present, are sigmas, microxeas, microstrongyles or toxas) making classification difficult compared to some other sponge groups e.g. Tetractinellida and Calcispongia (Chombard et al. 1998, Manuel et al. 2003), which have more distinct morphological features. More recently, genetic analyses using a range of markers have been employed to examine interspecific phylogenies to resolve the order Haplosclerida, which is currently divided into 2 to 4 suborders, but there are still major discrepancies between the molecular markers as to how many suborders there are within the order (McCormack et al. 2002, Itskovich et al. 2007, Redmond et al. 2011). At the species level there have been a number of examples of taxonomic confusion, for example in the genus *Haliclona*, where multiple samples of the same species, *H. oculata* and *H. cinerea*, both had individuals (presumed to be the same species) which were subsequently found to be genetically distinct and distantly related according to CO1 and 18s rDNA markers (Raleigh et al. 2007, Redmond & McCormack 2008). Therefore, it is clear that there are taxonomic problems and a lack of resolution at all levels of the order Haplosclerida, which complicates phylogeographic studies because even species identification is difficult.

The initial aim of this study was to investigate the potential introduction of *Haliclona caerulea* (order Haplosclerida) to the Central Pacific from the Caribbean (DeFelice et al. 2001, Coles et al. 2006; Appendix I). It is thought that *H. caerulea* was

introduced to Hawai‘i and then onto Palmyra Atoll, a near-pristine atoll in the Central Pacific, via fouling on ships and yachts (Godwin 2003b; Appendix I) or other structures (e.g. barges) as this was the primary pathway of introduction to Hawai‘i (Molnar et al. 2008), as Hawai‘i has long been the predominant shipping connection to Palmyra (Dawson 1959; Appendix I). The limited larval dispersal capabilities of *H. caerulea* (to several meters) (Maldonado & Young 1999) also suggests that the chances of this species naturally increasing its range from the Caribbean to the Central Pacific is unlikely. However, considering the limited micromorphologies of Haplosclerida species (Redmond et al. 2011), it is likely that there is undetected biodiversity (cryptic speciation) in this order, and it is at least possible that the sponge species in the lagoons at Palmyra are endemic as some suspected invasive species are actually not introduced. For example Concepcion et al. (2010) found that the invasive snowflake coral (*Carijoa riisei*), which overgrows black coral (*Antipathes* spp.) in Hawai‘i was believed to be introduced from the Caribbean turned out to be an endemic species. Therefore confirming that the same species is found in the Caribbean, Hawai‘i and Palmyra will be important in developing future management plans for Palmyra. Therefore this study aimed to use molecular tools to identify whether the Caribbean species *H. caerulea* was introduced to Palmyra Atoll via Hawai‘i or whether the regional populations represent cryptic species. Classical spicule (oxea) length measurements of *H. caerulea* from Palmyra and Hawai‘i were also examined to confirm whether morphological variability could be used to distinguish between the samples and test the utility of spicules to identify species or population differences. In order to examine the introduction hypothesis of *H. caerulea* to Palmyra from the Caribbean, four genes, both nuclear and mitochondrial, were examined: 18s rRNA, mtDNA *rnl* (large subunit rDNA) and the standard barcoding fragment, cytochrome oxidase subunit 1 (CO1) (Wörheide et al. 2007), along with the suggested extension, which is more variable and thought to provide greater resolution for intraspecific differences (Rot et al. 2006).

5.3 Methods

5.3.1 Study species

Haliclona caerulea (Hechtel 1965) (family Chalinidae), commonly known as the ‘Blue Caribbean sponge’, is found on rocks, dock pilings and mangrove roots in shallow water embayments, harbours and disturbed habitats, all with restricted water flow (Hechtel 1965, Cubit & Williams 1983, Wulff 1997, De Weerd 2000, DeFelice et

al. 2001). This species is also known as *Sigmatocia caerulea*, *Haliclona* (Gellius) *caerulea*, and *Haliclona* (Soestella) *caerulea*, but here I use *Haliclona caerulea*. *Haliclona caerulea* is characteristically blue, but the morphology can alter when it forms a mutualistic relationship with the red branching coralline alga *Jania adherens*, which is visible in individuals from the Pacific Coast of Panama (Wulff 1997), Mexico (Carballo & Avila 2004) and Palmyra Atoll (**Appendix I**). The megasclere spicules are bent oxeas, and the microscleres are C-shaped sigmas, which are less common throughout the purple mutualistic forms (Hechtel 1965).

5.3.2 Sampling design

In total 313 specimens were sampled from 7 locations in the Line Islands, Hawai‘i and the Caribbean. Samples were collected on SCUBA from Palmyra Atoll in the Line Islands and on snorkel in Hawai‘i: Kāne‘ohe Bay, Ke‘ehi Bay, Ala Moana and the Caribbean: St Thomas Island and St James Island in the US Virgin Islands and Puerto Rico (**Fig 5.1 and Table 5.1**), which at the furthest point are 10,572 km apart. All collected samples were of the blue colour morph with the exception of those from Palmyra, which were all purple with fragments taken from the base of the sponge where there is more blue sponge tissue compared to the associated *J. adherens* algae. On average 30–60 samples were collected from each location with the exception of Palmyra Atoll where 100 samples were collected and Ke‘ehi Bay where only 10 specimens were collected (representing all those individuals found). To avoid sampling clones, individuals were collected at least 2 m apart from each other and stored in individual 2 ml Eppendorf tubes. Immediately after collection, to reduce specimen atrophy, the seawater was replaced with dimethyl sulfoxide (DMSO), a saturated salt buffer for storage and a highly efficient storage solution for invertebrates, particularly in remote field locations (Dawson et al. 1998).

Figure 5.1 Map of the sampling locations for *Haliclona caerulea* in the Caribbean and Pacific along with the individual collection sites on O‘ahu, Hawai‘i: Ke‘ehi Bay (Ke), Ala Moana (Am) and Kāne‘ohe Bay (Kan).

Table 5.1 Location, code IDs and numbers of sequences included in the final analyses of *Haliclona caerulea* samples.

Sites	Code	Region	Latitude & Longitude	n
Ala Moana	(Am)	Hawaii	21°17'N, 157°50'W	7
Ke‘ehi Bay	(Ke)	Hawaii	21°19'N, 157°53'W	6
Kāne‘ohe Bay	(Kan)	Hawaii	21°25'N, 157°47'W	13
St John Island	(StJ)	Caribbean	18°19'N, 64°44'W	7
St Thomas Island	(StT)	Caribbean	18°18'N, 64°54'W	3
Puerto Rico	(PR)	Caribbean	18°17'N, 67°12'W	5
Palmyra Atoll	(PA)	Line Islands	05°53'N, 162°5'W	15
Total				56

5.3.3 DNA extraction and PCR amplification

The sponge samples were extracted using a Qiagen DNeasy Tissue Kit following the manufacturers protocol. After initial screening with twenty one primer sets from Jarman et al. (2002) and Redmond et al. (2007), four regions were successfully sequenced and all samples were screened with these primers. The primer regions included three mitochondrial regions: CO1 and CO1 extension (ext) (mtDNA) and *rnl* (rDNA) and the nuclear region 18s (rDNA) (see **Table 5.2**). CO1 is one of the three protein coding subunits in the cytochrome c oxidase complex involved in aerobic metabolism and *rnl* is a large ribosomal subunit (one of the two) in the metazoan mitochondrial DNA (the other is the small subunit *rns*) (Boore 1999). 18s is found in the small subunit 40s of eukaryotic ribosomal RNA.

Table 5.2 Primers used in the PCR and sequencing of *Haliclona caerulea* samples.

Primer name	Region	Primer sequence (5' - 3')	Reference
dgLCO1490	CO1	GGT CAA CAA ATC ATA AAG AYA TYG G	Meyer et al., (2005)
dgHCO2198		TAA ACT TCA GGG TGA CCA AAR AAY CA	
COX1-R1	CO1 ext	TGT TGR GGG AAA AAR GTT AAA TT	Rot et al., (2006)
COX1-D2		AAT ACT GCT TTT TTT GAT CCT GCC GG	
diplo-rnl-f1	rnl	TCG ACT GTT TAC CAA AAA CAT AGC	Lavrov et al., (2008)
diplo-rnl-r1		AAT TCA ACA TCG AGG TSG GAA AC	
18s_1-600_F2	18s	GCC AGT AGT CAT ATG CTT GTC TCA	This paper*
18s_1-600_R1		GAC TTG CCC TCC AAT TGT TC	

* modified from Redmond et al., (2007)

Due to difficulties in sequencing, instead of using the standard barcoding CO1 region from Folmer et al. (1994) I employed the degenerate primers developed by Meyer et al. (2005). Also, due to the lack of variability in the standard CO1 region in sponges (Duran & Rützler 2006, Wörheide 2006), I sequenced the suggested downstream extension region described by Rot et al. (2006), which includes an intron and has higher substitution rates (Erpenbeck et al. 2006). After alignment and trimming, the final sequence lengths were 554 and 318 base pairs (bp) for CO1 and CO1 ext, respectively. The Porifera-optimized primers diplo-*rnl*-f1 and diplo-*rnl*-r1 described by Lavrov et al. (2008) were used to amplify the mitochondrial *rnl* ribosomal RNA region ($\approx$ 700 bp), of which 311 bp were examined in this study. The 18s rRNA region is approximately 1,800 nucleotides long in Haplosclerida sponges (Redmond & McCormack 2008) and this study examined the first 407 bp after multiple sequence alignment and trimming.

PCR amplifications were performed in 15 μ l reactions consisting of: 8.5 μ l nanopure H₂O, 1.5 μ l 10x NH₄ buffer, 0.45 μ l MgCl₂, 0.30 μ l dNTP's (10 mM), 2.0 μ l 40x bovine serum albumin (BSA), 0.1 μ l *Taq* polymerase (Biolase, Bioline), 0.075 μ l of each primer and 2 μ l of DNA template with the following temperature profile: 95 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 58 °C (*rnl* and CO1) or 50°C (18s) for 45 s, 72 °C for 45 s and a final extension step at 72 °C for 10 min. The PCR products were then purified using ExoSAP-IT (USB Corporation) and sequenced using BigDye Terminators (PerkinElmer) at the EPSCoR core genetics facility at the Hawai'i Institute of Marine Biology on a ABI-3130XL automated sequencer.

5.3.4 Data analysis

All sequences were evaluated and aligned using Geneious Pro (v.5.3.6) (Drummond et al. 2011). The poriferan origin of the sequences was confirmed using the BLAST search engine in GenBank. For all data-sets, the outgroup taxon was *Amphimedon queenslandica*, also from the order Haplosclerida (van Soest & Hooper 2002). Those samples that did not produce comparable sequences across all regions, despite several attempts at sequencing, were removed. Simple Neighbour joining (NJ) trees (in Mega5 (Tamura et al. 2011) with a p-distance model, pairwise deletion of gap positions and 1,000 bootstrap replicates) were then produced to provide an initial overview of the data for each region. The number of samples were then reduced to improve the visualization of results and reduce analysis time while still representing the genetic diversity between samples, with up to 5 identical individuals selected per tree grouping per region resulting in 56 samples across all locations (see **Table 5.1**).

The mitochondrial regions (CO1, CO1 ext and *rnl*) for all 56 samples were then examined separately to determine if there were major differences in topologies between regions. The results indicated that there were no major conflicts in topology (**Fig 5.2**); therefore the mitochondrial regions were additionally concatenated to form a total evidence tree for the mtDNA to increase the resolution of information. To determine within and between species level differences between the subclades, the pairwise percent sequence divergence (P) (see **Table 5.3**) was calculated for all gene regions in MEGA5 as the proportion of variable nucleotide sites in each alignment.

Figure 5.2 Neighbour Joining phylogenetic trees of the 56 sequenced *Haliclona caerulea* samples along with the outgroup species *Amphimedon queenslandica* with mitochondrial gene region CO1, CO1 extension (ext) and *rnl*. with branch support from Neighbour Joining (NJ), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. Regional names (Hawaii, Palmyra and Caribbean) are followed by site names: Ke'ehi Bay (Ke), Ala Moana (Am) and Kāne'ōhe Bay (Kan). St James Island (StJ), St Thomas Island (StT) and Puerto Rico (PR) and then the sample number per region. The two clades are divided into A and B with all subclades numbered separately for each tree. Support values indicated as 100 represent identical support from each analysis (i.e. 100/100/1.0). Scale bar = 0.02 substitutions per 100 sites.

Table 5.3 Total number of base pairs (bp), samples (n), haplotypes (H) and pairwise per site, per alignment percentage sequence differences (P) for each sampling location and across all samples. ext = extension region.

Sites	Gene	Total length (bp)	n	H	P (%)
Palmyra Atoll	CO1	554	15	3	27
	CO1 ext	318	15	2	11
	<i>rnl</i>	366	15	1	0
	mtDNA	1182	15	4	16
	nDNA	407	15	3	11
Hawaii	CO1	554	26	3	31
	CO1 ext	318	26	3	15
	<i>rnl</i>	366	26	3	27
	mtDNA	1182	26	6	27
	nDNA	407	26	3	13
Caribbean	CO1	554	15	2	20
	CO1 ext	318	15	2	19
	<i>rnl</i>	366	15	2	22
	mtDNA	1182	15	3	21
	nDNA	407	15	3	10
All sequences	CO1	554	56	5	32
	CO1 ext	318	56	6	27
	<i>rnl</i>	366	56	3	27
	mtDNA	1182	56	12	31
	nDNA	407	56	6	14

Phylograms were created separately for each region (CO1, CO1 ext, *rnl* and 18s) as well as the concatenated mitochondrial region with NJ, Maximum Likelihood (MP) and Bayesian inference (BI) values supporting branch topologies. Maximum likelihood trees were generated by MEGA5 using the Nearest-Neighbour Interchange (NNI) heuristic method with gaps included at all sites and 1,000 bootstrap replicates. Neighbour Joining trees were also estimated by MEGA5 with the same conditions as described above. Bayesian analyses were conducted by MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) using the Markov Chain Monte Carlo (MCMC) analyses (Geyer 1991) for 300,000 generations, sampling 4 chains every 300 generations with the first 250 trees (75,000 generations) discarded at the burn-in period. All trees were based on the NJ topology and drawn by MEGA5.

To determine the best-fit nucleotide substitution model for each data set, corrected Akaike information criterion (AICc) model selections were performed by jModelTest v2.0 (Posada & Crandall 1998). The jModelTest results identified Hasegawa, Kishino and Yano HKY +G (Gamma) models for both CO1 regions and HKY for the *rnl* region as the best fitting prior models of evolution. Tranversion (TVM +G) and transitional models (TIM2ef +G) were selected for the concatenated mitochondrial and nuclear data sets, respectively. However, for the Bayesian and

Maximum likelihood analyses, TVM and TIM models were not available in MrBayes and MEGA5, therefore a General Time Reversible (GTR) model with Gamma rates (G) was used as it is the closest to the suggested models from jModelTest (Posada & Crandall 2001, Zakharov et al. 2009).

One voucher specimen sequence of the CO1 region (no extension) for *H. caerulea* from the Caribbean (ID# EF519619) (Erpenbeck et al. 2007) was found in GenBank and included in the analyses in order to confirm the identification of *H. caerulea* based on the sponge barcoding basic region.

5.3.5 Spicule analyses

To identify any local site level morphological differences between specimens, the oxea lengths from the Hawaiian and Palmyra atoll samples (**Fig 5.1**) were measured using ImageJ (Abràmoff et al. 2004) based on photographs taken under a light microscope. To prepare the samples, a 2 mm³ fragment was taken from each specimen and placed in a 1 ml Eppendorf tube filled with sodium hypochlorite to remove the tissue and leave only the silicate spicules. After 24 hours, the samples were rinsed with distilled water three times. The samples were then vortexed and a drop of water and spicules was pipetted onto a slide, with a cover slip and a drop of oil. Thirty to forty spicules were photographed under the microscope with the thirty clearest photos measured. A non-metric Multidimensional Scaling (nMDS) ordination (Anderson 2001) was executed on the Bray-Curtis similarity matrix to visualise the spicule length size groups, which was performed in PRIMER v6 (Clarke & Gorley 2006) with the PERMANOVA+ add-on (Anderson et al. 2008).

5.4 Results

5.4.1 Individual regions

When examining the CO1, *rnl* and 18s regions (excluding the CO1 extension) two clades were evident (**Fig 5.2**). The CO1 region identified five subclades with the voucher sample from Erpenbeck et al. (2007) nesting within clade B, which includes samples from all the sites, excluding St. Thomas. When examining the subclades in clade A with the barcoding region in the BLAST search engine, I found that the samples were most similar to *Halichondria* spp. (order: Halichondrida), while clade B was most similar to Haplosclerida species. Further resolution of clade B was not possible with the CO1 marker or the *rnl* region as all sequences were identical. The

CO1 extension resolved clade B more than any other marker (except the concatenated mtDNA region) resulting in 6 subclades in total with the subdivisions more consistent with geography than the other gene region.

The nuclear 18s marker was most congruent with the CO1 region indicating 6 groups in total; however, although the St James subclade (group 6) splits off it is not significantly supported by the NJ or ML analyses and when looking between samples there is only 1 bp (0.3% sequence divergence) difference between groups 5 and 6. Therefore the 18s nDNA region is more similar to the CO1 and *rnl* regions, indicating one Caribbean group, which is in sharp contrast to the CO1 ext, which splits off the Puerto Rican samples, which had a 60 bp difference (19% sequence divergence).

5.4.2 Concatenated mtDNA versus nDNA

There were no major discrepancies between the mtDNA and nDNA regions, (**Fig 5.3**) supporting two major clades (Halichondrida and Haplosclerida spp.) subdivided into a number of different terminal groups, all supported by congruent significant values from the NJ, ML and BI analyses. Therefore, the different analyses are in agreement, indicating that the trees provide a relatively robust visualisation of the data. The concatenated mtDNA tree was the most resolved, with the CO1 ext providing most of the polymorphism, yet not distinguishing all subclades. Overall the concatenated tree (mtDNA tree) revealed that there is fine-scale cryptic speciation that correlates to geographic differences. The mtDNA tree revealed a similar topography to the nDNA indicating two main clades, but with better-resolved subclades. The percentage sequence divergence between sites (**see Table 5.3**), however, was the lowest with nDNA (14%) compared to the mtDNA (31%) for all locations revealing slower rates of sequence evolution in the nDNA marker. Sequence divergence values also revealed both within and between species level differences among the Haplosclerida subclades. The only subclade in the mtDNA tree with samples from more than one geographic region was group 10 in clade B, which contained both Palmyra and Hawaiian samples. The next closest subclade (11) grouped Caribbean samples from St James Island, which had only 0.68% sequence divergence (8 bp different, all from the CO1 ext region) compared to subclade 10 (containing Palmyra and Hawai'i samples) also suggesting that are effectively the same species. The remaining subclades in clade B, 8, 9 and 12 had sequence divergence values of 4.0, 3.0 and 4.9% respectively, which may correspond to congeneric species-level differences, or populations that are highly

isolated. Overall, between all subclades in clade B the sequence divergence was 7.5% but 22.8% between all samples in clade A, suggesting that samples within clade B were more closely related and less divergent. The clades or individual subclades could not be identified further as the Genbank database is currently not comprehensive enough to provide such information.

Figure 5.3 Neighbour joining phylogenetic trees of the 56 sequenced ‘*Haliclonina caerulea*’ samples along with the outgroup species *Amphimedon queenslandica* with the concatenated total evidence mtDNA tree and the 18s nDNA trees with branch support from Neighbour Joining (NJ), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. Regional names (Hawaii, Palmyra and Caribbean) are followed by site names: Ke’ehi Bay (Ke), Ala Moana (Am) and Kāne’ohe Bay (Kan), St James Island (StJ), St Thomas Island (StT) and Puerto Rico (PR) and then the sample number per region. Overlaid on the mtDNA and nDNA trees are the oxea length groups for each sample from Hawaii and Palmyra. The two clades are divided into A (Halichondrida) and B (Haplosclerida) with all subclades numbered separately for each tree. Support values indicated as 100 represent identical support from each analysis (i.e. 100/100/1.0). Scale bar = 0.02 substitutions per 100 sites. % values represent the lowest sequence divergence values between subclades.

5.4.3 Morphological characteristics

The spicule length data formed three size classes: 60–75 μm , 95–120 μm and 200–250 μm , as indicated in the nMDS plot (**Fig 5.4**). The size classes when overlaid on the topographical trees revealed polyphyletic groups that were not consistent with the molecular data, including within subclades, with the exception of group 4 in the mtDNA tree, which confirmed that the Ke’ehi Bay individuals were significantly different from all other locations.

Figure 5.4 Non-metric multidimensional scaling (nMDS) ordination visualising grouped sponge oxea lengths (μm) over sites Ke’ehi Bay (Ke), Ala Moana (Am) and Kāne’ohe Bay (Kan) from Hawai’i and from Palmyra Atoll (PA) based upon a zero-adjusted Bray-Curtis similarity matrix.

5.5 Discussion

Cryptic speciation is a major problem when trying to understand the introduction of a species (Bickford et al. 2007) and can be particularly difficult when working with sponges, which are often difficult to identify in the field (Wulff 2001). Haplosclerida sponges are considered a taxonomically difficult order because of the low variability in morphological characteristics, but high levels of macromorphological plasticity; recent phylogenetic investigations suggests that this order needs revision at all taxonomic levels (Redmond et al. 2011). In particular, examining species level differences in this order is fraught with difficulties, including cryptic speciation and problems defining within or between species level differences (Raleigh et al. 2007,

Redmond & McCormack 2008). Establishing the level of within and between species differences between locations will determine whether a species is introduced (one species spread across several locations) or endemic (multiple species in their own locations). Therefore confirming the identity of a species is important when considering the management of potential introductions to non-endemic locations such as *Haliclona caerulea* to the isolated and near-pristine Palmyra Atoll (**Appendix I**).

This study found molecular evidence to indicate that *H. caerulea* is found at Palmyra Atoll, Hawai'i and the Caribbean; however, cryptic speciation complicates phylogeographical relationships between sampling regions. Overall, three of the four gene regions (CO1, *rnl* and 18s) provided low resolution between the samples and broadly grouped individuals into two order level clades (Halichondrida and Haplosclerida) (**Fig 5.2 and 5.3**) with almost no sequence divergence within the Haplosclerida clade B. Inclusion of the micromorphological characteristics did not improve the data resolution. Only the CO1 ext and the mtDNA trees increased the resolution within the Haplosclerida samples and revealed further evidence for cryptic speciation with up to twelve subclades that corresponded to geographic locations. Despite the high levels of cryptic speciation in the Haplosclerida clade (B), sequence divergence values revealed evidence of within species differences between the Caribbean, Hawai'i and Palmyra Atoll, which supports the hypothesis of introduction of *H. caerulea* to Palmyra via human transportation.

5.5.1 Gene regions

This study found considerable genetic variability within three mitochondrial and one nuclear DNA markers when examining samples of apparently morphologically similar *H. caerulea* and found that the two mtDNA markers, CO1 and *rnl*, provided the lowest resolution. Mitochondrial genes have long been used to study population genetics along with the identification of species because they have high mutation rates, shorter coalescence times and recombination is rare (an issue with nDNA) (Avice 2000a, Lavrov 2007). The sponge barcoding project uses the CO1 region as its basic identification region (Wörheide et al. 2007) and has been used successfully to resolve some sponges down to species level (e.g. Pöppe et al. 2010). However, often in sponges (and other non-bilaterians e.g. Cnidarians) the mutation rate in the mitochondrial genes is too slow to determine population structure or even species differences (Shearer et al. 2002, Duran et al. 2004b, Wörheide 2006). The issues regarding the use of

mitochondrial regions are evident in the low resolution of the *rnl* and CO1 trees. Therefore, the sponge barcoding project suggests the inclusion of an extension region to aid in the differentiation between species (Rot et al. 2006). In this study the CO1 extension provided the most resolved tree out of the 4 individual regions examined therefore supporting the use of this “add-on” in conjunction with the established barcoding region for identification of sponges down to species level. However, overall the best resolution was provided by the concatenated mtDNA tree, which had high support values for each subclade despite the disparity between individual regions, which if examined alone (**Fig 5.2**) may have resulted in different conclusions about the species identification between samples.

Classically, 18s rDNA is also slowly evolving and therefore used to resolve higher taxonomic levels (Peterson & Addis 2000, Borchellini et al. 2001), generally making it unsuitable for resolving finer population structure (Wörheide et al. 2002a, Wörheide et al. 2002b). When looking at the number of variable sites in *H. caerulea* the sequence divergence in the mtDNA was approximately 2 times higher compared to the 18s nDNA, an observation also noted by Lavrov et al. (2005), who found sequence divergence to be 4.3 times greater in mtDNA compared to nDNA in the sponges *Geodia neptuni* and *Tethya actinia*. The low sequence diversity in this 18s region concurs with my findings and is most likely due to higher divergence in mtDNA compared to 18s nDNA in sponges. The low level of polymorphisms in most mtDNA regions is consistent with other studies (Hudson et al. 1987, Shearer et al. 2002, Duran et al. 2004b); however, the relatively high levels of polymorphism in the CO1 ext offer a stark contrast.

The variable power of resolution between markers in this study is most likely due to different evolutionary pressures on each region resulting in varying levels of polymorphism and phylogenetic resolution between markers. For example, there is a disparity between the Palmyra samples, which group predominantly into one clade with the CO1 ext and *rnl* or two with the CO1 and 18s regions suggesting different mutational pressures or evolutionary histories acting on the different genetic markers. Genetic recombination is a possible explanation for these results; however it is rare in mitochondrial DNA and therefore unlikely to explain this pattern (Solé-Cava & Wörheide 2007). Mitochondrial pseudogenes, which result in non-functional copies of the mtDNA in the nDNA being sequenced, could also result in the sequencing of mtDNA of an ancestral origin (Bensasson et al. 2001) and a division of the Palmyra

samples into multiple subclades as seen in the CO1 tree. However, this is also an unlikely explanation of non-monophyly of the Palmyra samples with the CO1 marker, as the nDNA topology was broadly similar (Fukami et al. 2008). Natural selection may also result in different evolutionary pressures on particular regions, unfortunately most tests for selection or neutrality compare synonymous to nonsynonymous nucleotide substitutions, which is not applicable to the CO1 extension, since it is an intron and not a protein coding gene. Concatenating the mtDNA data resulted in a topology with high support values, which concurs with the nDNA but with higher resolution. This suggests that the data partitions are generally congruent and minor topological differences are primarily due to comparing highly conserved genes that lack phylogenetic resolution with an intron under relaxed selection pressures.

5.5.2 *Cryptic speciation within H. caerulea*

The genetic data indicate several hierarchical levels of cryptic speciation, which limits any interpretation of any phylogeographical findings because of the taxonomic difficulties. Initially, the CO1 data suggest at least two groups from the orders Halichondrida (clade A) and Haplosclerida (clade B), which have large geographic distributions and subpopulations, as individuals from all regions were found in both clades. However, the concatenated mitochondrial tree revealed that there may be up to 12 different isolated species (corresponding with the 12 geographic subclades). It is more likely that there are greater rather than fewer species due to the high support values for each of the subclades and number of substitutions.

Focusing on the target Haplosclerida order, the concatenated mitochondrial tree indicates that the same species is found at Palmyra Atoll and Hawai'i, with samples from both grouping in subclade 10 with 100% similarity over the 1,182 bp sampled. The Caribbean and Palmyra samples are very closely related with only 0.68% (8bp) divergence between groups 10 and 11. Huang et al. (2008) found that the intraspecific differences for sponges (taken from all CO1 sequences in GenBank in September 2006) were 0.60% (± 0.10) and 3.76% (± 0.57) for the closest congeneric interspecific distances. The percentage of divergence between groups 10 and 11 may therefore suggest that these subclades are from the same species. Looking at the next closest subclades to the Palmyra-Hawaiian group, 8, 9 and 12 had divergence values of 4.0, 3.0 and 4.9% respectively, indicating that samples in subclades 8 and 9 are consistent with cryptic congeneric species-level differences. However, Huang et al. (2008) focused on

sequences from the CO1 region in the analyses, most likely excluding the extension region identified by Rot et al. (2006) as the sequence data were collected before the publication of this study. In this study, with the CO1 marker there was 0% difference between the clade B samples, which included samples from all locations. However, but it was the CO1 extension which provided the greatest resolution of the data, which may have resulted in the higher values found between sequences in the concatenated tree. Therefore, it is unclear what the true species boundaries are between the subclades in clade B. When looking at the sequence divergence between the subclades 10 (clade B) and 6 (clade A) there is 13.9% difference between these groups, which supports the premise that these cryptic taxa represent higher level, distantly related taxonomic divisions (perhaps at the order level) that are presently morphologically indistinguishable from *H. caerulea*.

Although the possibility that *H. caerulea* is endemic but previously unrecognised in Palmyra cannot be entirely ruled out, it is exceedingly unlikely to observe such shallow geographic diversification across approximately 10,000 km, since *H. caerulea* larvae cannot travel far (Maldonado & Young 1999) without human transportation (DeFelice et al. 2001). Therefore it seems likely that *H. caerulea* has been transported to Palmyra Atoll from the Caribbean via Hawai'i. Unfortunately due to the high level of genetic variation among samples the data are not sufficient to draw any conclusions on connectivity patterns between the sampling regions. It therefore remains unknown whether *H. caerulea* was introduced just once or on multiple occasions. The overall large number of subclades may be partially explained by rapid diversification due to selection on the CO1 ext intron, however since rates of mtDNA evolution are low in sponges (Shearer et al. 2002, Duran et al. 2004b, Wörheide 2006), it is more likely that there were multiple introductions of diverse populations or closely related species from as of yet unsampled geographic locations in the Caribbean, which can be resolved in the future with additional geographic sampling.

5.5.3 Using morphological characteristics to identify *H. caerulea*

The spicule lengths grouped by the nMDS ordination (**Fig 5.4**) were not congruent to the tree topologies and did not help explain the variability between groups with the exception of the Ke'ehi Bay samples, which were distinctly longer (200–250 μm) than for all other locations. DeFelice et al. (2001) noted that *H. caerulea*'s oxeas in Hawai'i were 170–230 μm long and Cruz-Barraza and Carballo (2008) identified

lengths of 82–210 μm in Mexico, while the original description by Hechtel (1965) found that the oxea lengths in Jamaica varied between 117–200 μm depending on a location's water flow rates. Therefore we can assume that the micromorphology of Haplosclerida species is not a suitable tool for identification because it is either highly dependent on environmental conditions, has homoplastic non-distinct morphological features or both. Unfortunately, the Haplosclerida micromorphological traits may not even be useful on the order level as some Halichondrida species have similar oxeas to those seen in Haplosclerida (Erpenbeck & Soest 2002), which explains the lack of morphological resolution found in this study.

5.5.4 Conclusions

Understanding the scale of differentiation between samples (within and between species) is important to ascertain with regard to the management of introduced species particularly those which exhibit cryptic speciation and morphological plasticity. This study found surprising levels of genetic subdivision corresponding to geography; however, it is possible to suggest that there has been a movement of species across the Pacific with the same species found at Palmyra Atoll, Hawai'i and possibly even the Caribbean. This study forms a foundation for further exploring newly discovered cryptic speciation and it provides an example of why it is important to do so.

Chapter 6

General Discussion

The overall aims of this thesis were to examine the sponges in the WWII modified lagoons at Palmyra Atoll, and to establish their diversity (specifically NIS), impacts on the water-column and potential to extend onto the adjacent near-pristine reef. In summary, sponge diversity at Palmyra is similar to other atoll systems in the Pacific and includes non-indigenous species presumably introduced by ships. Based on the results of my thesis, I propose that the sponges in the lagoon are unlikely to extend onto the reef because: a) the environmental conditions in the lagoons are preferred; b) the assemblages appear temporally stable with sponge mortality and recruitment in equilibrium and c) their current distribution suggests that they can disperse effectively against the predominant water flow direction, but still have not extended onto the reefs. Also the impacts of the sponges on the water column appear negligible, if not beneficial, and the risk of their future influence on the water column seems low because recruitment and mortality are in equilibrium, so their overall abundance is probably stable. Here I discuss how my research contributes to the current body of knowledge of sponge ecology and invasion biology, as well examine the future of the sponges and the lagoons at Palmyra Atoll, and the implications of my results for other atolls or semi-enclosed systems such as harbours and bays.

6.1 Sponges: Wealth of benefits, paucity of knowledge

6.1.1 Diversity and distribution

Sponges are economically and ecologically valuable (see Duckworth et al. 2007, Becerro 2008, Bell 2008a), moreover, they are a highly diverse phylum with roughly 8,500 known sponge species, but an estimated 15,000 in total (Hooper & van Soest 2002, van Soest et al. 2012) compared to 5,600–8,000 for bryozoans (Ryland 2005) and 700 for corals (Cesar et al. 2003); however, sponges still remain relatively understudied. In general, the majority of tropical sponge research has focused on the Caribbean, with studies in the Pacific and Indo-Pacific mainly conducted around Australia, Mexico and Indonesia with very little information available on the Central Pacific. Compared to other tropical locations, the Central Pacific appears to have low

sponge diversity with 1 to 61 species per island or atoll in this region (**Fig 6.1**) (de Laubenfels 1949, 1950, 1951, 1954, 1955, Bergquist 1965, Chiriboga et al. 2011, van Soest et al. 2011). However, through this work 51 new species can now be added to the Line Islands ecoregion, which currently has only 10 recorded for this archipelago (**Fig 6.1**) (van Soest et al. 2012).

Despite these additions and the continued work in documenting the distribution of sponges, the global ecoregion diversity, although up to date, may not represent the true global diversity as the hotspots appear to relate to the location of institutes and the disproportionate research efforts in these locations (Hendriks & Duarte 2008). Therefore species diversity across the Central Pacific is likely to be higher as the number of publications for this area is low compared to other regions as it is difficult to access (Fisher et al. 2011). At Palmyra, the diversity is also most certainly higher than reported in this work (**Chapter 2**) as it was not possible to record all of the bioeroding sponges and those species found underneath the boulders reef crevices and deep waters in this thesis. The total number of sponge species is also likely to be higher at Palmyra and all other locations due to the potential high levels of cryptic speciation within sponges (Klautau et al. 1999, Blanquer & Uriz 2007, Hoshino et al. 2008, Xavier et al. 2010). In Chapter 5, I showed that at Palmyra *Haliclona caerulea* was potentially introduced to Palmyra from the Caribbean via Hawaii but cryptic speciation complicates this finding.

The identification of cryptic speciation across many phyla has been increasing over the years, particularly with the advent of molecular techniques (Bickford et al. 2007). Therefore, it seems highly likely that the total number of sponge species is nearer 15,000 (Hooper & van Soest 2002) compared to the current confirmed number (roughly 8,500) (van Soest et al. 2012). Cryptic species by definition are similar in phenotypic form and are often found in similar habitats in parallel evolution, resulting in cryptic speciation across different locations as described in **Chapter 5** where *H. caerulea* had cryptic species in the Caribbean, Hawai'i and Palmyra. Although it is unusual, two cryptic species can coexist at the same location and exploit the same resources (Zhang et al. 2004, De Meester et al. 2011). Coexisting cryptic species, although morphologically similar, can have disparate ecological interactions with e.g. predators (De Meester et al. 1995), food (McManus et al. 2010), intra and interspecifics (Zhang et al. 2004), parasites (Westram et al. 2011) and symbionts (Jaenike & Brekke 2011), and because they exploit alternate resources may influence food web dynamics differently

(Kaartinen et al. 2010). In the case of cryptic sponges they may appear similar because their functional morphology is constrained (Manuel et al. 2003, Botting et al. 2012) but they can in fact differ in other traits, for example chemical defences (Haber et al. 2011) and food source niche partitioning (Perea-Blázquez 2011), which allows them to coexist in the same habitat and increase the biodiversity.

Figure 6.1 Map indicating the global diversity of sponges in each of the 232 marine ecoregions of the world highlighting the Line Islands where Palmyra is located and Hawai‘i, in the Central Pacific. Modified from van Soest et al. (2012).

6.1.2 Filtration rates

To feed and respire, sponges filter vast quantities of water and can be particularly influential on semi-enclosed bodies of water (Peterson et al. 2006; Chapter 4). Gross morphology influences the amount of water filtered by a sponge over time by affecting the surface area to volume ratio and water flow over the surface of the sponge (Reiswig 1974, Frost 1980, Savarese et al. 1997, Osinga et al. 2001, Peterson et al. 2006). However, the majority of pumping rates recorded in the literature only report one or two species at a single location, with the majority being non-encrusting species (but see Peterson et al. 2006). Comparisons between morphologies are also difficult to make across studies as the site location may affect the pumping rates (even for sponges with the same morphology), as food availability and other environmental conditions (e.g. temperature and water flow) vary (Riisgard et al. 1993, Coma & Ribes 2003).

Therefore, the work in this thesis (**Chapter 4**) provides directly comparable information on filtration rates of sponges for a range of species and morphologies. Overall, I found that the average filtration rate per unit volume of lagoon sponge is comparable to other freshwater and marine species (Reiswig 1974, Frost 1980, Savarese et al. 1997, Osinga et al. 2001, Peterson et al. 2006), but with lower variability between species (e.g. Reiswig 1974, Peterson et al. 2006). This low variability in sponge filtration rates across morphologies at Palmyra suggests a link to low variability in environmental conditions in the lagoons (**Chapter 2**). This low variability in environmental conditions therefore made it possible to compare filtration rates between species and morphologies accurately. I found that morphology was more important than species level differences in affecting the filtration rates, as multiple species could be grouped by morphology (**Table 4.1**).

This research was only the third example (the other two were: (Peterson et al. 2006, Perea-Blázquez et al. 2012) examining the impact that the filtering capabilities of an entire sponge assemblage can have on the water column in a habitat, but was the first to examine the impacts of sponges on the removal of DOC and dissolved oxygen. This work was also the first to highlight the importance of incorporating the propagation of errors when scaling up recorded measurements and values e.g. from sponge filtration rates to area and volume of the lagoons. This is also one of the first studies to use propagation of errors in a marine case study to answer an ecological question, as typically this work has focused on terrestrial systems (Deutschman et al. 1999, Ruckelshaus et al. 2003, Chave et al. 2004). Propagation of errors allows us to incorporate the variability in calculations which have more than one measured variable and gives the maximum possible error around the mean, so providing greater confidence in the results. Knowing the maximum and minimum filtration capabilities of sponge assemblages illustrates that even assuming the lowest filtration rates that the sponges still turnover all the water in the lagoons every six days, which means they still roughly filter all of the available water in the lagoons at least three times before it leaves. Therefore, it seems likely that sponge assemblages, particularly in a semi-enclosed body of water, play an important role in water column filtration. Understanding the impacts that entire assemblages can have on a system can be complex and not initially apparent. Palmyra Atoll provides an excellent model site to not only understand the current biological impacts that species can have on habitats but

also how historical anthropogenic perturbations can affect a system long after it has happened.

6.1.3 Phase shifts

Chapter 3 indicates that there is strong evidence that a coral to sponge dominated phase shift has occurred in the lagoons at Palmyra. Phase shifts as a result of anthropogenic disturbances have often been noted in marine systems, which can lead to a loss of coral cover and species diversity (McManus & Polsenberg 2004, Mumby 2009). The majority of case studies observe dominance shifts from coral to algae; however this is not the only trajectory that reefs may follow after anthropogenic pressures alter habitats (Norström et al. 2009). A number of studies have observed phase shifts from coral to corallimorphs (Work et al. 2008), soft corals (Stobart et al. 2005), anemones (Chen & Dai 2004), ascidians (Bak et al. 1996) and sponges (Aronson et al. 2002, Rützler 2002, Ward-Paige et al. 2005). Despite the number of coral to sponge phase shifts already documented, this is the first research to demonstrate this outside of the Caribbean. Describing this phase shift is particularly interesting because Roff and Mumby (2012) predict that the Caribbean is predisposed to phase shifts in comparison to Pacific reefs, which are more resilient to these events. Palmyra Atoll is considered a near-pristine reef (Sandin et al. 2008) and therefore more resilient to perturbations like bleaching and coral disease (Bellwood et al. 2004, Côté & Darling 2010, Williams et al. 2010, Williams et al. 2011b); however, the lagoons are heavily degraded making them more susceptible to introduced species (**Appendix I**) and phase shifts (**Chapter 3**). Therefore the resilience of an ecosystem is not just regionally dependent but also strongly influenced by local levels of degradation. It is also commonly accepted that one mechanism of phase shifts is the loss of consumer predators (Bellwood et al. 2004). However, at Palmyra there is a high abundance of apex predators (DeMartini et al. 2008, Sandin et al. 2008); therefore it is unlikely that this is a mechanism which perpetuates the phase shift within the lagoon. Instead the primary mechanism was the dredging of the lagoons in WWII, but the intact food web at Palmyra should assist with the eventual recovery of this degraded habitat.

Overall the work at Palmyra Atoll adds to the body of knowledge that not all current assumptions on sponge ecology need necessarily apply to all locations, particularly those found in different oceans (e.g. Motta 1989, Becerro et al. 2003). The

work at Palmyra Atoll has increased our knowledge on the diversity of sponges in an area where there is very limited information on sponges. It revealed that the number of species is comparable to other locations (**Chapter 2**); however, the diversity is likely to be higher not only at Palmyra Atoll or the Central Pacific but globally as well. In particular, sponge diversity is greatly underestimated due to the potential for high levels of cryptic speciation (**Chapter 5**) and through species introductions (**Appendix I**). Although introduced species may increase diversity they can also threaten native species diversity. However, as the results of this thesis indicate (**Chapter 3** and **4**) it may be unimportant at Palmyra that the sponges are introduced if they are not having a negative impact on the atoll system.

6.2 Are all marine “invasions” bad?

There is considerable evidence to support the negative impacts of invasive species on recipient ecosystems and biota (Briggs 2012, Simberloff et al. 2012). Invasive species are purported to cost the United States US \$120 billion a year in damage (e.g. through predation, extinctions, spread of pathogens) and control measures implemented to remove them (Pimentel et al. 2005), which because of logistical reasons are particularly costly in remote locations like Palmyra Atoll (Wegmann et al. 2011). However, controversially it has been suggested that not all alien species have negative impacts. Gurevitch and Padilla (2004) suggested that only 6% of threatened taxa will face extinction due to invasive species and < 2% of extinctions are a result of alien species. There are also a number of reports indicating that species diversity has even increased with the introduction of non-native species (McKinney 2002, Tecco et al. 2006).

Unfortunately, introduced species are often found in association with habitat disturbance or loss, making it unclear whether any negative impacts on native species are due to invasive species or simply correlate with the effects of habitat disturbances (Didham et al. 2005, MacDougall & Turkington 2005). Within the lagoons at Palmyra, the sponges are the dominant fauna on the hard substrate with few coral species (Williams et al. 2008; see Chapter 2); however, pre-WWII corals were purportedly prominent in the lagoons before the military modifications (**see Chapter 3**) (Anon. 1947). Artificial substrates, like those in the lagoons at Palmyra, often harbour introduced fouling communities including sponges (Sutherland & Karlson 1977, Tyrrell & Byers 2007). The structures and the inevitable disturbance to the environment during

the military construction most likely shaped the subsequent communities at these sites by creating ecosystems better suited to fouling communities and reducing the capability of the native fauna to compete (Vitousek et al. 1997, Mooney & Cleland 2001, Tyrrell & Byers 2007), even potentially resulting in a complete phase-shift (**see Chapter 3**). Palmyra Atoll is therefore an extreme case of a habitat subjected to a rapid pulse disturbance, but it still raises the question as to whether the “newly” dominant sponges are having or will potentially have a negative/positive impact on the atoll ecosystem directly and/or indirectly.

Direct competition of the sponges at Palmyra with endemic species was not studied in this thesis although I did observe (**Chapter 4**) a potentially positive impact on the water column by the sponges through the removal of DOC. High concentrations of DOC can cause coral disease and death, and is worryingly the highest on the reefs at Palmyra compared to the rest of the Line Islands (Dinsdale et al. 2008). Other than removing DOC sponges can also reduce harmful bacteria (Fu et al. 2006, Stabili et al. 2006) and phytoplankton levels (Peterson et al. 2006) in semi-enclosed bodies of water as well as accumulate heavy metals including iron, copper, nickel, lead, zinc and cadmium making them useful biological filters and biomonitors of water quality (Patel et al. 1985, Hansen et al. 1995, Cebrian et al. 2007, Padovan et al. 2012). At Palmyra the sponge assemblage filters the lagoon water every 3–6 days and can remove up to 19% of DOC which suggests that sponges also have the ability to reduce the risk of phytoplankton blooms as suggested by Peterson et al. (2006), which in extreme cases causes eutrophication and hypoxia (Cloern 2001). Gordon et al. (1971) noted that in coral reef lagoons it is the residence time of the water that will influence whether phytoplankton blooms will occur because it will determine the level of nutrients. Delesalle and Sournia (1992), however, found no link with nutrients and residence time, but did find a linear relationship with chlorophyll-*a* levels. Therefore based on the chlorophyll-*a* levels in the lagoon it was estimated that the mean residence time in the lagoons at Palmyra was 22 days (± 13). Considering that the residence time is longer than the time taken for the sponges to filter all of the flowing water in lagoons (3–6 days) and the high retention rates of sponges, it seems likely that the sponges have the potential to regulate the lagoon ecosystem by preventing eutrophication events (Grall & Chauvaud 2002).

The US construction of a military base at Palmyra in WWII potentially all but removed the native species from the lagoons and changed the environment (e.g. by

removing the hard substrate and reducing water flow) (Dawson 1959, Collen et al. 2009) so it was no longer suitable for the re-establishment of endemic species such as corals and clams. The proliferation of the sponges in the lagoons, a number of which are non-indigenous, however may not be a negative side-effect. If the sponges are vigorously filtering the water while not competing with native species and not currently extending onto the adjacent near-pristine reef, then they should not be viewed in a negative light. However, on an ecological timescale the time since the modifications is fairly short and it is unknown what will happen to the sponge assemblages in the future. There is still a chance that they could extend onto the reef if environmental conditions change to become more similar to that of the lagoons (**see Chapter 2**). However, the sponges may also play a key role in the natural succession and recovery of the lagoons. Assuming that the dredging removed the majority of reef-forming species and left relatively clear boulders, the sponges have already increased the complexity of the benthos since the WWII modifications. The added structure of the sponges also creates turbulence (e.g. small eddies) and reduces water flow over the substrate, therefore increasing residency time of particles in their locality and allowing more time for particle consumption by all suspension feeders (Gili & Coma 1998). With the addition of more energy to a system through increased water flow (i.e. with the break through of the north-south causeway at Palmyra (**Chapter 1**)) species complexity can again increase due to a greater availability of food particles and therefore available niches (Margalef 1997). Generally, fouling communities will be the first to settle on a cleared substrate, which are generally encrusting species (like the majority (79%) of the sponges in the lagoon), but over time there might be an increase in digitate and massive species (currently 21% of sponge species) (Richmond & Seed 1991). Although it should be considered that not all introductions have negative impacts, fouling communities often consist of potentially harmful introduced species, a threat which increases with the prospect of global climate change (Sorte et al. 2010). Therefore, establishing which species are introduced and the path of introduction may be important to the future management of Palmyra Atoll as well as other locations that undergo high levels of disturbance such as bays and harbours.

Molecular tools are often useful in understanding the unintentional introduction of species, including confirming the species identification and indicating the path of introduction (Lee 2002, Molnar et al. 2008, Wilson et al. 2009), which can then be managed (e.g. via hull cleaning) to reduce the influx of further non-endemic species

(Godwin 2003a). Establishing the path of introduction can yield surprising and informative results important to the future management of remote sites. For example Concepcion et al. (2010) found that the snowflake coral (*Carijoa riisei*) in Hawai‘i, once believed to be an introduced species from the Caribbean was in fact an endemic species and most likely introduced to the Caribbean from Hawai‘i, which resulted in the cessation of attempts to remove it from the commercially important Antipathidae black coral.

Globally it is also becoming clear that despite the lack of conventional barriers to gene flow in marine systems (Bierne et al. 2003), a number of species are more structured and isolated than previously thought (López-Duarte et al. 2012, Schulze et al. 2012) and the once considered cosmopolitan marine species (with high intra-species plasticity) are often complexes of cryptic species (e.g. Klautau et al. 1999, Blanquer & Uriz 2007). Molecular analyses have aided in the clarification of cryptic speciation (Boury-Esnault et al. 1992, Muricy et al. 1996, Blanquer & Uriz 2007), which is required for the identification and management of invasive species (Bickford et al. 2007). Species identification using genetics is particularly useful with sponges due to their high morphological plasticity and low number of informative characteristics (Erwin & Thacker 2007), which hinders taxonomic clarity (Hooper & van Soest 2002). Chapter 5 supports the notion that *H. caerulea* was likely brought to Palmyra from the Caribbean, but the interpretation was complicated by the number of cryptic species. Therefore the number of total species is likely to be higher than originally identified in Chapter 2. Also, more importantly it demonstrates the ability for species to be introduced to the lagoons at Palmyra, a number of which may have been introduced by the more recent (post-WWII) visits by yachts to the atoll (see **Appendix I**). Therefore, the potential for further introductions to the lagoons at Palmyra is likely and should be closely observed and managed, as even though the sponges are currently not a threat other introductions might be.

6.3 The future for Palmyra’s lagoons and sponges

6.3.1 Planned removal of the north-south causeway

In 2008 the USFWS outlined a proposal to encourage the restoration of Palmyra’s lagoons by removing a section of the causeway to restore water flow across the Atoll (**Chapter 1**). It is predicted that removing the causeway will increase the east to west water flow in the lagoons and relieve the sediment stress on the eastern backreefs, but

will also potentially have further ecological consequences for the lagoon and western reef (Williams et al. 2011c). If turbidity increases substantially in the lagoons there is also a possibility that not only coral species will be affected, but the sponges as well. Sediment loading on sponges could affect them in the short and long term via reducing sponge metabolism (Bannister et al. 2011) and pumping rates (Gerrodette & Flechsig 1979, Tompkins-MacDonald & Leys 2008) as well as reproductive output (Roberts et al. 2006, Whalan et al. 2007) and larval survival (Maldonado et al. 2008, Wahab et al. 2011). Despite the unknown origin of all of the sponge species in the lagoon, their highly efficient water filtering capabilities might be having a positive influence in returning the lagoons to their pre-war state. For example, Peterson (2006) found that sponges have the potential to control nuisance phytoplankton blooms in the Florida Keys. Increased flow between the lagoons, when the north-south causeway was breached in 1979 (Collen et al. 2009) appears to have started the natural restoration of the lagoons. Dawson (1959) noted that soon after the military modifications, the lagoons were essentially dead with no living coral worth mentioning, and the East Lagoon was filled with sediment, was stagnant and often smelt of hydrogen sulphide. However, currently more than 7 genera of coral (Williams et al. 2008) inhabit the lagoons, already indicating an improvement in the water conditions. The increase in sediment due to the removal of the causeway would not only smother the corals already in the lagoon but further impact larval settlement and survival (Hodgson 1990, Connell et al. 1997). Coral larvae in particular cannot settle in shifting sediments (Rogers 1990). This would suggest that with the limited hard substrate and high sediment in the lagoons, the time it would take for corals to re-establish might increase by preventing coral and sponge larval settlement and survival (Hodgson 1990, Connell et al. 1997, Maldonado et al. 2008). Therefore I suggest, along with Williams (2011c), that any removal of the causeway be taken in small incremental steps with monitoring of the reef and lagoon species, because if the sponges are removed the conditions of the lagoons could worsen (**see Chapter 4**) as well as inhibit the current natural restoration of coral species already taking place.

6.3.2 Shipwrecks and military debris

The ability of the sponges to accumulate heavy metals may also form an important functional role at Palmyra because the military construction activities (Dawson 1959) and the shipwrecks (Work et al. 2008) may have led to higher levels of

heavy metals such as iron into the surrounding environment (Youssef & El-Said 2011). The long-line shipwreck at Palmyra on the western terrace, which grounded on the reef in 1991, is of particular concern as it is strongly associated with a phase-shift from a coral to corallimorph (*Rhodactis howesii*) dominated reef (Work et al. 2008). It is speculated that as the steel ship corrodes it leaches iron, which is normally a limited resource for many marine organisms (Butler 1998). This leaching may therefore be resulting in the evident dominance of *R. howesii* around the wreck as well as sites with the steel chains marking mooring buoys (Work et al. 2008). Also at Palmyra, considering that there are a number of iron and steel structures across the lagoons, levels of heavy metals including iron are likely to be higher than if no modifications occurred to the atoll. Therefore the sponges may be playing an important role in removing these heavy metals from the water (Perez et al. 2005, Padovan et al. 2012). The sponges could also even be used to assess the levels of other heavy metals across the lagoons (Fichez et al. 2005, Perez et al. 2005, de Mestre et al. 2011) left over from the military modifications. Although there is currently no evidence of heavy metal contamination when examining bivalve shells at Palmyra (Collen et al. 2011), the sponges may provide a different result because all marine invertebrates accumulate trace metals in different concentrations in different tissues/cells (see Rainbow 2002). The sponges may also reveal the metabolically available metal compared to the stored detoxified metals in bivalve shells (see Rainbow 2002, Rainbow 2007). Also because only trace levels of iron may be required for *R. howesii* to proliferate, they may be too low to be considered as contamination, but are still influencing the corallimorphs. Therefore the sponges may be helping to reduce these trace elements from the water column including those from the atoll alterations.

6.3.3 Future identification of sponge extension on to the reefs

Although it currently seems unlikely that the sponges will extend onto the reefs, some invasive species can have lag times before they become more aggressive (Crooks 2005) and therefore the sponges should still be considered a potential risk. Also the time since the military modifications is not long on an evolutionary time scale. Fortunately, detecting the movement of the sponges may be as simple as identifying an indicator species (Zacharias & Roff 2001). Throughout this thesis I have identified *Iotrochota protea* as a potential indicator species because identification is fairly easy compared to other sponges as it stains the skin purple, it is the most dominant species in

the lagoons and therefore can clearly establish on newly available substrates, and it also recruits to the top surface of boulders so it is visible without the need to turn over rocks or look in the reef matrices. Due to the dominance of *I. protea* over any other species in the lagoon it is also the greatest species of concern, particularly as it is likely that it is an introduced species from Hawai‘i where it is endemic (see **Chapter 2**). Although this currently cannot be confirmed because there are no sponge species lists for the Line Islands and *I. protea* has only been recorded in the Hawaiian archipelago (van Soest 2002) and has not been noted at other islands or atolls in the Line Islands including Kingman, which is only 67 km away (pers obs G. Williams). *Iotrochta protea* also generally inhabits sandy or rubble substrates in sheltered bays (Hoover 2006) and has very poor dispersal ability (a few minutes to a few days; (Maldonado 2006)). Natural colonisation between the islands therefore seems unlikely.

6.4 Concluding remarks

Sponges have been studied for well over 100 years with considerable advances made in this field; however, relatively little of this research has been conducted in the Central Pacific. This thesis reveals that the species diversity in the Central Pacific can be considerable even in remote locations and highlights the important filtering role that sponges can have on an ecosystem even if the assemblage includes introduced species. Molecular examination into the introduced species, *H. caerulea*, suggests that it was introduced to Palmyra from the Caribbean via Hawai‘i, most likely via boating activity. The genetic analyses also revealed high levels of cryptic speciation, which indicates that the identification of sponge species introductions using *in situ* identification can be difficult. Ecologically, my work at Palmyra demonstrated that environmental conditions alone can maintain sponge assemblages in one location. It was also found that locations other than the Caribbean have undergone a rapid coral to sponge phase shift; however it is important to note that not all introductions are negative as they may eventually result in the natural succession of a system back to its climax community with time.

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Appendix I

Published manuscript: Records of non-indigenous marine species at Palmyra Atoll in the US Line Islands.

Records of non-indigenous marine species at Palmyra Atoll in the US Line Islands

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Globally, introductions of non-indigenous species have caused dramatic economic and ecological damage. Most research on marine invasions has occurred in locations easily accessible by researchers, but much less is known about introductions to remote islands. In the central Pacific, Palmyra Atoll represents one of the last remaining quasi-pristine reef systems left in the world. In the 1940s the Atoll underwent extensive military modifications, potentially making it susceptible to invasive species. Here we describe the presence of five non-indigenous invertebrate and algal introductions at Palmyra, including two sponges, Halidona (Sigmadocia) caerulea and Gelliodes fibrosa; one bryozoan, Zoobotryon verticillatum; one hydroid, Pennaria disticha and one macroalga, Acanthophora spicifera. The Hawaiian Archipelago is the most likely source of the introductions via shipping or yachting activity to the Atoll. Currently, the impacts of these introductions remain unknown although future monitoring will assess the influence of these non-indigenous species on this remote reef system.

Keywords: non-indigenous, introduction, sponge, algae, hydroid, bryozoan, lagoon, coral reef, Palmyra Atoll

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INTRODUCTION

The historic isolation of many Pacific island marine communities generally provides these with greater protection from direct anthropogenic impacts compared to coastal communities. However, many of these island ecosystems such as Johnston and Wake Atolls, and Howland and Baker Islands were drastically altered during World War II due to military construction work (Maragos *et al.*, 2008a). Both the alteration of habitats and the inadvertent and intentional transport of terrestrial and marine non-indigenous propagules began the transition of these habitats away from their pristine state.

Palmyra Atoll (Figure 1) in the US Line Islands is part of the Pacific Remote Island Areas (PRIAs) Marine National Monument created in 2008 and is administered by the US Fish and Wildlife Service of the US Department of Interior. The reefs surrounding Palmyra are richly diverse with high coral biodiversity (Williams *et al.*, 2008) and large numbers of apex predators, providing one of the last remaining baselines for a 'near pristine' reef system (Sandin *et al.*, 2008b). This atoll is a suitable site for studying historical alteration as it was developed into a military installation in the early 1940s, with upwards of 6000 servicemen stationed there during World War II (Dawson, 1959). Prior to development by the military, Palmyra was an undisturbed atoll with numerous small islets forming a perimeter around three distinct

lagoons (Dawson, 1959). The military drastically altered the atoll by using dredged material from the lagoon to build up the islets, and create airstrips and connecting causeways. The causeways formed a perimeter road around the lagoons bisecting the eastern and central sections, essentially creating a fourth lagoon at the west end of the atoll and drastically altering the circulation patterns within the interior of the atoll (Dawson, 1959; Maragos, 1993; Maragos *et al.*, 2008a, b; Collen *et al.*, 2009). In addition, a navigation channel was excavated in the south-western end of the atoll to allow ships to enter the lagoon (Dawson, 1959). Therefore, the recent history of Palmyra Atoll is one of severe habitat alteration and potential exposure to terrestrial and marine non-indigenous species through anthropogenic transport mechanisms.

Dredging characteristically reduces habitat complexity and availability (Auster, 1998; Auster & Langton, 1998) heavily impacting the macrobenthos (Rosenberg, 1977; Wassenberg *et al.*, 2002), changing species composition, diversity and richness (Jones, 1992; Wassenberg *et al.*, 2002; Hiddink *et al.*, 2006). The dredging across the lagoons may initially have left non-indigenous species unchallenged due to the removal of the native biota as well as inadvertently creating conditions more suitable for introduced species over the recipient biota (Vitousek *et al.*, 1997).

The terrestrial non-indigenous species at Palmyra have been documented extensively (Dawson, 1959; Flint, 1992), but no non-indigenous marine species, which may threaten the reefs, have yet been described. Here we present details of the recently reported non-indigenous species at Palmyra Atoll. To date, one macroalgae species and four marine invertebrates have been documented as non-indigenous to this

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Fig. 1. Location of Palmyra Atoll within the Central Pacific Ocean. CI, Cooper Island.

remote atoll and include a red alga, two sponges, a hydroid and a bryozoan.

MATERIALS AND METHODS

Initial surveys for marine non-indigenous species began in 2002 through to 2009 under the auspices of inventory and monitoring activities conducted at Palmyra Atoll by the US Department of Commerce, National Oceanic and Atmospheric Administration (NOAA), Pacific Islands Fisheries Science Center and Coral Reef Ecosystem Division (CRED) (Godwin, 2002). These surveys examined both natural coral reef habitat and altered habitats in the lagoon including sea walls, docks and dredge spoil areas. Additional surveys began in 2008 by researchers from Victoria University of Wellington, New Zealand and the Scripps Institution of Oceanography associated with the Palmyra Atoll Research Consortium (PARC).

Surveys

The surveys for non-indigenous species at Palmyra Atoll were conducted by three institutes with independent objectives and therefore different, but appropriate, sampling methods were used for each.

ALGAE

An initial qualitative survey of *Acanthophora spicifera* (Børgesen, 1910) (see Figure 2) was conducted at a number of sites in 2002 and again in 2008 (as part of a lagoon restoration experiment). Regular annual quantitative monitoring takes place using $10 \times 1 \text{ m}^2$ quadrats along each of $2 \times$

25 m transect lines. Photographs of the benthos are taken and species abundance and composition are determined using image analysis software Photogrid[©] v1.0 (Bird, 2003). During 2008 a number of sites spanning from the western to eastern lagoons at 1–5 m depth were also surveyed.

NON-SPONGE INVERTEBRATES

The CRED surveys were conducted using two $25 \times 2 \text{ m}$ belt transects at each site. Suspected non-indigenous species were sampled and preserved for later taxonomic analysis.

SPONGES

Surveys for the non-indigenous sponges were conducted in 2008 across all four lagoons at twelve sites using $10 \times 1 \text{ m}^2$ quadrats subdivided into 20 cm^2 squares at depths of 6–8 m. Within each square, per species, we counted numbers of individuals, estimated the percentage area cover of both sponges and hard substratum and adjusted all data to the percentage of available substrate.

RESULTS AND DISCUSSION

Acanthophora spicifera

The red alga *Acanthophora spicifera* is currently the only known non-indigenous seaweed species present in the waters surrounding Palmyra Atoll. During surveys conducted in 2002, *A. spicifera* was not found around the atoll despite sampling a large number of sites including both lagoon and reef habitats (Godwin, 2002). However, in 2008 specimens of the alga were found conspicuously attached to several of the mooring buoys anchored in the western lagoon near

Fig. 2. Photographs of identified non-indigenous species at Palmyra Atoll. (A) *Acanthophora spicifera*; (B) *Pennaria disticha*; (C) *Zoobotryon verticillatum*; (D) *Gelliodes fibrosa*; (E) *Haliclona (Sigmadocia) caerulea* purple morph; (F) *Haliclona (Sigmadocia) caerulea* blue morph.

Cooper Island (see Figure 1), the current location of the field station. After this initial discovery, qualitative surveys were conducted around the lagoon and samples were found at the locations shown in Figure 3. *Acanthophora spicifera* is native to the western and south Pacific, the Indian Ocean and the Caribbean, but is a known invader in the Hawaiian Islands (Guiry & Guiry, 2010).

Acanthophora spicifera was originally introduced to the Hawaiian Islands in the 1950s via hull fouling on a barge that was brought from Guam to Oahu (Doty, 1961; Russell, 1992). This species has now spread throughout all of the main Hawaiian Islands and has most likely been transported via hull fouling of small vessels (Smith *et al.*, 2002). Privately owned yachts are allowed entrance to Palmyra's lagoons for short periods of time with special permission and permitting through the USFWS. Therefore, it is likely that *A. spicifera* was transported on the hull of a yacht or small vessel from Hawaii to Palmyra sometime between 2002 and 2008. It likely first established on a mooring near Cooper Island and either through sexual reproduction or asexual fragmentation spread throughout much of the shallow water habitats (>1 m, <10 m) in the lagoon. While

A. spicifera is a red alga its coloration can be highly variable ranging from purple to brown and even yellow. The alga attaches to the substrate with a single holdfast and the upright portion of the thallus has an alternate branching pattern. All of the main branches are covered with short thick spines that radiate sporadically off of the algal thallus. On Palmyra this alga is primarily found on limestone flats or rubble zones spanning in depth from 1–5 m. There are currently no data indicating the ecological impacts (if any) of this algal invader on the already highly degraded lagoon ecosystem of Palmyra, but observational evidence suggests that numerous green sea turtles found on the atoll are consuming it (E. Sterling, personal communications). Specimens of fertile female plants were collected in July 2010 suggesting that *A. spicifera* is now reproducing sexually, which may help facilitate its spread around the atoll. To date this alga has not been found on the outer reef habitats, but future survey efforts should pay special attention to this and other algal invaders as they have the potential to alter the coral and coral-line algal dominated fore reef and reef terrace habitats (Sandin *et al.*, 2008a). Documentation of the spread and ecology of *A. spicifera* around Palmyra Atoll could be very useful for

Fig. 3. Locations of non-indigenous species found across the lagoons at Palmyra Atoll. ● *Acanthophora spicifera*; ● *Pennaria disticha*; ● *Zoobotryon verticillatum*; ● *Haliclona (Sigmadocia) caerulea*; and ● *Gelliodes fibrosa*.

understanding the invasion ecology of this species in other locations. Finally, if any management or control strategy is going to be developed for *A. spicifera* on Palmyra a targeted monitoring programme should be established.

Pennaria disticha (hydroid) and *Zoobotryon verticillatum* (bryozoan)

The hydroid *Pennaria disticha* (Goldfuss, 1820) and the bryozoan *Zoobotryon verticillatum* (Della Chiaje, 1822) (see Figure 2) are the two recorded non-sponge invertebrate introductions to Palmyra Atoll.

Pennaria disticha, also known as the Christmas tree hydroid, is native to the western Atlantic and has now established within the western lagoon of Palmyra (see Figure 3). This species has become cosmopolitan in tropical and warm temperate seas and is a common sessile organism in harbours, natural lagoons and embayments. It has also been documented at remote coral reef habitats in the Hawaiian Archipelago (Godwin, 2006). *Pennaria disticha* has been observed to be an aggressive competitor for space in both man-made and natural habitats in the Hawaiian Archipelago (Godwin, personal observation). This behaviour has proved to be unpredictable with this species and could be associated with variations in environmental factors, which has been shown to be the case for hydroids in other locations (Boero, 1984).

Zoobotryon verticillatum is a stoloniferous bryozoan forming translucent colonies on hard substrates in bays and harbours. The colonies can form streaming masses and tangled mats that have been confused with macroalgae (Coleman, 1999). This species is found in many tropical and warm temperate waters around the world and has an uncertain home range (Carlton & Eldredge, 2009). *Zoobotryon verticillatum* is considered high risk by management agencies in the United States based on documented economic and ecological impacts (Nagy, 2006; Williams, 2007). Its presence

was first recorded at Palmyra Atoll in 2008, at which time it was forming large bushy aggregations at a long-term monitoring site established by CRED in 2002 within the main navigation channel (see Figure 3). These concentrated aggregations have the potential to overgrow coral communities if they spread to fore reef locations and create habitat changes in the shallow subtidal areas of the lagoon. Pederson & Peterson (2002) showed that stoloniferous bryozoans create an ephemeral habitat in shallow subtidal zones that can cause shifts in species assemblages.

The distributions of *P. disticha* and *Z. verticillatum* can be attributed to their ability to foul boat hulls, which is well-documented in remote tropical ecosystems, such as the Hawaiian Archipelago (Godwin, 2003; Godwin *et al.*, 2004) and the Azores (Amat & Tempera, 2009). Palmyra Atoll receives maritime vessel traffic in the form of commercial re-supply vessels from Hawaii and sporadic visits from sailing yachts that have frequented ports in the tropical Pacific. Therefore, identifying the source populations for *P. disticha* and *Z. verticillatum* found at Palmyra Atoll would be difficult.

Haliclona (Sigmadocia) caerulea and *Gelliodes fibrosa*

The two non-indigenous sponge species in the lagoons at Palmyra Atoll (see Figure 3), are the Demosponges *Haliclona (Sigmadocia) caerulea* (Hechtel, 1965), and *Gelliodes fibrosa* (Wilson, 1925) (see Figure 2) from the order Haplosclerida. Both species are found around the main Hawaiian Islands (De Felice *et al.*, 2001; Coles *et al.*, 2006) usually in shallow water embayments, harbours and disturbed habitats, all with restricted water flow (De Felice *et al.*, 2001).

Haliclona caerulea (family Chalinidae), commonly known as the 'Blue Caribbean sponge' originates from the Caribbean Sea where it is found on rocks, dock pilings and mangrove

Fig. 4. Sponge densities (right hand side of graph), and area cover (cm^2) (left hand side of graph) of *Haliclona* (*Sigmadocia*) *caerulea* (H) and *Gelliodes fibrosa* (G) $10 \times 1 \text{ m}^2$ quadrats at each of the 12 sites across the Western (1–6), Central (7–8), Eastern (9–11) and Turtle Pool (12) lagoons at Palmyra Atoll.

roots (Hechtel, 1965; Cubitt & Williams, 1983; Wulff, 1997; De Weerd, 2000). *Haliclona caerulea* is pale blue with a beige interior and is soft and easy to tear. Interestingly, the majority of individuals at Palmyra are found in association with the red branching coralline alga *Jania adherens*. The alga rarely protrudes from the sponge tissue and provides skeletal structuring and rigidity (Carballo & Avila, 2004). This mutualistic relationship also exists in individuals from the Pacific Coast of Panama (Wulff, 1997) and Mexico (Carballo & Avila, 2004), and changes the colour of the sponge from its characteristic pale blue to purple, making the sponge brittle, but still easy to tear. The purple forms retain some blue tissue, normally at the base of the sponge aiding in the identification of this species. *Haliclona caerulea* develops into thickly encrusting or massive forms approximately 40 cm in diameter and 2–15 cm in height. The oscules (0.5–1.5 cm diameter) are on top of thickly walled irregular volcano-like protrusions (1–5 cm in height). The megascleres are bent oxeas (170–230 μm), and the microscleres are C-shaped sigmas (25 μm) (De Felice *et al.*, 2001). The sigmas are common throughout the blue tissue, but less common in the purple forms (Hechtel, 1965).

Gelliodes fibrosa is characterized by its dark grey, blue/black appearance, and tissue, which is fibrous, hard to tear, and if repeatedly squeezed turns beige. It forms thick encrusting mats or branches with a smooth to conulose surface and regular extruding fibres. The oscules, usually found on the flatter encrusting individuals, indent before forming the opening. The megascleres are curved oxeas (160–180 μm) and the microscleres are sigmas (15 μm). *Gelliodes fibrosa* was originally described from the Philippines and was introduced to Hawaii in the 1990s and has now also been documented in Guam (De Felice *et al.*, 2001).

Spatial distribution patterns of *H. caerulea* indicate higher densities at sites across the northern edge of the western lagoon, the centre of the eastern lagoon and the eastern edge of Turtle Pool (see Figure 4). *Gelliodes fibrosa* was primarily located on the southern edge of the western lagoon, and the central lagoon. The abundance of both species was lower in the eastern lagoon, but interestingly *H. caerulea* also had high abundance in the far east of Turtle Pool. The reduction in sponge densities from the west to east lagoons may relate to the reproductive strategies of the sponges. Sponges can either fragment over short distances and/or produce larvae. The larvae are predominantly anchiplan, remaining in the water column for only minutes to days, thus having limited dispersal capability (Maldonado, 2006). Increased densities of *H. caerulea* in the far eastern Turtle Pool suggests that sponge distribution patterns across Palmyra may not be limited by dispersal capabilities, but also influenced by environmental conditions across the lagoons.

It is most likely that *H. caerulea* and *G. fibrosa* were introduced to Palmyra from Hawaii, via fouling on ships and yachts (Godwin, 2003) or other structures (e.g. barges), as Hawaii has always been the predominant shipping connection to Palmyra (Dawson, 1959). Neither De Laubenfels (1950) or Bergquist (1967) recorded either species during their respective sponge surveys in Hawaii, therefore they are considered to be recent introductions.

The ecological impacts of *H. caerulea* and *G. fibrosa* at Palmyra are currently unknown; however, potential impacts include spatial competition with native fauna and a possible threat to corals (De Felice *et al.*, 2001). Sponges are also highly efficient suspension feeders, pumping up to 24,000 l of water a day (kg^{-1} of sponge) (Vogel, 1977) and removing carbon resources, such as plankton, as well as oxygen and

nitrogen from the water column (Reiswig, 1971, 1974). The vast quantity of water sponges pump each day means that in high densities they can significantly impact surrounding pelagic ecosystems (Bell, 2008), even potentially controlling phytoplankton levels (Peterson *et al.*, 2006).

CONCLUSIONS

The modifications to the lagoons at Palmyra during World War II have likely created an environment with reduced water quality and lower circulation now better suited to fouling communities. The presence of these non-indigenous species at such a remote atoll is most likely attributable to man-made transport mechanisms in the form of vessels arriving from harbours and coastal areas such as Hawaii where all five species are also listed as non-indigenous. Future monitoring to assess the invasive potential of all the non-indigenous species established at Palmyra is important to ensure their impacts on the lagoon and adjacent reefs are limited and not progressive.

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Appendix II

Published manuscript: Effect of depth on sponge assemblage structure at Palmyra Atoll, Central Pacific

Effect of Depth on Sponge Assemblage Structure at Palmyra Atoll, Central Pacific

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Abstract: We examined the influence of depth on sponge assemblages across three lagoons at Palmyra; a remote atoll situated in the Central Pacific. The atoll is unusual in that it was heavily altered during WWII resulting in an atypical lagoon system. We conducted surveys at both 6-8m and 12-14m at 3 sites at the atoll and recorded sponge densities, area cover and available substrate for the 29 species found at these sites. Multivariate analysis of sponge densities and area cover revealed significant variability in sponge assemblages between both site and depth. Univariate analysis also showed significant differences in area cover between depths, but not between sites. Diversity indices and the average number of species at each site showed an increase in diversity across lagoons from West to East. This study indicates that despite the small size of the atoll, depth is a factor that shapes sponge assemblages at Palmyra Atoll. The heavy alterations to the lagoon, particularly the area near the channel entrance may be influencing the sponge assemblage structure, however, other environmental variables still need to be investigated.

Keywords: Sponge, depth, Palmyra Atoll, sedimentation, Central Pacific, flow rate.

INTRODUCTION

To examine and identify the ecological processes that shape assemblages, species abundance and distribution patterns must first be described. Sponges are an important component of benthic fauna that are highly diverse as well as being an ecologically important invertebrate taxon. Despite their importance, sponges are vastly and notoriously understudied [1, 2] even though on some reefs, sponges can even exceed the species diversity and biomass of the scleractinian corals [3]. Understandably, due to the remoteness of some locations, such as Palmyra Atoll (located in the Central Pacific), accessibility for researchers can seriously limit our understanding of these assemblages. In the case of Palmyra Atoll, it is only through its recent co-ownership by The Nature Conservancy (TNC) and the U.S. Fish and Wildlife Service (USFWS) in 2001, that this remote atoll can now be extensively studied. Palmyra is of particular interest to scientists because it remains one of the few 'near pristine' reef systems left in the world [4].

Palmyra consists of many islets with shallow lagoons surrounded by an extensive reef system. The lagoons were essentially an unaltered system until the Second World War when the U.S. Navy occupied the atoll making major alterations. The work included enlarging the islets, creating an entrance channel for boats, dredging the lagoons and building a central causeway to connect two islets, effectively interrupting the water flow between lagoons [5]; this would likely have had serious impacts on the marine fauna of the lagoons.

Sponges form an important part of subtidal benthic communities and inhabit most marine ecosystems [6, 7]. The widespread distribution of this phylum is most likely due to a number of adaptive features including efficient suspension feeding capabilities [8, 9] and the ability to regenerate [10, 11]. Depth has long been recognised as a strong influencing factor on marine benthic community structure [12, 13] and therefore an important factor to consider when conducting surveys of new locations to understand assemblage structure.

The atoll system at Palmyra, like the rest of the Line Islands, is unusual in that large macro-invertebrates including sponges are uncommon on the outer reef systems (S. Godwin *pers. Obs.*) [4], usually an ecosystem abundant in such fauna e.g. in the Caribbean and the Indo-Pacific [3, 14, 15]. The reef system is considered 'near pristine', however, the lagoon is far from its original state. Lagoons, are generally low current systems and act like sediment 'sinks' and suffer from high levels of sedimentation [16]. Sponges, like many marine invertebrates are suspension feeders and as such obtain oxygen and food in the form of organic carbon from the water column [17]. High levels of sediment may clog the delicate filtering apparatus of sponges [18, 19]. However, they have been commonly documented inhabiting areas of high sedimentation [14, 16, 20] [21, 22] and it is therefore of considerable interest to understand the distribution patterns of sponges within the lagoon system at Palmyra Atoll, particularly as it is an altered environment.

Understanding the diversity and abundance of sponges in the lagoons at Palmyra is also very important from a management perspective because the diversity, distribution and abundance of sponge assemblages is presently unknown, despite sponges appearing to be the dominant fauna of the lagoon environment. The lagoons at Palmyra are densely populated with sponges, however, little is known of their potential impact on the surrounding ecosystem or whether

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they are even native to Palmyra or were introduced during military occupation. Furthermore, another important reason to survey and study the sponges at Palmyra is that the lagoons are very shallow in places; areas of low water flow and the high sponge biomass could potentially remove large quantities of nutrients and oxygen from the water column [23, 24]. Therefore, changes in sponge assemblages have the potential to affect the environment and organisms in the lagoons and even the reef as the water flows out across the reef systems [25].

The aim of this study is to determine the effect of depth on sponge assemblages at three different sites in the lagoon system at Palmyra Atoll. We quantified the sponge species density, cover, diversity and evenness, relative to the available boulder substrate at these different sites and depths.

METHODS AND MATERIALS

Study Site

Palmyra Atoll is located at the North-western end of the Line Islands ($5^{\circ}53'N$, $162^{\circ}05'W$), 1930 km south of Hawaii (Fig. 1). The atoll was designated a National Wildlife refuge in 2001 is now jointly owned by The Nature Conservancy and U.S. National and Fish and Wildlife. Palmyra is listed as one of the U.S. Pacific Remote Island Areas (PRIAs) and in 2009 became part of the Pacific Remote Islands Marine National Monument (8336).

Sampling Design

Surveys were conducted using SCUBA within the lagoons at Palmyra Atoll (See Fig. 1) between June to July and October to November 2008. Sponge assemblages were sampled at three sites across the three main lagoons at shallow (6-8m) and deep (12-14m) water sites. Fig. (1) indicates the location of the sites within the three lagoons. Site 1 is located in the Western lagoon adjacent to Strawn

Island ($05^{\circ} 53'.318''N$, $162^{\circ} 05'.860''W$). Site 2 is in the Central lagoon on the south west side of the North-South causeway ($05^{\circ} 53'.246''N$, $162^{\circ} 03'.880''W$) and site 3 is north east of the Eastern lagoon just south of Aviation Island (see [5] for all island names); from here on each site is referred to by the name of the lagoon in which it is found, although it is not necessarily considered to represent the lagoon as a whole. The sites are characterised as having sandy slopes ($40-50^{\circ}$) with small boulders generally < 1 m with negligible current flow rates ($< 0.05 \text{ cm}^2 \text{ sec}^{-1}$) and low boulder disturbance levels.

At each of the three sites, $10 \times 1 \text{ m}^2$ quadrats (divided into $20 \times 20 \text{ cm}$ sub-sections) were haphazardly placed over clusters of boulders and surveyed by counting the number of individual sponge patches (sponge density) and estimating the percentage area covered and available substrata for each species per sub-section. Sponge density and cover values were then adjusted to the total amount of available substrate to provide sponge abundance data per m^2 of available substrate. Each species was given a number ID based on preliminary laboratory analysis (although these species have been distinguished they have not currently been identified).

Statistical Analyses

To characterise sponge assemblages, and compare them between sites and depths a number of indices were calculated including: number of species (S); Hill's numbers N_1 and N_2 and the modified Hill's ratio (N_2/N_1) [26]. The Hill's N_1 and Hill's N_2 assess species diversity by examining the influence of rare and dominant species on assemblage structure, respectively. The modified Hill's ratio examines species equitability (evenness).

Spatial patterns of sponge densities and area occupied across the factors 'sites' and 'depths' were examined using a 2 factor nested permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations [27] based on a zero-adjusted Bray-Curtis similarity matrix. The

Fig. (1). Map of Palmyra with sites surveyed across the 3 lagoons at Palmyra Atoll. Site: 1= Strawn (Western lagoons), 2= South Cut (Central lagoon), 3= Aviation (Eastern lagoon).

nature of the nested design used in the PERMANOVA test means any significant difference between depths is strictly relative to the sites within which they are nested. Site was a random factor (3 levels) with depth a fixed factor (2 levels) nested within sites. Pairwise *post hoc* tests were also carried out on significant factors from the main test. Univariate analyses of sponge densities and area covered at the different sites and depths were also conducted using PERMANOVA. All data was transformed using the dispersion weighting pre-treatment, which negates the effect of spatial clustering [28], a characteristic of phyla (such as sponges) where species are known to reproduce asexually through budding, fragmentation and gemmule formation [29]. All analyses were conducted using the PRIMER v6 statistical package [26] in combination with the PERMANOVA+ add-on package [30].

RESULTS

A total of 29 distinct species were identified across all sites and depths; 15 species were found at both depths at the Western lagoon site, 19 species were found at Eastern lagoon site at both depths, while 15 species were found at the shallow site and 19 at the deep site at the Central lagoon site. Despite the similarity in the total number of species between sites and depths, assemblage composition differed considerably. The mean sponge densities ($\pm$ SE) are shown in Fig. (2) and range from 37 (± 5.9) (Western-deep) to 72 (± 13.7) (Eastern-shallow) sponges m^{-2} , with a mean of 51 (± 9.6) sponges m^{-2} across all three sites. The mean average sponge percentage cover across all sites was 15.2% (± 2.8) with a range of 10.6% (± 1.8) (Western-deep) to 20.8% (± 1.9) (Western-shallow). Analysing sponge densities with a PERMANOVA test showed no significant differences between sites or depths (pseudo- $F=2.19$; $p=0.07$, pseudo- $F=2.09$; $p=0.06$ respectively). Sponge percentage cover also showed no significant difference between sites (pseudo- $F=1.31$, $p=0.26$), but there was a significant difference between depths ($p<0.05$).

Fig. (2) shows that the Western lagoon site had the lowest mean number of species ($S=7-8$), and the Eastern lagoon site had the highest number of species ($S=9-12$), while the central lagoon had $S=8-10$. The Hill indices N_1 , N_2 and the modified ratio N_2/N_1 in Fig. (2) indicate similar diversity patterns amongst sites and depths. The Western lagoon site (shallow and deep water sampling locations) had the lowest diversity and the Eastern lagoon site (shallow and deep sampling locations) the highest.

Sponge assemblages were significantly different between the 2 factors, 'site' and 'depth' when analysing both sponge counts and area cover (PERMANOVA, $p<0.001$). Furthermore, PERMANOVA pairwise comparisons also found significant ($p<0.001$) differences between all sites and depths.

DISCUSSION AND CONCLUSION

The total number of species was similar at all sites, but sponge assemblages were significantly different between all sites and all depths (with depths nested within sites) across the lagoon system at Palmyra Atoll. We found no significant difference for univariate analyses of sponge densities, but we did find a significant difference between depth and area occupied. The average number of species along with the species diversity indices; N_1 , N_2 and the modified ratio N_2/N_1 , indicate an increase in species numbers and diversity from the Western to Eastern lagoon sites.

Sponge assemblages vary considerably across environmental gradients [21, 31-33], therefore it is unsurprising that this study has shown differences in assemblage structure and area occupied with changes in depth even on a local-scale across lagoons in an atoll 6 km long. Previous studies have shown that sponge species distribution and abundance often vary on small spatial scales [6, 29, 31]. The explanation for the variability in assemblage structure with depth in our study is, however, unlikely to be a simply result of depth, but

Fig. (2). Measures of sponge diversity (mean $\pm$ SE) at the three sites and two depths in the lagoons at Palmyra Atoll. Shallow sites are between 6-8m and deep sites are between 12-14m. $N=10$ for each site. Sponge densities and percentage area occupied are adjusted to available boulder substrate.

the result of a combination of environmental factors which co-vary with depth [32]. Site-specific ecological processes that may account for the sponge assemblage structure identified in our study include; light attenuation [22], water flow regime [6], temperature [34] and oxygen concentrations [35].

Species diversity indices and species number averages indicate an increase in diversity (low to high) across the atoll from West to East. Historically, and more recently, the Western lagoon was the location of the highest levels of anthropogenic disturbance particularly during WWII [5]. The work included extensive dredging, increasing the land mass of the islets and excavating the channel through which large vessels could enter the lagoon and make safe passage to the nearby wharf (just 100m from the Western lagoon site). Habitats subject to dredging are heavily altered, removing complexity and available substrate [36, 37]. Impacts on the macrobenthos is also well documented [38, 39]. Dredging causes changes in species composition, diversity, richness [39-41] and potential permanent faunal changes [40], sometimes even resulting in complete phase shifts [42]. We suggest that the relatively higher level of disturbance at the Western lagoon has potentially lead to a site, with reduced available substrate and created conditions which is habitable by fewer species, since it has the lowest diversity, density and average number of species. The Western lagoon site, however, has the highest sponge area cover (per m² available substrate), which suggests that those species able to deal with the conditions in this environment have proliferated. Environmental factors, as mentioned above, may also provide a further insight into the diversity distribution of sponges across the lagoons and will be investigated in the future.

Diving restrictions at Palmyra (given it is a very remote site) limit deeper surveys, however, we have found that where there is available substrate in the lagoon at shallow depths there are abundant sponge assemblages. Therefore, we have no reason to believe this is not the case in deep waters, however, the deepest parts of the lagoons extend down to 50m and are therefore unlikely to support sponges as the waters here become anoxic (pers. comm. John Collen & Jonathan Gardner). This raises an interesting question as to the impact these abundant sponge assemblages may be having directly, as competitors, or indirectly through their efficient water filtration capabilities on other organisms. The ability of sponges to filter large quantities of water [9] has the potential to affect oxygen and nutrient levels in the lagoon, potentially influencing water quality as it leaves the lagoon and flows on to the surrounding reef. Future work will focus on broader-scale surveys of the sponge assemblages in the lagoons at Palmyra, the influence of environmental variables on distribution and the presence of sponges before the alterations.

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Appendix III

Photographs of collected and identified sponges from the lagoons and reefs at Palmyra Atoll.

Methods to identify surveyed sponges:

All samples were collected on SCUBA and each novel specimen was sampled at least 3 times. A minimum of one sample remained in New Zealand at Victoria University of Wellington, one was sent to the National Autonomous University of Mexico for identification by Dr José Luis Carballo and Dr José Antonio Cruz-Barraza and a third set was deposited in the Bishops Museum, Honolulu, Hawaii in 2012. If a sponge was suspected of being a novel species (even though similar in appearance to an already collected specimen) it was sampled separately. Fourteen specimens were later combined and identified as eight species. In the field each specimen was given a number ID in order to consistently identify the same specimens for data analysis before the identifications were confirmed. Labels refer to those used in the species list in chapter 2 (**Table 2.2**).

Appendix IV

Further ecological and morphological information for the Palmyra Atoll sponge species in Table 2.2

Table IV.1. Information on the collected sponge specimens in the list from Table 2.2

ID	List	Date collected	Site	Habitat	Size (cm)	Depth (m)	Colour in life	Colour in alcohol	Form	Texture	Osculum size	Ostia size	Surface	Internal colour
A	cf. <i>Aphysilla rosea</i>	07.06.08	horizontal, slope	on boulders	<5	4-15m	rosy pink	pink	thin encrusting, <5cm diameter	soft, slimy, easy to tear	small, <5mm	not clearly defined	clear of sediment	rosy pink
B	<i>Dysidea</i> sp.1	14.06.08	horizontal, slope, wall	predominantly on boulders, but also on the Ripple Wharf and in association with <i>Caulerpa</i> algae	<15	4-15m	brown	pale cream	small massive	rough with flexible spikes, fairly easy to tear	large, up to 10mm, thin membrane round osculum	not clearly defined	conulose, holds sediment	brown
C	<i>Dysidea</i> sp.2 cf. <i>atherea</i>	13.06.09	horizontal, slope, wall	predominantly on boulders also found on man-made iron wharf	<15	4-15m	pale blue	pale cream	small massive	rough with flexible spikes, fairly easy to tear	large, up to 10mm, thin membrane round osculum	not clearly defined	conulose, holds sediment	pale blue
D	<i>Spirastrella</i> sp.	14.06.08	horizontal, slope	on boulders	<100	4-15m	dark orange	pale beige/orange	thick encrusting	leathery, soft, cushioned, compressible, easy to tear	large at the end of small mounds and at the centre of converging veins	not clearly defined	hold sediment, veins mostly visible	dark brown turning to orange
E	<i>Scopolina</i> sp.	06.06.08	horizontal, slope, wall	on boulders	<15	4-15m	orange	light beige/brown	thick encrusting to small massive	soft, easy to tear, forming small prominent mounds	large, thin membrane round osculum	not clearly defined	lattice membrane	orange
F	<i>Halichondria</i> sp.1	14.06.08	horizontal, slope	on boulders	<30	4-15m	brown	dark brown/grey	digitate	smooth/soft, easy to tear, creates biosurfactant	large, small thin membrane round osculum	not visible	holds sediment	dark brown
G	<i>Halichondria</i> sp.2	11.06.08	horizontal, slope, wall	on boulders	<15	4-15m	yellow		thin encrusting	soft, slimy, easy to tear	small	not visible	veins only visible when sediment on surface	yellow
H	<i>Hymeniacidon</i> sp.1	13.06.08	horizontal, slope, wall	predominantly on boulders also found on man-made iron wharf	<30	4-15m	brown/orange/green	dull white (initially turned blue)	thick encrusting, often found sticking debris together	fleshy, soft, velvety, lumpy (rugose) surface easy to tear	small and on end of small mounds	not clearly defined	various shades, dark green to bright yellow orange	yellow
I	<i>Halichondriidae</i> sp.1	08.06.08	horizontal, slope, wall	on boulders	<15	4-10m	yellow	pale beige	thickly encrusting	soft, easy to tear, with occasional tube worms making mounds	small, <5mm, thin membrane round osculum	not clearly defined	lattice membrane	yellow
J	<i>Halichondriidae</i> sp.2	11.06.08	horizontal, slope, wall	on boulders but completely endopasmmic (under sand)	<20	4-15m	orange/yellow w	dull white	encrusting	soft, velvety, easy to tear	small, barely visible	not visible	like orange peel, holds fine sediment even after wafting	yellow/orange
K	<i>Chalinula</i> sp.	08.06.08	slope	on boulders and in association with <i>Caulerpa</i> algae (algal balls)	<10	4-10m	cream	translucent light beige	encrusting, <5cm diameter	translucent, velvety, easy to tear	small, barely visible	not clearly defined	holds sediment	cream

Table IV.1. Continued

ID	List	Date collected	Site	Habitat	Size (cm)	Depth (m)	Colour in life	Colour in alcohol	Form	Texture	Osculum size	Ostia size	Surface	Internal colour
L	<i>Haliclona caerulea</i>	14.06.08	horizontal, slope, wall	predominantly on boulders also found on man-made iron wharf	<25	4-10m	purple (pale blue without algae)	dull white	small massive	fine sandpaper/rough, easy to tear	large and prominent on top of chimneys with a rim of thin tissue at the top	not clearly defined	<i>Jania adherens</i> algae visible	dirty beige
M	<i>Haliclona</i> sp.1	11.06.08	horizontal, slope	on boulders	<30	4-15m	purple	pale beige	small massive	soft, easy to tear	medium size, 5-10mm, irregular on surface, sometimes on top of small mounds	not clearly defined	holds some sediment	purple
N	Challyspongiidae sp.	08.06.08	horizontal, slope	on boulders	<20	4-10m	rosy pink	light beige (loses colour very quickly)	thick encrusting	soft, velvety, easy to tear	small, <5mm	not clearly defined	clear of sediment	rosy pink
O	<i>Gelliodes fibrosa</i>	13.06.08	horizontal, slope, wall	predominantly on boulders, also found on man-made iron wharf and in association with <i>Caulerpa</i> algae	<30	4-15m	grey/blue/beige	grey	small massive	rough, cartilagineous, hard to tear	small to medium, indented before forming, thin membrane round osculum	not visible	holds sediment	grey
P	Niphatidae sp.1	11.06.08	horizontal, slope	on boulders	<20	4-15m	grey/blue to beige	pale beige	small cushion	fine sandpaper, spongy hard to tear	5-10mm, irregular on surface, sometimes on top of small mounds	not clearly defined	sediment particles can attach to surface	pale blue to beige
Q	Haplosclerida sp.	04.06.08	horizontal, slope	on boulders	<15	4-10m	purple	translucent pale beige	thin encrusting	soft, slimy, easy to tear	large and infrequent	not clearly defined	some small veins visible	purple
R	<i>Acarnus</i> sp.1	04.06.08	horizontal, slope	in and on boulders and dead <i>Tridacna maxima</i> (small giant clam) shells	<50	4-15m	orange	pale brown/orange	excavating with small irregular chimney extrusions, in groups or spread out	soft, easy to tear, creates chambers within the dead coral	small <5mm, protrude approx 5mm from surface, smaller osculum can be latticed (clathrate)	not visible	irregular protrusions (1-10mm), mostly clear of sediment	orange
S	<i>Acarnus</i> sp.2	11.06.08	slope	in boulders	?	4-15m	orange	pale cream	excavating	follows contours of rock, so rough, no mucous	not visible	not visible	like rock	orange
T	Chondropsidae sp.	04.06.08	horizontal, slope, wall	predominantly on boulders and in association with <i>Caulerpa</i> algae	<40	4-15m	grey/brown/blue to dark olive green/orange	pale cream	thickly encrusting to small massive and digitate	soft, easy to tear, fleshy with polychaete worms forming mounds	large and small randomly scattered (1-10mm), thin membrane round osculum and at the centre of converging veins	not clearly defined	holds sediment, veins mostly visible	grey/brown/blue to yellow/orange
U	<i>Isorochata protea</i>	13.06.09	horizontal, slope, wall	predominantly on boulders also found on man-made iron wharf	<100	4-15m	dark purple	dark purple	thin encrusting to small digitate form	slimy, stains hands purple, fibrous when digitate, otherwise very thin	small, about <5mm on top of small chimney	not visible	sediment on surface shows up	dark purple
V	Isorochotidae sp.	06.06.08	slope	on boulders	<10	4-10m	pink/peach	pale cream	encrusting, <5cm diameter	soft, slimy, tears easily	small, barely visible	not clearly defined	clear of sediment	pink
W	<i>Clathria</i> sp.	07.06.08	horizontal, slope, wall	predominantly on boulders also found on man-made iron wharf	<40	4-15m	orange	pale brown	thin encrusting	slimy smooth, rubs off boulder fairly easily	at the end of a small mounds and at the centre of converging veins	not clearly defined	veins clearly visible	orange
X	<i>Hexadella</i> sp.	14.08.08	horizontal, slope, wall	in rock/boulder crevices	<15	4-15m	pale pink	light beige/brown	thin encrusting	soft, slimy, easy to tear, top lattice layer peels off	small <5mm, across entire surface	not visible	white lattice pattern on top of peachy pink flesh	pink

Table IV.1. Continued

ID	List	Date collected	Site	Habitat	Size (cm)	Depth (m)	Colour in life	Colour in alcohol	Form	Texture	Osculum size	Ostia size	Surface	Internal colour
AA	<i>Darwinellidae</i> sp.	12.11.08	slope	on boulders	<15	10-15m	yellow	brown	encrusting	soft, easy to tear	medium at the end of veins	not clearly defined	prominent veins	pale yellow
AB	<i>Cliona</i> sp.1	25.07.08	slope	on boulders	<20	4-15m	yellow/dark green	dull white	encrusting	fleshy, soft, easy to tear	large, irregular, sponge becomes paler yellow near osculum	not clearly defined	holds sediment in areas	green
AC	<i>Cliona</i> sp.2	12.11.08	horizontal	in boulders	?	10-15m	rosy pink to white/beige	pale blue/green turning to grey eventually	excavating	soft, easy to tear	generally the only visible part, osculum form white chimneys	not clearly defined	holds some sediment	rosy pink
AD	<i>Suberitidae</i> sp.1	22.10.09	horizontal, slope	on boulders	<20	4-15m	orange/lime green	pale beige	encrusting	soft, tears easily	irregular, medium, small thin membrane round osculum	not clearly defined	holds some sediment	orange/yellow
AE	<i>Suberitidae</i> sp.2	16.07.09	horizontal	in boulders	?	4-15m	orange/yellow	dull white	excavating	unknown	not clearly visible	not visible	surface of dead coral,	orange/yellow
AF	<i>Hymeniacidon</i> sp.	12.11.08	slope	on boulders	<40	10-15m	lime green/blue-pale yellow orange	lime green/blue-pale yellow to cream	encrusting, occasionally forming long thin papillae like structures	spongy, soft, fleshy (approx. 3mm thick), easy to tear	large, small thin membrane round osculum	not clearly defined	mostly clear of sediment and veins sometimes visible	blue/green
AG	<i>Halichondriidae</i> sp.3	12.11.08	horizontal, slope	on boulders	<40	4-15m	beige to orange/lime green	pale beige	excavating and encrusting, can be found joining debris together (like glue)	soft, fine sandpaper, easy to tear	large chimneys > 1cm	not clearly defined	holds sediment, slightly translucent	beige-orange/yellow
AH	<i>Haliclona</i> sp.2	06.08.08	horizontal	on boulders	<10	4-10m	pale blue	pale beige	thin encrusting, <5cm diameter	soft, slimy, easy to tear	small, <5mm, but prominent	not clearly defined	slight curling up at edges	pale blue
AI	<i>Niphariidae</i> sp.2	23.11.08	slope	on boulders	<20	10-15m	purple/blue	turns pale beige quickly	small massive	soft, easy to tear	large, small thin membrane round osculum, often at the end of mounds	not clearly defined	holds some sediment	beige
AJ	<i>Microcionidae</i> sp.	14.07.09	horizontal	in boulders	?	4-15m	pale dirty yellow/orange	dull white	excavating	excavates into dead coral only up to 1cm	not clearly visible	not visible	thin layer on the surface of dead coral, holds sediment	pale dirty yellow/orange
AK	<i>Myxillidae</i> sp.	12.07.09	horizontal	in boulders	?	4-15m	orange	dull white	excavating	creates chambers within the dead coral	not visible	not visible	irregular but frequent small holes (1mm)	orange
AL	<i>Poecilosclerida</i> sp.	22.11.08	horizontal, slope	small debris	<30	10-15m	orange/lime green	pale beige	encrusting, generally found joining debris together in sandy areas, translucent	soft, tears easily	not visible	not visible	debris, clear of most sediment	orange/yellow

Table IV.1. Continued

ID	List	Date collected	Site	Habitat	Size (cm)	Depth (m)	Colour in life	Colour in alcohol	Form	Texture	Osculum size	Ostia size	Surface	Internal colour
BA	Astrophorida sp.	13.07.09	Fore reef	in dead coral	?	>10m	brown at edge, paling in	translucent pale brown	excavating	creates chambers within the dead coral	not visible	not visible	irregular protrusions of varying size ranging from 1-5mm	pale orange
BB	Jaspis sp.	17.07.08	Back reef	multiple small protrusions in dead coral e.g. Pocillopora	1-10cm	<10m	dark brown	dark grey surface, pale grey interior	small thick cushions	velvety, hard to tear	one or two large prominent oscular	not visible	small amounts of sediment often attach to the surface	pale brown/mushroom brown
BC	Halisarca sp.	01.11.08	Fore reef	dead coral	2-3cm	>10m	orange	light beige/brown	thinly encrusting	soft slimy	small, scattered oscular	not clearly defined	thin veins visible	orange
BD	Clathrina sp.c	20.08.08	Fore reef	on red cyanobacteria: <i>Lyngbya bouillonii</i> , which grows in coral reef crevices	1-2mm	>10m	translucent white	pale beige/white	small massive	calcareous, brittle	one or two prominent oscular on top	not clearly defined	spicules protrude slightly	white
BE	Hyatella	21.08.08	Fore reef	on dead coral, nudibranch found feeding on this species	3cm	>10m	brown	yellowy/brown	small massive	fibrous, hard to tear	row of oscular on top	not clearly defined	spicules protrude slightly	brown
BF	Cliona sp.3	13.07.09	Fore reef	in dead coral	?	>10m	dark brown	dull white	excavating	unknown	not visible	not visible	irregular small protrusions (1-2mm) lattice of small hexagons, slightly translucent	pale orange
BG	Thetys sp.	17.07.08	Back reef	within reef matrices	1-2cm	<10m	crème	dull white	small ball	tough, squidgy, slightly fuzzy, hard to tear	<3mm, do not protrude	not visible	internal lattice structure visible	orange/green
BH	Callyspongia sp.	16.08.08	Fore reef	in crevices in dead reef matrices	2-5cm	>10m	blue	translucent pale yellow	small massive	tough to tear, fibrous	large, prominent	not clearly defined	visible over entire surface of the sponge	translucent pale blue
BI	cf. Chalinidae sp.	17.07.08	Back reef	only sample found growing on CCA	2-3cm	<10m	brown	dark brown	thinly encrusting	soft slimy	large, scattered across the sponge	entire surface of the sponge not clearly defined	smooth albeit the presence of the oscular and ostia	brown
BJ	Haliclona sp. 4	24.10.09	Back reef	under dead coral ledge	10-30cm	<10m	rosy pink, paling at the edge	pale beige/white	thickly encrusting thickly	velvety firm	scattered on surface, on small mounds	not clearly defined	short squiggle pattern and fine sediment on surface	white/creme
BK	Haliclona sp.3	09.08.08	Fore reef	within reef matrices	15cm	>10m	lilac purple	white	encrusting with prominent thickly	velvety, easy to tear	large, prominent on mounds. Top of mound is paler purple/white	not clearly defined	small amounts of sediment on surface	purple
BL	Niphatidae sp.	19.09.08	Fore reef	on reef, found occasionally competing with coral	50-150cm	>10m	orange/brown	brown	encrusting, irregular	firm, easy to tear	large, on top of chimneys and in the valleys	not clearly defined	thin residue (biosurfactant) left on hands	crème interior
BM	Plankinidae	31.07.08	Fore reef	predominantly on dead coral fragments-bioeroding	?	>10m	sulphur yellow	dark blue (turns blue in contact with	bioeroding	cannot feel texture, rough because within the dead coral	not visible	not visible	within the dead coral fragment	dark blue
BN	Crella (Crella) sp.1	16.08.08	Back reef	under dead coral ledges grows up to edges but never on top	<1m	<10m	orange	pale beige	thickly encrusting	leathery, thick, easy to tear, compressible	large and prominent, at the centre of junction of veins	not clearly defined	large veins, darker brown orange in between veins	darker brown/orange
BO	Crella (Crella) sp.2	20.08.08	Fore reef	on dead coral and CCA in crevices and under ledges, sprawling	5-30cm	>10m	white/pink	pale beige/orange	thickly encrusting	spongy, slightly cushioned, thin layer on top, easy to tear	large prominent, darker around the edge, variety of sizes	not clearly defined	colour not even	pale orange brown pink

Table IV.2. Continued

Microscleres (um)														Megasccleres (um)				Fibres (um)
ID	List	Isochelae	Oxeas 1	Oxeas 2	Oxeas 3	Sigmas 1	Sigmas 2	Spiraster 1	Toxas 1	Styles	Subtylostyle	Acanthostyles	Tylostroangyle	primary fibers				
AA	<i>Darwinellidae</i> sp.													40				
AB	<i>Cliona</i> sp.1		355-395 x 5-9	205-275 x 2.5-4	115-150 x 2-2.5													
AC	<i>Cliona</i> sp.2							10_15			325-400 x 5-15 x 7.5-1.5							
AD	Suberitidae sp.1										290-500 x 2.5-5.0 x 2.5-6.0							
AE	Suberitidae sp.2										260-360 x 4-5 x 6.0							
AF	<i>Hymeniacion</i> sp.									210-435 x 2.5-7.5								
AG	Halichondriidae sp.3		175-305 x 2.5-5.0	375-540 x 5.0-9.0														
AH	<i>Haliclona</i> sp.2		100-120 x 1.25-2.5															
AI	Niphatidae sp.2		162.5-197.5 x 2.5-4.5															
AJ	Microcionidae sp.	10_15							20-65	137.5-275 x 2.5-5 x 3-6.25								
AK	Myxillidae sp.	25-30				30-55						65-215 x 5-12.5	225-280 x 2.5-5 x 4-6					
AL	Poecilosclerida sp.					25-100	7.5-12.5					60-165 x 1-7.5						

Appendix V

Taxonomic histories of *Gelliodes fibrosa* (Wilson 1925) and *Iotrochota protea* (de Laubenfels, 1950)

Gelliodes fibrosa (Wilson, 1925) (accepted name)

Citation:

Wilson, H.V.P (1925). Silicious and horny sponges collected by the U.S. Fisheries Steamer 'Albatross' during the Philippine Expedition, 1907-10. pp. 273-532, pls 37-52. *In*: Contributions to the biology of the Philippine Archipelago and adjacent regions. Bulletin of the United States National Museum, 100 (2, part 4).

Type specimen information:

Original names: *Gellius varius* (Bowerbank, 1875), var. *fibrosus*, new variety and *Gellius fibrosus*.

Collection sites: (during Albatross Philippines expedition 1907-1910), station D5626 Kayoa, Indonesia; Togian Bay, Togian Island, Gulf of Tomini; Celebes (Sulawesi), Indonesia (identifications were made from 2 dried and 2 alcohol preserved specimens).

Morphology: flattened or branching. Branches reach 30-40 cm and are cylindrical or flattened often terminating in pointed extremities. The sponge is firm but brittle (when dry).

Ectosome: Pores are uniformly distributed and close together, oscula are 2 mm in diameter, abundant and situated in well-defined depressions. Numerous small afferent canals (≈ 0.5 mm) extend radially from the surface of the sponge giving a porous appearance.

Endosome and skeleton: Consists of a renieroid reticulum and longitudinal polyspicular tracts. The tracts (often compact enough to be called fibres) are 20-100 μm in diameter and consist of closely packed spicules in approximately 3-12 rows which branch and anastomose obliquely forming a coarse and rather vague network with elongated meshes. The longitudinal tract distribution varies across the sponge. The remainder of the skeleton is made up of a renieroid reticulum with 3 or 4 sided meshes (one to two spicules long) made up of one to four spicules arranged into lines or fibres that are radial to the surface. Spongin is exiguous and only clearly apparent in the

angles of the skeletal and dermal reticulum. Spicules can radiate outward from the nodes of the dermal reticulum. The tracts attach to the reticulum at nodes where several spicules converge.

Spicules: Oxea megascleres, which are smooth and slightly curved and taper evenly at both ends (160-180 μm long and 8-9 μm wide) and sigma microscleres (18-20 μm long), which are common in the interior and dermal membrane.

Notes: When dried the sponge may be light grey or light yellowish brown; in alcohol it can be reddish brown. Identifications were made from dried and preserved samples, therefore *in situ* descriptions were not provided.

Recent published/unpublished records:

Godwin, L. S. (2003). Hull fouling of maritime vessels as a pathway for marine species invasions to the Hawaiian Islands. *Biofouling*, 19(S1), 123-131.

Eldredge, L. G., & Smith, C. M. (Eds.). (2001). *A guidebook of introduced marine species in Hawaii*. Bishop Museum and the University of Hawai'i.

Coles, S. L., DeFelice, R. C., Eldredge, L. G., & Carlton, J. T. (1999). Historical and recent introductions of non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. *Marine Biology*, 135(1), 147-158.

de Laubenfels, M. W. (1935). A collection of sponges from Puerto Galera, Mindoro, Philippine Islands. *Philippine Journal of Science*, 156(3), 327-336.

***Iotrochota protea* (de Laubenfels, 1950) (accepted name)**

Citation:

de Laubenfels, M.W. (1950) The Sponges of Kaneohe Bay, Oahu. *Pacific Science* 4(1): 3-36.

Type specimen information:

Original name: *Hiattrochota protea*

A new genus described as having smooth strongyles as special ectosomal spicules, smooth styles as endosomal megascleres, and birotulates or amphidiscs among the microscleres, without chelas.

Collection sites: Coconut Island (Kaneohe Bay, Oahu), Kaalualu Bay (Oahu) and Honaunau (Kona, Hawaii)

Morphology: Massive, velvety black, somewhat elastic, not remarkable, smooth surface, oscula 1-3 mm and located on the summit of a rounded dome or lobe ($\approx$ 1cm diameter and 0.5 m high)

Spicules: Smooth endosomal styles (135-180 μ m long and 7-10 μ m wide), smooth ectosomal strongyles (140-205 μ m long and 3-6 μ m wide) and amphidisc or birotulate microscleres (12-15 μ m long) with greater than 4 clads at each end and a potentially spiny shaft.

Ectosome: Definite dermis over subdermal spaces, not very fleshy

Endosome and skeleton: Microcavernous with a skeleton similar to *Isodictya* species. Few vague spicular tracts diameter $\approx$ 50-100 μ m.

Notes: in preservative it retains its colour but stains the alcohol dark brown.

Recent published/unpublished records:

Smith, B. D., Donaldson, T. J., Schils, T., Reyes, A., Chop, K., & Dugger, K. (2009). Marine Biological Survey of Inner Apra Harbor, Guam. *University of Guam Marine Laboratory Technical Report*, (126).

Van Soest, R. W. (2002). Family Iotrochotidae Dendy, 1922. In *Systema Porifera* (pp. 594-601). Springer US.

Dollar, S. J. (1979). WRRCTR No. 124 Ecological Response to Relaxation of Sewage Stress off Sand Island, O'ahu, Hawai'i.

Appendix VI

Fish predation trial experiment

In order to examine whether fish predation could be maintaining the sponges in the lagoon I conducted a trial transplantation experiment where sponge species were taken from the lagoon and moved to the backreef to observe whether fish would predate on the sponges. Seven species of sponge ($\approx 5 \text{ cm}^2$) were collected from the lagoon and photographed next to a scale bar for later reference (**Fig. VI.1**). The seven species were (B) *Dysidea* sp.1, (D) *Spirastrella* sp., (F) *Halichondria* sp. 1, (L) *Haliclona caerulea*, (O) *Gelliodes fibrosa*, (U) *Iotrochota protea* and (W) *Clathria* sp. (ID letters taken from the species list in Table 2.2). The lagoon sponges were predominantly encrusting species and therefore if possible were collected on small rock fragments. All other organisms were removed from the rock fragments during the collection process in the lagoon. Removal of organisms only occurred if they could be taken off without damage. If this could not be achieved, the rock was replaced and another was selected. All material was returned to the site of collection after the experiment.

The sponges were then attached to a weighted PVC pipe (**Fig. VI.1**) using zip-ties threaded through pre-drilled holes (which were trimmed before placement on the reef) and placed in a container with seawater during transit to the reef. The water from the container was returned to the lagoon after the experiment. The experiments were set up in areas such as sand/shale patches or areas with dead coral all at backreef sites. If the trial was successful then the sponge samples would have been weighed on scales in a pre-tarred container of water before and after the fish predation experiment. However, for the trial the samples were not weighed as the aim of the trial was to observe whether any fish would consume the lagoon sponge species.

Preliminary trials were conducted 6 times at the backreef sites: Penguin Spit Inner, Middle (x2) and Outer Buoys, the Western Terrace and Tortugonas. These trials were conducted with diver and video surveillance to assure the quality of the experimental equipment. The experiment was in place for 1 h or theoretically for as long as it took for a considerable amount, but not all, of the sponge to be consumed. Predation generally occurs very rapidly on translocated sponges (Dunlap & Pawlik 1996, Wulff 2000). During these trials divers remained at least 10 m away from the

experiment but within visual range to observe fish feeding behaviour and intervene and remove the experiment if fragmentation occurred. The samples were encrusting or low lying massive species and therefore consumed by fish either by biting or scraping, not tearing (Dunlap & Pawlik 1996). Fragmentation is unlikely, but all samples were handled with great care to avoid this as I did not want any chance of introducing any lagoon sponges to the reefs at Palmyra Atoll. Upon successful completion of the preliminary trials, the experiment would have been filmed with no divers present. It was believed that the presence of divers would not inhibit the fish feeding behaviour because they did not appear overly concerned by the presence of divers during the numerous dives prior to this trial.

All videos were watched afterwards despite divers observing the experiment throughout time of filming. The videos were double checked to ensure no fish predation occurred as the divers were in a different location to the camera. Unfortunately no fishes were observed predated on any of the lagoon sponges during these trials, therefore the experiment was not taken any further.

Figure VI.1. Fish predation trial setup with a) sponges photographed adjacent to a scale bar (see arrow) prior to deployment on the backreef, b) the placement of the weighted PVC pipe in a cleared area at Penguin Spit Inner Buoy with the video camera positioned nearby (see arrows) and c) the seven sponge species attached to the weighted PVC pipe with zip ties (untrimmed).